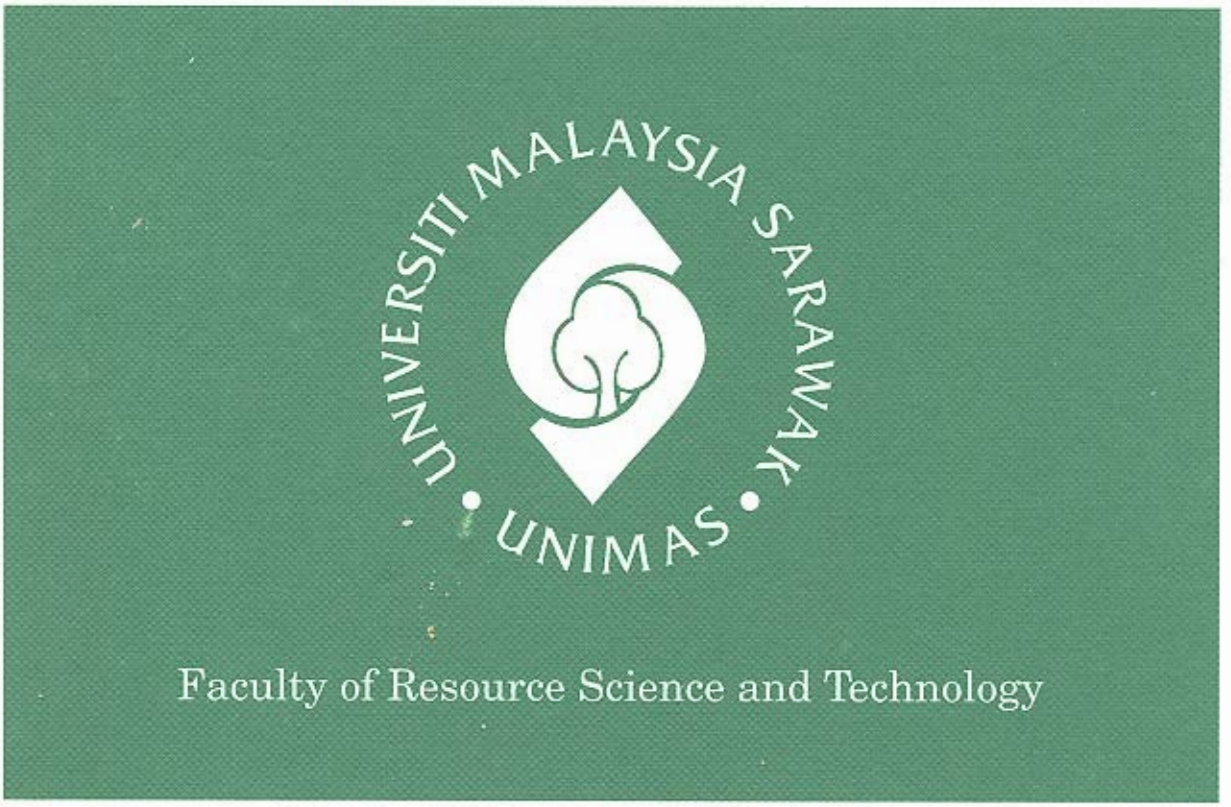

FUNGI CONTAMINANT IN BEANS

Siti Ranieyah Binti Merzuki

Bachelor of Science With Honours
(Plant Resource Science and Management)
2004

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Fungi contaminant in beans from market

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ABSTRACT

Eleven types of bean were used in this study. Seven species fungi were found contaminating the beans. They were *Aspergillus niger, A. flavus, A. fumigatus, A. versicolor, Trichoderma sp., Penicillium sp.* and *Rhizopus sp.* The highest percentage of occurrence of the fungi was *Rhizopus sp.* It was found on 54% of the total 1100 beans examined. Effects of temperature and pH on growth for every type of fungi were also observed. The optimum temperature for the fungi to grow was different depending on the species. The fastest growth of *A. niger* and *A. versicolor* was at 30°C while *A.fumigatus* and *Trichoderma sp.* was at 25-35°C, *A. flavus* was at 30-35°C, *Penicillium sp.* was at 15-25°C and *Rhizopus sp.* was at 15°C. The pH value for the optimum growth of the fungi were at pH 4 and pH 7.

Keywords: Beans, contaminant fungi, temperature, pH

ABSTRAK

Sebelas jenis kacang telah digunakan dalam kajian ini. Tujuh species kulat telah dijumpai mencemari kacang-kacang tersebut. Species-species kulat tersebut ialah Aspergillus niger, A. flavus, A. fumigatus, A. versicolor, Trichoderma sp., Penicillium sp. dan Rhizopus sp. Peratusan kehadiran kulat yang paling tinggi ialah Rhizopus sp. Ia ditemui pada 54% daripada 1100 jumlah keseluruhan kacang yang digunakan. Kesan suhu dan pH ke atas pertumbuhan setiap jenis kulat juga dikaji. Suhu optimum bagi pertumbuhan kulat adalah berbeza bergantung kepada species kulat. Pertumbuhan terpantas pada suhu 30°C adalah A. niger dan A. versicolor manakala A.fumigatus dan Trichoderma sp. adalah pada suhu 25-35°C, A. flavus pada suhu 30-35°C, Penicillium sp. pada 15-25°C dan Rhizopus sp. pada suhu 15°C. pH yang terbaik bagi pertumbuhan kulat adalah pada pH 4 dan pH 7.

Kata kunci: Kacang, kulat pencemar, suhu, pH

INTRODUCTION

There are 300 species of plants that have been used as a main food for human and livestock at all over the world (Macrae *et.al.,* 1993). Cereals are the largest group followed by legumes in terms of global production. Leguminoceae is a family of plant that includes legume and bean, is a great importance resource to provide some world's nutritious crops. They are rich in protein, contain almost two or three time higher than cereals.

In Malaysia, beans crop productions are not satisfactory if compared with other countries like China, Thailand, Burma, Philippine, Taiwan and Indonesia (Normah *et.al.,* 1982). Problems occur during preharvest as well as after harvest. Under favorable conditions, the disease can causes severe defoliation and deteriorate the beans. Factors that influence post-harvest losses of beans are moisture, temperature, pH, spoilage and others. Infected kernels caused early splits and staining shells, thus can reduce marketable yields (Aradhya *et.al.*, 2001). Beans are host to many pathogens, mostly of fungi. Sharma (1989) reported that the common food or plants spoiling fungi are *Aspergillus sp., Rhizopus sp., Penicillium sp.* and *Mucor sp.*

Fungi are achlorophyllous, heterotrophic (saprophytic, parasitic, symbiotic or hyperparasitic) eukaryotic and multicellular and spore bearing organisms surrounded by well defined cell wall made up by chitin (Gerard *et.al.,* 2001). Fungi have their own specialty, for example they consists of feeding system of colorless thread, mycelium and a branch of mycelia or hyphae (Ingold and Hudson, 1993). Fungi obtain food by absorption, except a few lower groups where they take in food by ingestion (Sharma, 1989).

Fungi play an important role in our daily life and ecology. It can give advantages and disadvantages to the world. For example, in medicine, *Penicillium notatum* produces antibiotic while *Aspergillus flavus* can contaminate the foods.

The factors that have crucial effects on fungal growth in beans are moisture, pH and temperature (Macrae *et.al.*, 1993). Moisture and temperature in storage are also the important factors determining species composition of mycoflora on beans, which in turn, affected the seed nutritive value. High seed moisture content could increase fungal infestation, particularly by *Aspergillus spp* (Lokesh and Hiremath, 1993).

The objectives of this study are to determine the types of fungi that contaminating the beans and also to investigate the effect of pH and temperature on growth of the fungi.

MATERIAL AND METHODS

Isolation and identification of contaminant fungi in beans

Eleven types of bean bought from market in Kuching were used in this study. They were pigeon pea or dhall bean (*Cajanus calan*), soybean (*Glycine max*), chick pea (*Cicer arietinum*), mungbean or green bean (*Vigna radiate*), black gram or black bean (*Phaseolus mungo*), blanched groundnut, peanut or groundnut (*Arachis hypogaea*), red bean (*Phaseolus calcaratus*), sword bean (*Canavalia gladiata*) and white bean.

All of the samples were selected randomly, washed with distilled water for three times and dried with filter paper. 100 samples from each type of the bean were inoculated on artificial media, Potato Dextrose Agar (PDA) and water agar. There were 10 replicates for each bean and five beans in every plate. All the plates were numbered by name of the beans, date and number of replicate.

All the inoculated plates were incubated at room temperature for 4 to 7 days. Then, the plates were examined for fungal occurrences. The percentages occurrence of the fungi in the bean on each media were calculated by using the formula:

$$\text{Percentage (\%) occurrence} = \frac{X}{50} \times 100$$

The percentages occurrence of overall fungi in all types of beans were calculated by using the formula:

$$\text{Percentage (\%) occurrence} = \frac{\text{Total of specific fungi in all type of beans}}{\text{Total of all the fungi in all types of beans}} \times 100$$

Pure culture of each fungus was prepared. It was done by observing the fungal colonies on beans under microscope then the young hyphae and spores of the fungi were taken and transferred onto a new agar.

Identification of the fungi was done based on the morphological structures. Slides for each type of the fungus were prepared then examined under light microscope at 10X, 40X and 100X magnifications.

Growth of fungi at different temperature

The tested temperatures were 15°C, 20°C, 25°C, 30°C and 35°C. The pure cultures of 4 to 7-day-old of the isolated fungi on PDA were used as inoculum. The media used for this study was Malt extract Agar (MEA).

Block of 3 mm diameter agar containing mycelia of the tested fungus was inoculated on MEA in 9 cm diameter Petri dish. Three replicates were prepared for each type of fungus and for each temperature. Average colony diameter was determined by measuring two colony diameters, which were perpendicular to each other. The average growth rates of the fungi were calculated.

Average colony diameter, $Di = \frac{d_1+d_2}{2}$

Growth rate (G.R) = $\frac{(D_2-D_1)+(D_3-D_2)+(D_4-D_3)+(D_5-D_4)+(D_6-D_5)+(D_7-D_6)}{N-1}$

Where d1 & d2 = Colony diameter

N = days

D_1, D_2, D_3 ... = day 1, day 2, day 3 ...

Growth of fungi at different pH

The pH values used were 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5. The pure cultures of 4 to 7-day-old of the isolated fungi on PDA were used as inoculum. The potato broth was used as growth media in this test.

The media broth was prepared as following: 200 g of peeled and sliced potatoes were washed in cool tap water for several times until the water was clear. Then, the sliced potatoes were cooked with 1 litre distilled water until tender for about 1 hour. Then, the liquid of the boiled potatoes was filtered and collected. 20 g of glucose were added into the liquid and the mixture was stirred until the glucose dissolved. The required pH was adjusted with Natrium Hydroxide (NaOH) or Hydrochloric Acid (HCl). Every 100 ml conical flask, 15 ml of the liquid was transferred. Then, the mixtures were autoclaved for 20 minutes at 15 psi.

After autoclaved, all the broths were cold at room temperature. Then a block of 3mm diam agar containing mycelia of the tested fungus was inoculated into the conical flask. Three replicates were prepared for each fungus. All the inoculated conical flasks were covered with aluminium foil to prevent any contamination and were incubated at 30°C for 7 days.

The cultures were filtered after 7 days using known dry weight filter paper. Then, the filtered mycelia were dried in oven at 50°C for 2 days. The dried weight of each fungi were calculated by using the formula:

Mycelia dry weight = X-Y

Where; X was dried mycelia + weight of filter paper

Y was known dry weight of filter paper

Data Analysis

All data was analyzed statiscally using One Way ANOVA in SPSS program.

RESULT

Fungal occurrence

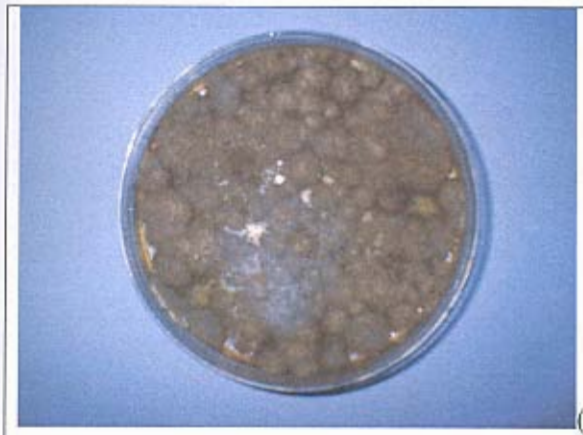
Seven types of fungi were found contaminating in 11 types of beans bought from markets in Kuching. The fungi were identified based on morphological structure. The morphological descriptions of the fungi are shown in Table 1.

Table 1: Description of morphological structures of fungi isolated from 11 types of beans.

Fungi species	Description
Aspergillus niger (Figure 1.1)	The colonies initially white and then become black. The mycelia consist of many branches and septate. Conidiophore were unbranched, aseptate and colourless. The vesicles were round and radiate head shape. Phialides were unicellular, while the conidia were large, roughly and uninucleate.
Aspergillus flavus (Figure 1.2)	The colonies initially white to yellow and then become yellowish-green. The mycelia consist of many branches and septate. Conidiophores were colourless, roughened and unbranched. The vesicles are round and radiate head shape. Phialides were unicellular while the conidia were round, small, globose and multinucleate.
Aspergillus fumigatus (Figure 1.3)	The colonies initially white to blue green and then become gray. The mycelia consists of many branches and septate. Conidiophore were commonly smooth, unbranched,

	aseptate and colourless. The vesicles were round and radiate head shape. Phialides were unicellular while the conidia were round, small, multinucleate and not formed as cylindrical segments.
Aspergillus versicolor (Figure 1.4)	The colonies initially white then turn yellow and pale green. The mycelia were branches and septate. Conidiophore were unbranched, aseptate and colourless. The vesicles were round and had no radiate head shape. Phialides and mostly unicellular while the conidia were round, small and globose.
Rhizopus sp. (Figure 1.5)	The colour of colonies was initially white and then turned to gray. Colonies grew rapidly, filled the 9 cm diameter Petri dish within 2 days and the appearance typically cotton candy like. The mycelium was branched, filamentous hyphae, no septate, rhizoid and stolon present. The rhizoids were repeatedly branched and penetrated the substratum. Stolons were grew above and bend down into the substratum. The sporangiophore was erect, aerial, unbranched and developed from mycelia. The columella was large and some were invaginated. The sporangia were multinucleate, non-motile and produced in round black bodies.

*Penicillium sp. (*Figure 1.6)	The colonies were initially white and become blue gray or olive in time, surrounded by white mycelia. The mycelium were uninucleate, well branched, many septate hyphae, flat, filamentous and velvety. The conidiophores were septate, erect and branched terminate into cluster of phialides. Conidiophores with phialides and terminal chains appear like artist's brush. There was no vesicles. The phialides were oval or bottle-shaped. The conidia were globose, uninucleate and rough.
Trichoderma sp. (Figure 1.7)	The colonies were initially white then turn to dark green. The conidiophores were long, smooth, typically branched with cluster of phialides at the end. There was no vesicles. Phialides were straight, tapering slightly from base to tip. Conidida were smooth and colourless.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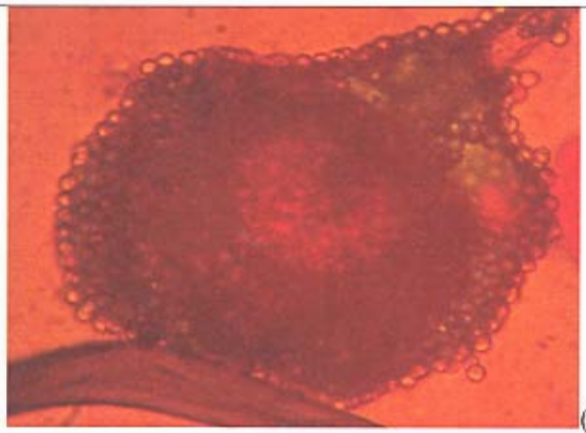
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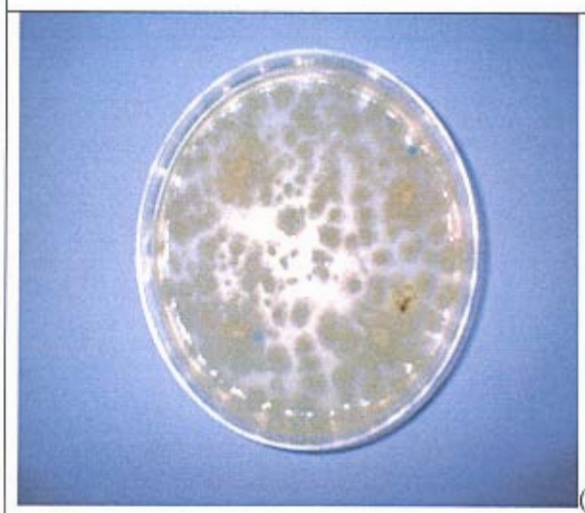
Figure 1.1: *A.niger* (i) Colony on MEA (ii) Conidiophores with conidia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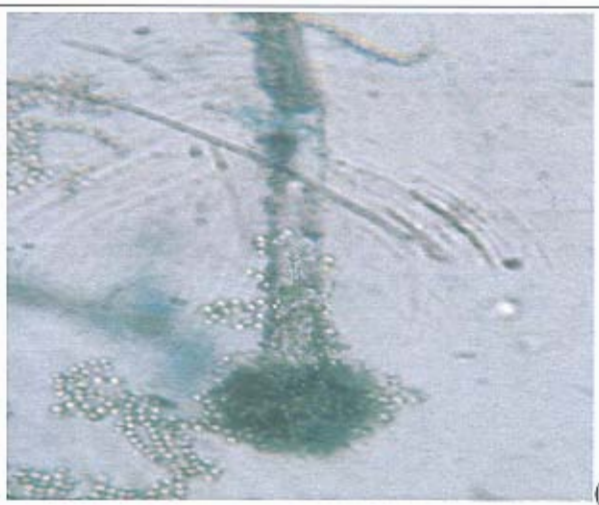
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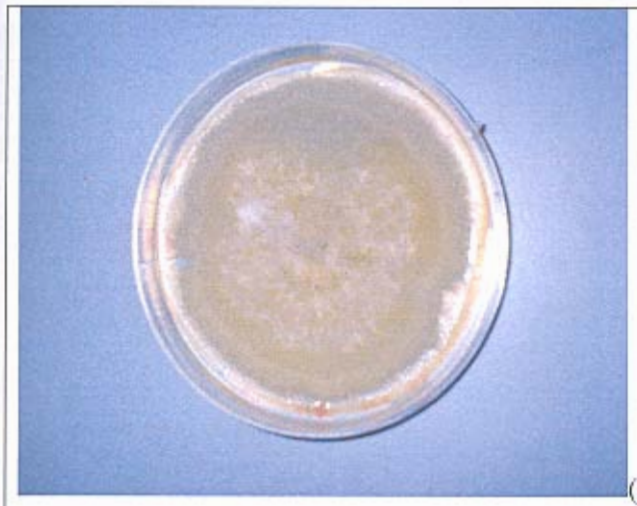
Figure 1.2: *A.flavus* (i) Colony on MEA (ii) Conidiophores with conidia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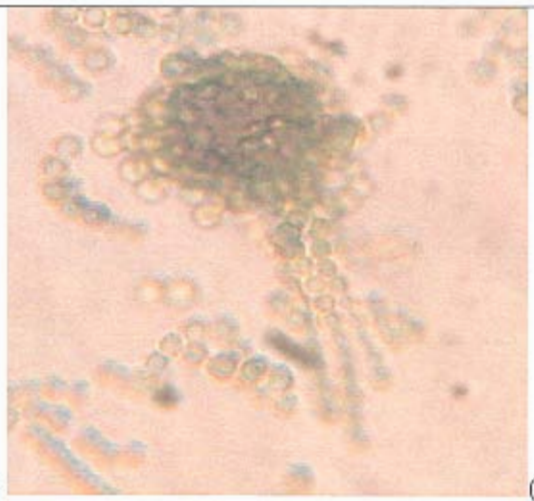
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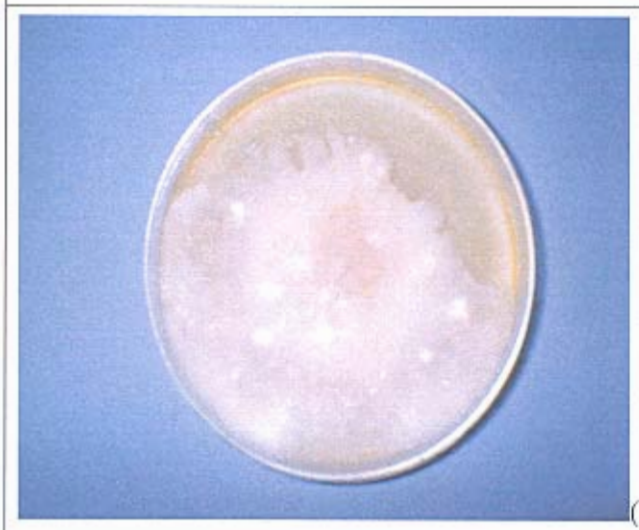
Figure 1.3: *A.fumigatus* (i) Colony on MEA (ii) Conidiophores with conidia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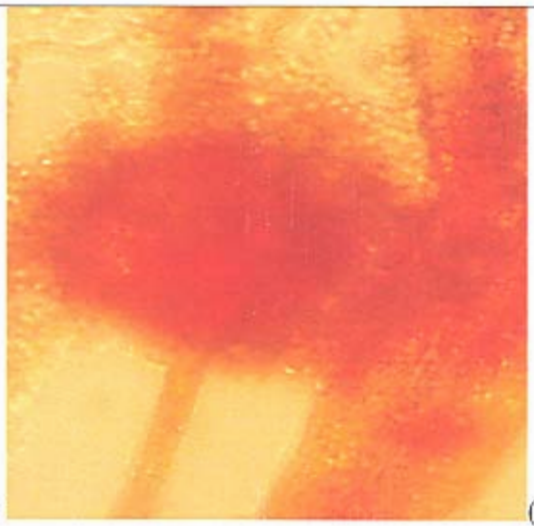
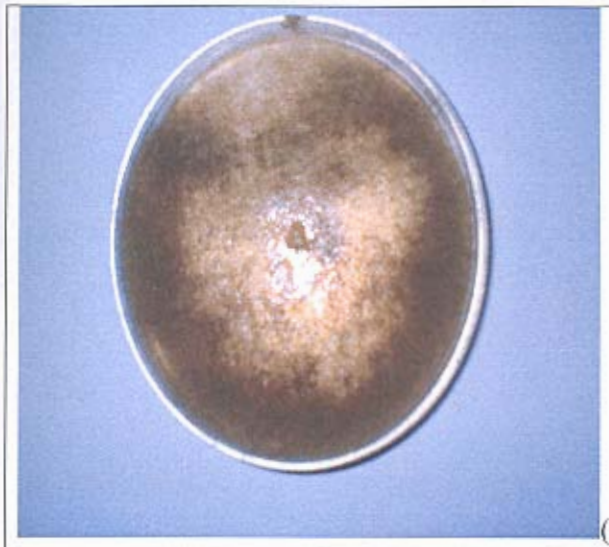

(ii)

Figure 1.4: *A.versicolor* (i) Colony on MEA (ii) Conidiophores with conidia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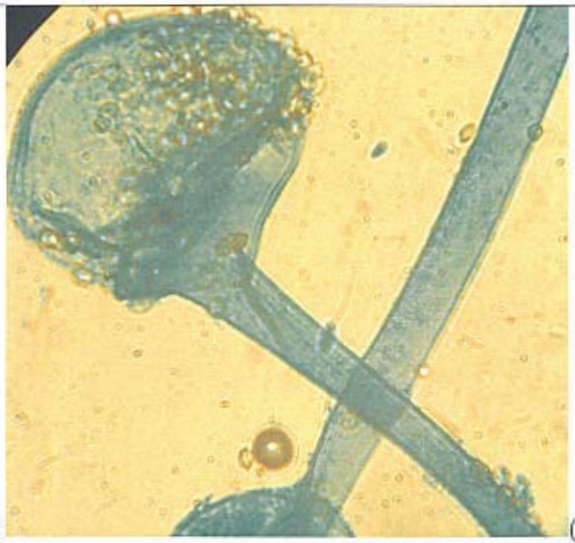
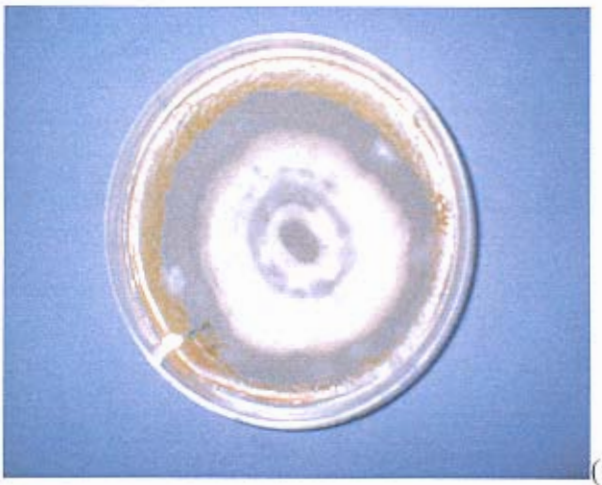

(ii)

Figure 1.5: *Rhizopus sp.* (i) Colony on MEA (ii) Sporangiophore with spores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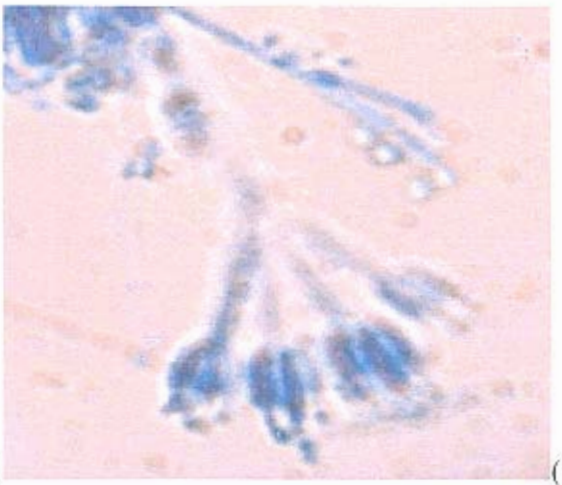
(ii)

Figure 1.6:*Penicillium sp.* (i) Colony on MEA (ii) Conidiophores with conidia

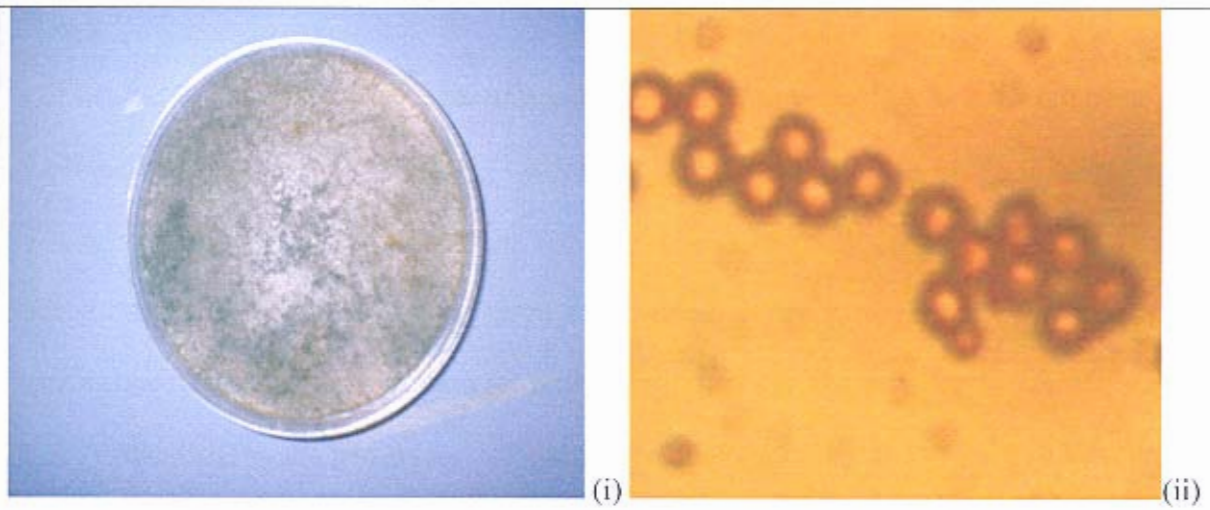

Figure 1.7: *Trichoderma sp* . (i) Colony on MEA (ii) Conidia

Table 2 shows the percentages of occurrence of the fungi on the beans. Sword beans were contaminated by all types of the fungi except of *Trichoderma sp.* Most of the fungi except for *Trichoderma sp* and *A. fumigatus* were found in black beans. Blanched groundnut was also contaminated by most of the fungi except of *A. flavus* and *A. fumigatus*. Less species of fungi was found on small red bean. Only three types of the fungi were encountered.

Table 3 shows the percentages of occurrence of overall fungi that were found on all types of beans. In general, the highest percentages occurrence of fungi on the beans was *Rhizopus sp.* It was found on 54% of the beans. The lowest percentage of occurrence of the fungi was of *A. fumigatus* found only on 1.2% of the beans.

Table 2: Percentages (%) of occurrence of fungi in all types of beans on the different media.

	Fungi species																					
	A.niger			*A.flavus*			*A.fumigatus*			*A.versicolor*			*Rhizopus sp*			*Penicillium sp*			*Trichoderma sp*			**Total no. of isolated fungi**
Types of Beans	**PDA**	**WA**	**AVR**	**PDA**	**WA**	**AVR**	**PDA**	**WA**	**AVR**	**PDA**	**WA**	**AVR**	**PDA**	**WA**	**AVR**	**PDA**	**WA**	**AVR**	**PDA**	**WA**	**AVR**	
Dhall bean	36	12	24	0	12	6	0	8	4	0	0	0	50	38	44	0	2	1	0	0	0	**5**
Soybean	18	20	19	0	0	0	0	2	1	0	0	0	74	36	55	0	2	1	0	0	0	**4**
Mungbean	46	16	31	22	24	23	0	0	0	18	10	14	0	0	0	16	8	12	0	0	0	**4**
Chick peas	4	8	6	0	0	0	0	0	0	0	0	0	100	78	89	0	10	5	0	0	0	**3**
Red bean (large)	14	0	7	0	0	0	0	0	0	4	0	2	100	90	95	12	4	8	14	0	7	**5**
Red bean (small)	0	0	0	0	0	0	0	0	0	0	0	0	100	32	66	8	12	10	14	16	15	**3**
Groundnut	32	14	23	0	0	0	0	0	0	0	0	0	76	72	74	12	8	10	0	0	0	**3**
Blanched groundnut	28	10	19	0	0	0	0	0	0	4	2	3	100	74	87	64	50	57	14	8	11	**5**
Black bean	14	0	7	56	54	55	0	0	0	6	32	19	36	30	33	16	2	9	0	0	0	**5**
Sword bean	48	66	57	24	8	16	14	6	10	4	24	14	86	40	63	10	4	7	0	0	0	**6**
White bean	20	12	16	0	0	0	0	0	0	6	4	5	100	84	92	4	0	2	2	0	1	**5**
Average (%) of occurrence	**19**			**9.1**			**1.4**			**5.2**			**63.5**			**11.1**			**3.1**			

Table 3: Average percentages (%) of occurrence of the fungi that were found in 1100 beans used in this study.

Fungi species	Average total
Aspergillus niger	**16**
Aspergillus flavus	**7.6**
Aspergillus fumigatus	**1.2**
Aspergillus versicolor	**4.7**
Rhizopus sp	**54**
Penicillium sp	**9.5**
Trichoderma sp	**6.8**

Growth of fungi at different temperature

All the seven types of fungi were used in this test. Temperature had significant effect on growth of *A. niger*. *A. niger* was able to grow at 15 °C to 35 °C (Figure 4.1). The highest growth rate of *A.niger* was at 30°C. The growth rate of the fungus was 1.14 ± 0.03 cm / day, which was significantly higher at P=0.001 than growth rate of the fungus at other temperatures. The growth rate was reduced as the temperature decreased or increased.

There was significant effect of temperature on growth of *A. flavus*. *A. flavus* was able to grow at 15 °C to 35 °C. *A. flavus* grew fast at 30°C to 35°C and slower growths were observed as the temperatures were decreased. There was no significant different at P=0.05 in the growth of *A. flavus* at 30°C and 35°C (Figure 4.2).

A. .fumigatus was also able to grow at 15 °C to 35 °C (Figure 4.3). The growth rates of the fungi at 25 °C, 30 °C and 35 °C were 1.03 ± 0.15 cm/day, 1.07 ± 0.02 cm/day and 0.98 ± 0.12 cm/day respectively. There were no significant different at P=0.05 in the growth rates of *A. fumigatus* at 25 °C to 35 °C. The growth rate was reduced as the temperature decreased.

Temperature also had significant effect on growth of *A. versicolor*. *A. versicolor* was able to grow at 15 °C to 35 °C (Figure 4.4). The highest growth rate of *A. versicolor* was at 30°C. The growth rate of the fungus was 1.74 ± 0.05 cm/day, which was significantly higher at P=0.001 than growth rate of the fungus at other temperatures. The growth rate was reduced as the temperature decreased or increased.

There was a significant effect of temperature on growth of *Rhizopus sp*. *Rhizopus sp.* was able to grow at 15 °C to 35 °C. The fastest growth of *Rhizopus sp* was at 15 °C and slower growth was observed as the temperature increased. There was no significant different at P=0.05 of growth rates of the fungus at 20 °C to 30 °C (Figure 4.5).

Penicillium sp. was able to grow at 15 °C to 30 °C (Figure 4.6). At 35°C, there was no growth of this fungus. There was significant different at P=0.001 of growth rates of the fungus at 35 °C and the growth rate of the fungus at other temperature. However, there was no significant different at P=0.05 of the growth rates at other temperature.

Temperature has significant effect on growth of *Trichoderma sp. Trichoderma sp.* was able to grow at 15 °C to 35 °C (Figure 4.7). The highest growth rate of the *Trichoderma sp.* was at 35°C . Growth rate of the fungus was reduced as the temperature decreased.

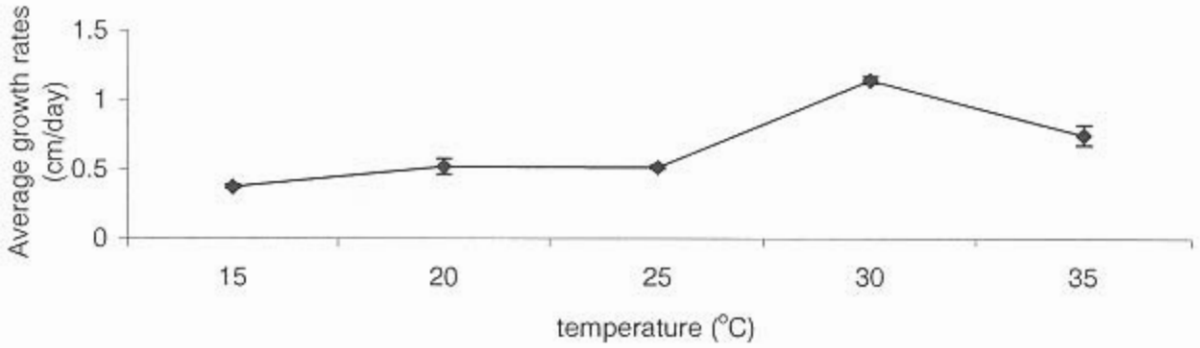

Figure 4.1: Average growth rates of *Aspergillus niger* at different temperature.

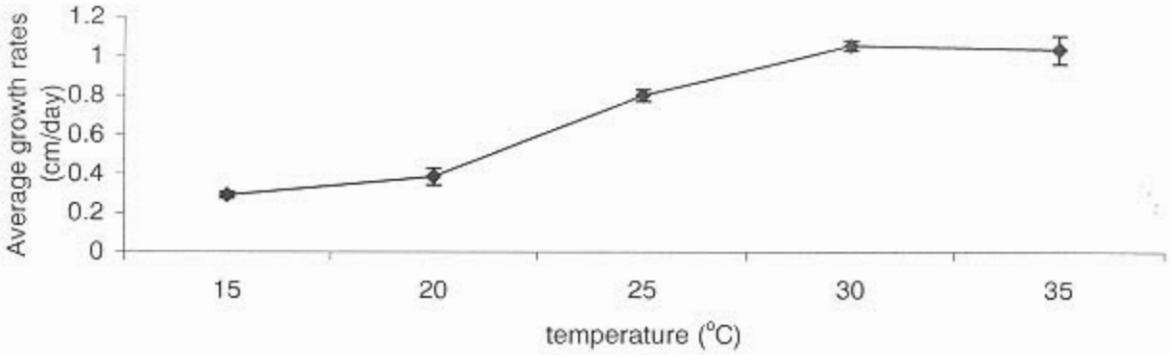

Figure 4.2: Average growth rates of *Aspergillus flavus* at different temperature.

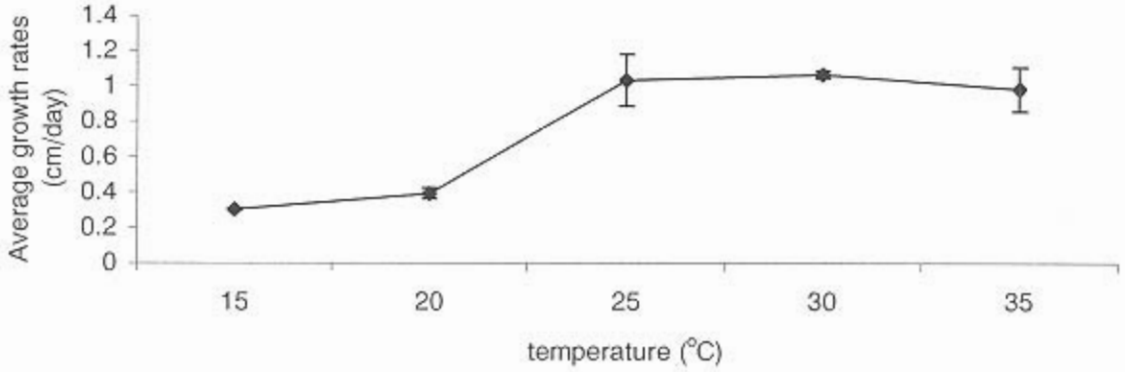

Figure 4.3: Average growth rates of *Aspergillus fumigatus* at different temperature.

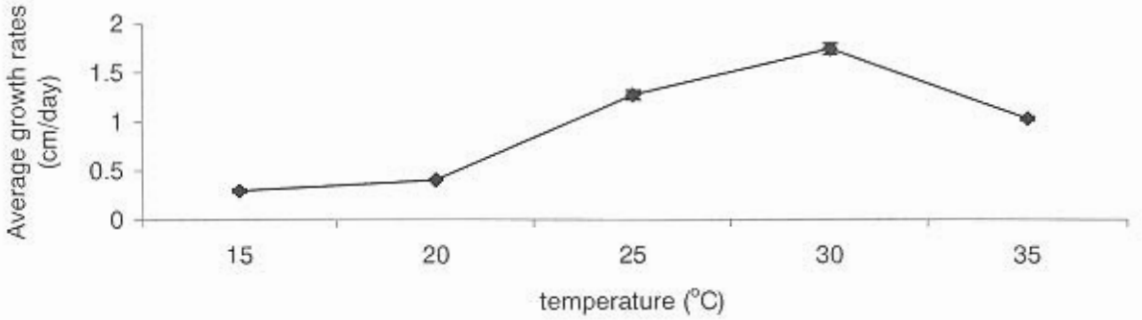

Figure 4.4: Average growth rates of *Aspergillus versicolor* at different temperature.

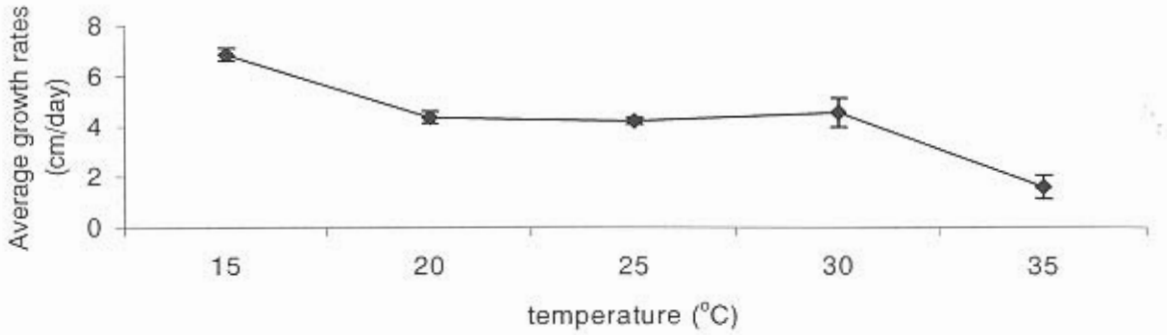

Figure 4.5: Average growth rates of *Rhizopus sp.* at different temperature.

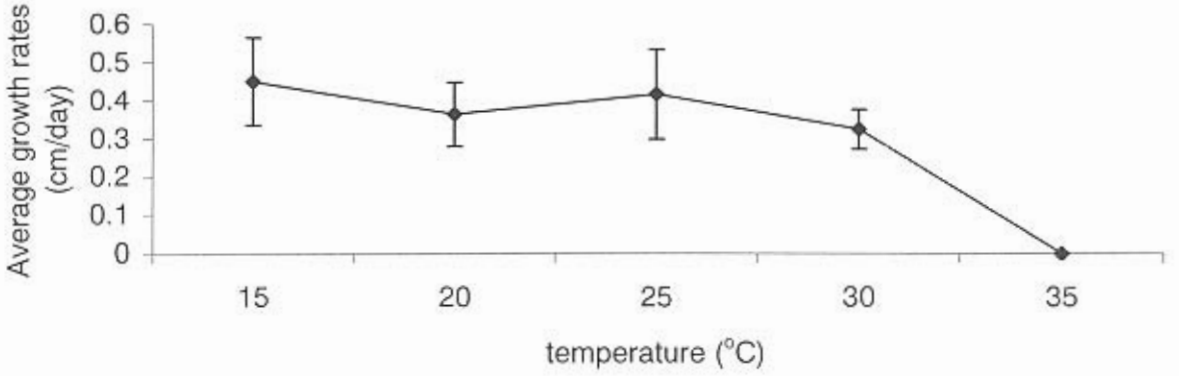

Figure 4.6: Average growth rates of *Penicillium sp.* at different temperature.

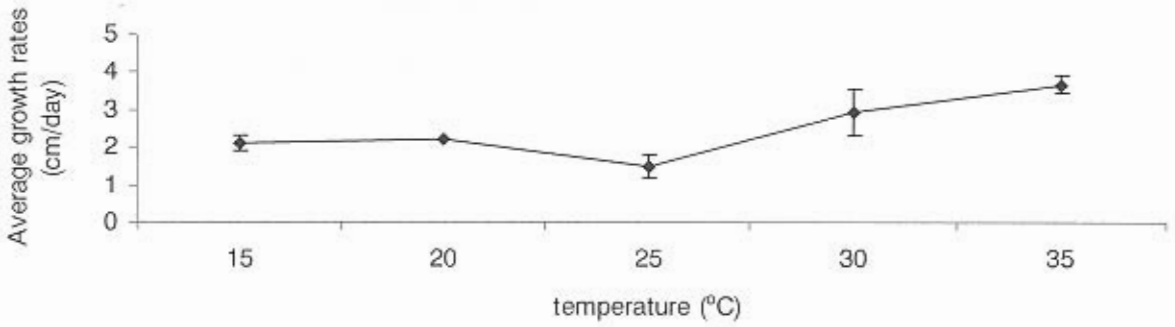

Figure 4.7: Average growth rates of *Trichoderma sp.* at different temperature.

Growth of fungi at different pH

All the seven types of fungi were used in this test. There was significant effects of pH at P=0.001 on growth of *A. niger* (Figure 5.1). Good growth of the fungus was obtained at pH 4.0 and pH 7.0. There was no significant different at P=0.05 of mycelial dry weight of the fungus on the media in these two pH values. Less mycelial dry weights were obtained at other pH values.

There was significant effect of pH at P=0.001 on growth of *A. flavus* (Figure 5.2). Good growth of the fungus was also obtained at pH 4.0 and pH 7.0. There was no significant different at P=0.05 of mycelial dry weight of the fungus on the media in these two pH values. Less mycelial dry weights were obtained at other pH values.

There was significant effect of pH at P=0.001 on growth of *A. .fumigatus* (Figure 5.3). Good growth of the fungus was obtained at pH 7.0. The mycelia dry weight of the fungus was 0.43 $\pm$ 0.04 g, which was significantly higher at P=0.001 than mycelia dry weights of the fungus at other pH values.

There was significant effect of pH at P=0.001 on growth of *A. versicolor* (Figure 5.4. Good growth of the fungus was obtained at pH 7.0. The mycelial dry weight of the fungus was 0.29 $\pm$ 0.06 g, which was significantly higher at P=0.001 than mycelial dry weights of the fungus at other pH values.

There was a significant effect of pH on growth of *Rhizopus sp.* (Figure 5.5). Good growth of the fungus was obtained at pH 4.0 and pH 7.0. There was significant different at P=0.001 of mycelial dry weight of the fungus in the media of these two pH values. The highest mycelial dry weight however was at pH 7.0. The mycelial dry weight of the fungus was 0.48$\pm$0.01 g.

There was significant effect of pH on growth of *Penicillium sp.* (Figure 5.6). Good growth of the fungus was obtained at pH 4.0 and pH 7.0. There was no significant different at P=0.001 of