



Comparative Analysis of Antifungal Properties of Different Oils on *Aspergillus spp.* Isolated from *Vigna unguiculata* And *Pisum sativum* L. Seeds

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Abstract: Present study was conducted to isolate seedborne fungi associated with *Vigna unguiculata* and *Pisum sativum* L. seeds detected using one of the seed health testing techniques as recommended by ISTA viz; Agar plate method. Many different species belonging to 5 fungal genera were isolated and identified. Before isolation of seedborne fungi the *Vigna unguiculata* and *Pisum sativum* L. seeds were stored under different condition i.e. plain seeds, seeds with Oil, seeds with Bay leaf, seeds with Black cardamom, seeds with Star anise for varying period of time, to observe the effect of spices on the seed mycoflora. Pathogenic fungi like *Aspergillus*, *Fusarium*, *Cladosporium*, *Penicillium* and *Rhizopus* were isolated through agar plate method. *Aspergillus flavus* followed by *Aspergillus niger* was the most dominant seedborne fungi. Later, Agar well diffusion method was performed to observe the antifungal properties of different oils on *Aspergillus spp.* isolated.

Index Terms - *Vigna unguiculata*, *Pisum sativum* L., *Aspergillus*, seedborne fungi, oils, storage conditions.

INTRODUCTION

Pea (*Pisum sativum* L.) belongs to Leguminosae family, which has an important ecological advantage for it contributes to the developments of low-input farming systems by fixing atmospheric nitrogen and it serves as a break crop which further minimizes the need for external inputs. Legumes constitute the third largest family of flowering plants, comprising more than 650 genera and 18,000 species (Lewis et al., 2005).

There are several factors which are responsible for their low production, among them diseases which played an important role (Nine 1986). Cowpea (*Vigna unguiculata* L. Walp) is a large seeded legume grown for its rich green pods, grains and stover by resource-poor farmers of under developed and developing countries of Africa and Asia. It is evident by the survey of literature showed both pathogenic and saprophytic fungi associated with cowpea seeds. Many workers have reported the association of *Fusarium oxysporum*, *F. equiseti*, *F. verticillioides*, *Aspergillus niger*, *A. flavus*, *Penicillium digitatum*, *P. chrysogenum*, *Rhizopus arrhizus*, and *Rhizoctonia solani* with seeds of cowpea crop (Kritzinger, 2003; Mogle and Maske, 2012; Makun et al., 2012).

Sonawane et al. (2004) reported that seed samples of some pea cultivars collected from India were analyzed by agar plate or blotter method for the presence of seed borne fungi. *Aspergillus flavus*, *Aspergillus niger*, *Fusarium moniliforme* (*Gibberella moniliformis*), *F. roseum*, *Macrophomina phaseolina*, *Mucor globosus*, *Helminthosporium tetramera* and *Rhizopus nigricans* (*R. stolonifer*) were dominant. The agar plate method yielded a higher number of fungi than the standard blotter method.

Various seed-borne fungal species have been reported to be associated with cowpea seeds in different parts of the world. *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporum*, *Alternaria alternata*, and *Penicillium sp.* were found to be associated with cowpea seeds in India (Zanjare et al., 2020).

Seed borne fungi affect adversely to nutritive value of pulses. Biodegradation of protein content of pulses by their common and dominant seed borne fungi like *Aspergillus flavus*, *Aspergillus fumigatus*, *A. niger*, *Drechslera tetramera*, *Fusarium moniliforme*, *Rhizopus stolonifer* etc. has been reported through artificial infestation of the pulses like green gram, black gram, chickpea and pigeon pea. Results reveal considerable degradation in protein content of the test pulses (Kandhare, 2014).

Many fungal genera; *Alternaria*, *Aspergillus*, *Cladosporium*, *Curvularia*, *Fusarium*, *Penicillium*, *Rhizopus* and *Trichoderma* known to invade stored grains and pulses (Schafer and Kotanen, 2003). Fungal infestation of cowpea seed is a major issue, resulting in decreased quality and nutritional value and toxic to human consumption for cattle feed (Castillo et al., 2004).

A. flavus found to affect lipids and carbohydrates contents of plant seeds in addition to the aflatoxin production (Aziz and Mahrous, 2004).

In different parts of the world, plant parts like bark, leaf, flower, seed powder, and derivatives like ash, vegetable oil, spice oil have been in used to control insect and microbial growth since ages. Several researches have also highlighted the advantages of using the botanical derivatives as chemical fumigants and contact pesticides. Some of these plant products that have lower phytotoxicity are easily biodegradable and has traditionally been used in some parts of the world (Dubey et al., 2008).

Several households in India apply vegetable oil on their stored grains for preventing damage caused by insects and fungus. Oil layer on the grains reduces the fungus to grow, as it limits the oxygen supply and works as a thin barrier for the moisture to seep into the grain. Many studies indicated the effectiveness of vegetable oil in controlling both fungal and insect growth in grains, which can be a low-priced and effective method for safe grain storage (**Hall and Harman, 1991a, b**).

II. RESEARCH METHODOLOGY

2.1 Collection of Samples

Vigna unguiculata and *Pisum sativum* L. seeds were purchased from local market in Jabalpur (Madhya Pradesh).

2.2 Physical appearance/Quality of Seeds

The seeds with good physical quality were selected. The seed with physical quality should have uniform size, weight, and color. It also should be devoid of shriveled, diseased mottled, molded, discolored, damaged and empty seeds.

2.3 Storage Conditions

Vigna unguiculata and *Pisum sativum* L. seeds were stored in clean airtight containers in different conditions as follows:

SEEDS→	<i>Vigna unguiculata</i>	<i>Pisum sativum</i> L.
Container 1	Plain seeds	Plain seeds
Container 2	Seeds coated with cooking oil	Seeds coated with cooking oil
Container 3	Seeds with Bay leaves	Seeds with Bay leaves
Container 4	Seeds with Black Cardamom	Seeds with Black Cardamom
Container 5	Seeds with Star Anise	Seeds with Star Anise

Agar plate method: In Northern Ireland, Musket (1948) first used this method for seed health management (**Musket et al., 1948**). In this method, pre-sterilized petri plates were poured with 15ml of the autoclaved media. After the solidification of medium, 4 seeds per plate of the sample to be studied were equidistantly placed aseptically and incubate at 27±1°C.

2.4 Isolation and Identification of Fungi

Every week, seeds were sampled, and on SDA medium, fungi were isolated from various stored seeds. On SDA, the isolated fungi were kept alive with frequent subculturing for further tests. On the basis of morphological and microscopic analysis, along with literature consultation, fungi were identified.

2.5 Determination of Percent Frequency

The number of times a specific fungal species appeared out of all observations was counted in order to determine the frequency of appearance of that species. The percentage of observations in which a species appeared relative to all observations was then used to calculate percentage frequency.

III. RESULTS AND DISCUSSION

3.1 TABLE NO.1

ISOLATED FUNGI	NO. OF COLONIES	% CONTRIBUTION
<i>Aspergillus</i>	12	35.2%
<i>Fusarium</i>	08	23.5%
<i>Penicillium</i>	07	20.5%
<i>Cladosporium</i>	05	14.7 %
<i>Rhizopus</i>	02	5.8%

From the previous study (**Khare et al., 2016 and Shahnaz Dawar et al.,2015**) it was clear that *Aspergillus spp.* are the dominant fungal flora in seeds. The isolated *Aspergillus* genera were again studied and found out that different *Aspergillus* species were present as seedborne mycoflora. They are *Aspergillus niger* and *Aspergillus flavus*. Further study was carried out with these two species as they are the predominant flora of the seeds.

3.2 Microscopic Identification

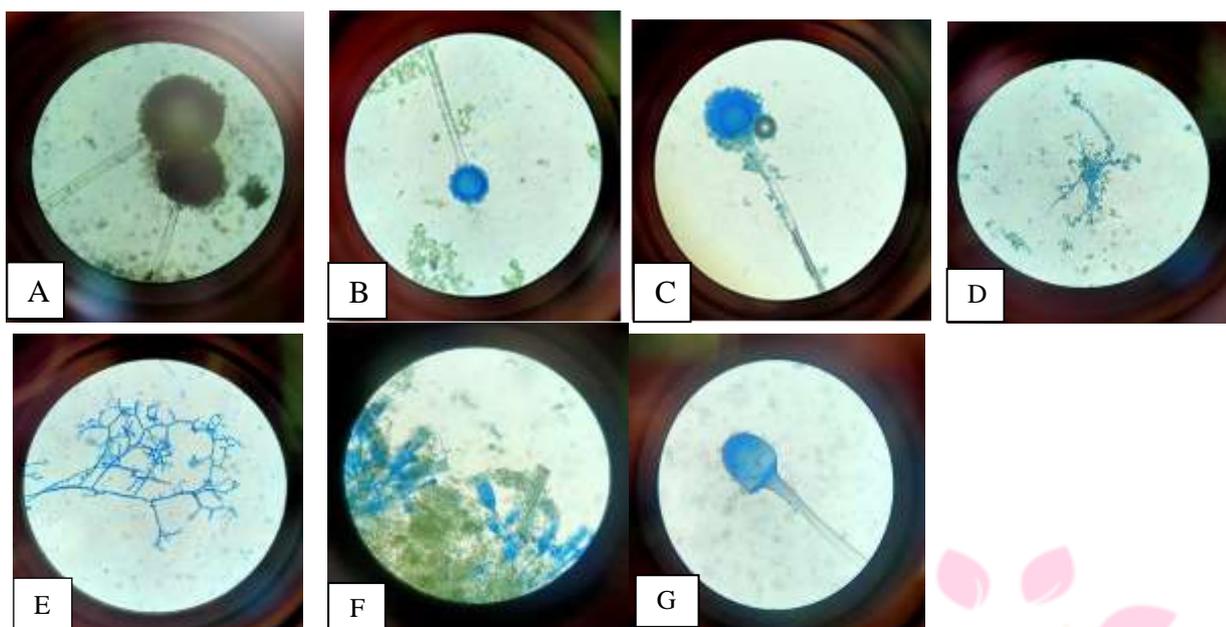
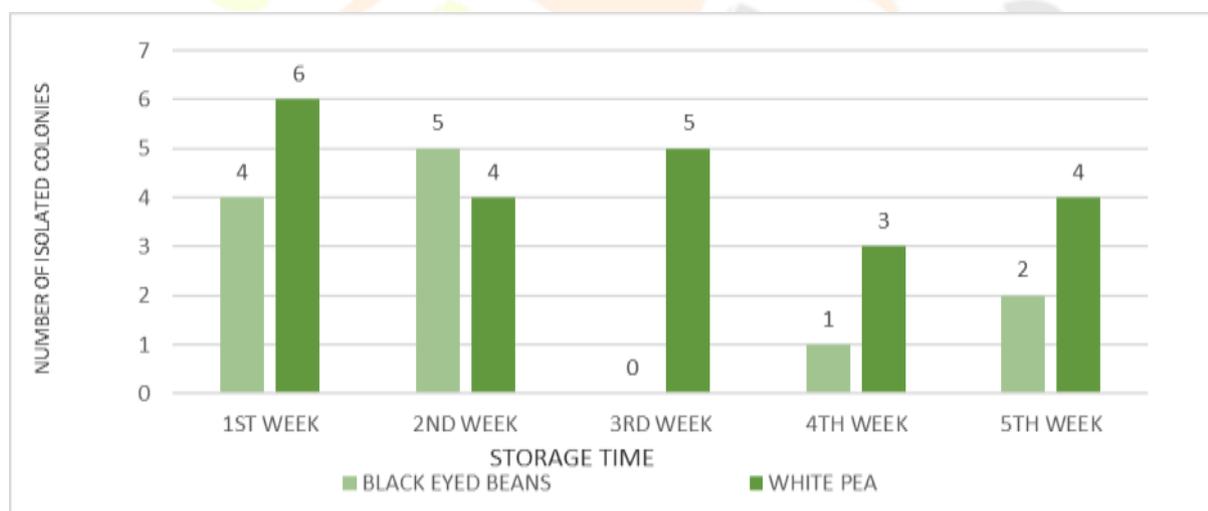


FIG. (A), (B), (C) *Aspergillus*, (D) *Cladosporium*, (E) *Fusarium*, (F) *Penicillium*, (G) *Rhizopus*
 A total of 34 fungal colonies was isolated from 5-5 samples of *Vigna unguiculata* and *Pisum sativum* L. Among them, the highest percentage is of *Aspergillus* i.e. 35.2%, followed by *Fusarium* with 23.5%, followed by *Penicillium* with 20.5%, followed by *Cladosporium* with 14.7% and *Rhizopus* with 5.8%.

3.3 GRAPH NO.1

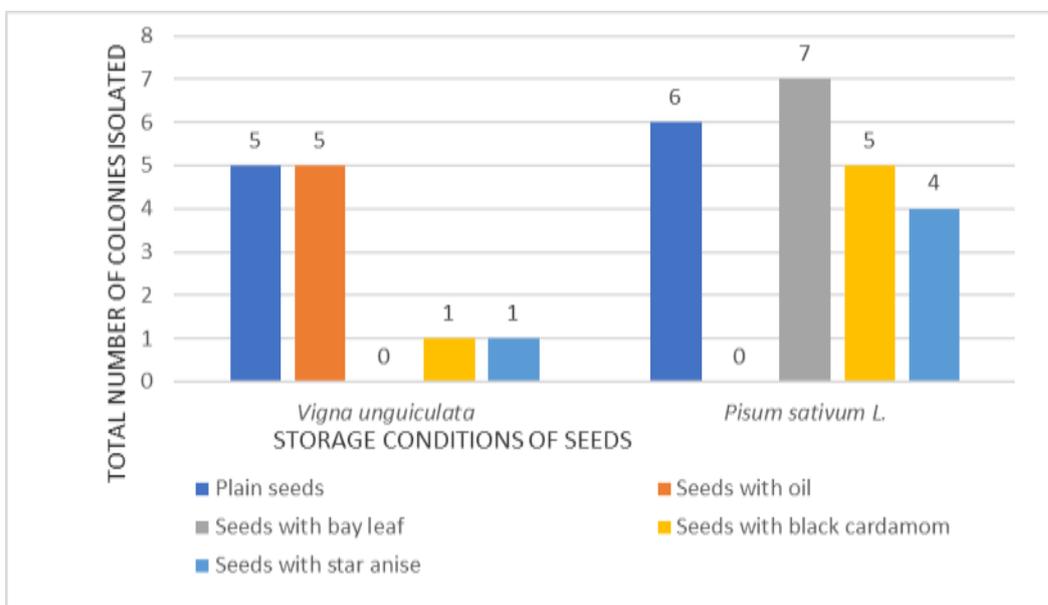
A comparative of storage period and number of colonies isolated from *Vigna unguiculata* and *Pisum sativum* L.



A total of 12 fungal colonies isolated from *Vigna unguiculata* seeds in 5 weeks in different storage conditions. A total of 22 fungal colonies were isolated from *Pisum sativum* L. Number of colonies decreases as the storage period increases.

3.4 GRAPH NO. 2- NUMBER OF COLONIES ISOLATED FROM *Vigna unguiculata* AND *Pisum sativum* L. SEEDS UNDER VARIOUS STORAGE CONDITIONS

This graph shows the number of fungal colonies at different storage conditions. Plain seeds along with 4 different substances were used with seeds which were used traditionally in India. The different storage material used along with seeds are oil, Bay leaf, Black cardamom and Star anise. The seeds which were stored without any material show a total of 5 fungal colonies in *Vigna unguiculata* and 6 fungal colonies in *Pisum sativum* L. Seeds coated with oil shows a very good result in *Pisum sativum* L., no fungal colonies were isolated in 5 Week time whereas only 5 fungal colonies were isolated in *Vigna unguiculata*. Seeds of *Vigna unguiculata* stored along with Bay leaf shows a very good effect as no fungal growth was seen after incubation for 3 days. Even seeds stored with Black cardamom and Star anise was good as only 1 fungal colony was isolated after incubation. While effect of Bay leaf, Black cardamom and Star anise is not effective for *Pisum sativum* L. as 7, 5 and 4 fungal colonies were observed respectively.



3.5 TABLE NO.2

DETERMINATION OF ANTIFUNGAL ACTIVITY OF DIFFERENT OIL AGAINST *Aspergillus* spp. BY AGAR WELL DIFFUSION METHOD

OILS	<i>Aspergillus niger</i>	<i>Aspergillus flavus</i>
COCONUT OIL	-	-
MUSTARD OIL	20	45
CLOVE OIL	30	45
SUNFLOWER OIL	-	-
SOYBEAN OIL	-	-
GROUNDNUT OIL	-	-
SESAME OIL	-	-

NOTE: (-) indicates NO ZONE, values in mm.

From the Graph 2, it was clear that Oil and Bay leaf are effective to control seedborne mycoflora. As we cannot take Bay leaf further, we studied to compare the antifungal activity of different oils used in home against *Aspergillus niger* and *Aspergillus flavus*. Seven different oil samples were collected. They are Coconut oil, Mustard oil, Clove oil, Sunflower oil, Soyabean oil, Groundnut oil and Sesame oil. These oils are tested against *Aspergillus niger* and *Aspergillus flavus*. Among these seven oil samples, only two oils i.e. Mustard oil and Clove oil gives 45mm zone against *Aspergillus flavus* whereas Clove oil gives 30mm and Mustard oil gives 20mm zone against *Aspergillus niger*.

CONCLUSION

In the present study about the seedborne fungi isolated from seeds of *Vigna unguiculata* and *Pisum sativum L.*, it was observed that there are wide range of mycoflora present on the seed samples. The isolated fungal genera are - *Aspergillus*, *Penicillium*, *Cladosporium*, *Fusarium* and *Rhizopus*. Among these isolated fungal genera, *Aspergillus* was found as the predominant genus. Among the isolated genera, *Aspergillus* is present with the highest percentage contribution (35.2%) and is best known for its colonization of cereal grains, legumes and tree nuts. Two different species of *Aspergillus* were isolated namely, *Aspergillus niger* and *Aspergillus flavus*.

Out of all the storage conditions provided, the best fungal growth inhibiting activity was observed in seeds stored with oil and bay leaves. Therefore, for the further study of seedborne mycoflora, tests were conducted to observe the antifungal activity of different oils on the most predominant mycoflora namely, *Aspergillus niger* and *Aspergillus flavus*.

Agar well diffusion test was performed to test the antifungal activity of seven different oil particularly: Coconut oil, Mustard oil, Clove oil, Soybean oil, Sunflower oil, Sesame oil and Groundnut oil, out of which only Mustard oil and Clove oil inhibited the growth of *Aspergillus niger* and *Aspergillus flavus*. Mustard oil and Clove oil inhibited the complete growth of *Aspergillus flavus*

with zone of 45mm for both. Whereas, Mustard oil and Clove oil was effective against *Aspergillus niger* with zone of 20 mm and 30 mm respectively.

This information can be useful for the future study related to management of seed mycoflora by the use of natural products such as spices and oils to reduce the ill effects of these mycoflora on humans and animals.

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