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EFFECTS OF SEED BORNE MYCOFLORA ON SUGAR, OIL AND FATTY ACID COMPOSITION OF THREE VARIETIES OF MUSTARD (*BRASSICA COMPESTRIS*) VIZ,BASANTI, KALASONA, KAVERI AK-47.

WANI MUBASHIR*, SAJAD HUSSAIN BAKSHI [1], NAZIR AHMAD BHAT[1] AND WAHEED-U-ZAMAN[1].

** Institue of Basic Science, Department of Botany, Bundelkhand University Jhansi, U.P.*
1. Government Degree College Boys Anantnag,Jammu and Kashmir.India.

ABSTRACT

The present study was conducted to explore the seed born mycoflora or fungi associated with seeds of mustard. A total 7 fungi were isolated among the isolated fungi in all replications. *Aspergillus flavus* was most dominant species, so further investigated were carried out with *Aspergillus flavus.* Mass multiplication of the pathogen was done and *Aspergillus flavus* was cultured on PDA plates. The spores were collected after 5 days and a spore suspension was prepared. The culture filtrate was used to infest the healthy seeds of mustard varieties Basanti, Kalasona, Kaveri AK-47. After infestation for 48 hours the studies were conducted to check the impact of *Aspergillus flavus* on the biochemical parameters as well as Vigour of the seed. The oil content of both the samples (healthy and infested) was studied for all three varieties, colour, odour and quantity of oil was taken into consideration. There was reduction in oil quantity, color and odour in case of infested seeds as compared to healthy seeds.

KEYWORDS: Mustard *(Brassica), Aspergillus flavus,* seed borne fungi, PDA plates, oil quality, sugar content, fatty acids.

Corresponding author

WANI MUBASHIR
Institue of Basic Science, Department of Botany, Bundelkhand University Jhansi, U.P.

INTRODUCTION

Mustard (Brassica sps.) belongs to family Brassicaceae, is an important oil seed crop of the world. The rapeseed and mustard are most important oilseed crops in India preceded by groundnut. The genus Brassica contains over 150 species cultivated worldwide as oil seed crop or vegetables. Mustard oil possess 60% monounsaturated fatty acids of which 42% Erucic acid and 12% Oleic acid, it has also 21% polyunsaturated of which 6% is the omega-3 alpha-Linolenic acid and 15% omega-6 linoleic acid along with 12% saturated fats. A large number of fungi are known to bring about several biochemical changes in mustard seeds and degrade seed constituents (Rai and Saxena, 1980). Mustard is one of the important oilseed crops in India which is consumed by human beings in the form of oil and condiments. It is also feed to cattle as a feed supplement in the form of oil cake. At the same time mustard seeds also harbor a number of saprophytic and parasitic microorganisms which thrive at a cost of host substrate as externally or internally seed born inocula. Amongst various microorganisms invading the seeds, fungi stand at the forefront. Some of the common detriments that can directly be attributed to fungal association include lowering of seed quality, deterioration of nutritional components, and failure of seed germinibility and elaboration of toxic metabolites (Ahmad and Sinha 2002).

MATERIALS AND METHODS

Seeds of three accessions of Mustard namely Basanti, Kalasona and Kaveri AK- 47 were collected from germplasm collection of National Bureau of Plant Genetic Resource, New Delhi. The seeds were then placed in B.O.D for 24 hrs at 25^0c. Seeds of each accessions were examined under binocular microscope after incubating on moist blotter for presence of mycelium or fruiting body of fungi on seed surface. The identification of fungi was made according to Ainsworth and Bisbys (1973) .A large number of seed born mycoflora was obtained from seeds placed in Petri plates in incubator. Out of these seed born mycoflora obtained *Aspergillus flavus* was used to study the effect of this very fungus on the quality of mustard. For that *Aspergillus flavus* was cultured on Potato dextrose Agar for 5 days at room temperature to obtain large number of spores. The seeds of each variety were infested with the solution of *Aspergillus flavus* and observation was made in 5,7,12 days. 100 grams of each variety of mustard both infested and control were grinded with the help of grinder and oil of each accession was obtained through Soxlet apparatus for 9 hours per sample using Hexane as solvent (Jham *et al* 1982). The oil obtained was put in specific viols that were already sterilized. The oil obtained through Soxlet apparatus was then subjected to GLC analysis to study variation in free fatty amino acid (Uppstrom *et al* 1978). Estimation of Sugar was done by method evolved by Dey (1990). Sugar was extracted from grinded seeds (1gm) both infested and control in ethanol (90%) .It was kept in oven at 60^oc for 1 hour and final volume was made to 25ml. To 1 ml aliquot of 5% phenol was followed by addition of 5ml sulphuric acid. Optical density was read at 485 nm, the concentration were determined against curve by using glucose.

RESULTS

The effect of seed borne mycoflora on the quality of mustard was carried out to study its various parameters like Sugar content, Oil Content, Rate of Germination, Vigor index and percentage of fatty acids. The results given below for all three accessions of mustard (Basanti ,Kala-Sona and Kaveri Ak-47) are mostly expressed by taking their mean values into consideration from the observation tables. *Table 1* **r**epresents the comparison among the sugar content of the Control and infested

verities of the mustard .The maximum sugar content among control samples was recorded in Kaveri Ak- 47(46.1±2.77),followed by Basanti (28.95±2.19) and Kala-Sona (28.1±2.15) respectively. While in case of the infested samples the maximum sugar content was recorded in case of Kaveri –Ak -47 (33.5±2.36) followed by Basanti (24.00±1.99) and Kala –Sona(14.8±1.57) respectively.

Table1

Sugar content of three different varieties of Mustard with mean and standard error in tabulated form.

Variety	Sugar Content mg/gm fresh weight.	
	Control (Mean±S.E)	Infested (Mean±S.E)
Basanti	28.95±2.19	24.0±1.99
Kalasona	28.1±2.15	14.8±1.57
Kaveri Ak-47	46.1±2.77	33.5±2.36

Table 2 represents the comparison among the oil content of the three sleeted verities of Brassica. .The maximum oil content among control samples was recorded in Kala –Sona (34.95±2.40) followed by Kaveri Ak -47(27.12±2.12) and Basanti (24.6±2.02) respectively. While in case of the infested samples the maximum oil content was recorded in case of Basanti (9.46±1.55) followed by Kala – Sona(7.33±0.90) and Kaveri –Ak -47 (2.46±0.46) respectively.

TABLE 2

Oil content of three different varieties of Mustard with mean and standard error in tabulated form

Variety	Oil Content in grams.	
	Control (Mean±S.E)	Infested (Mean±S.E)
Basanti	24.6±2.02	9.46±1.55
Kalasona	34.95±2.40	7.33±0.90
Kaveri AK-47	27.12±2.12	2.46±0.64

Table 3, 4 and 5 depicts percentage of fatty acids and variation shown by these fatty acids in three varieties of mustard (Basanti, Kalasona, Kaveri AK-47) in controlled and infested oil. In Variety Basanti the percentage of Palmitic acid in controlled oil was 2.82% while in infested oil It was 2.97%, the Oleic acid in controlled oil was 7.5% while in infested oil it was 8.2%, on the other hand percentage of Linoleic acid in controlled oil was 18.2% while in infested oil it was 19.9% and the percentage of Erucic acid in controlled oil was 37.1% while in infested oil it was 38.1%. In Variety Kalasona the percentage of Palmitic acid in controlled oil was 2.92% while in infested oil it was 3.7%,

the Oleic acid in controlled oil was 14.28% while in infested oil it was 14.37%, on the other hand percentage of Linoleic acid in controlled oil was 17.3% while in infested oil It was 17.79% and the percentage of Erucic acid in controlled oil was 50.2% while in infested oil It was 52.7%. In Variety Kaveri AK-47 the percentage of Palmitic acid in controlled oil was 1.94% while in infested oil it was 2.42%. The Oleic acid in controlled oil was 12.27% while in infested oil it was 13.2%, on the other hand percentage of Linoleic acid in controlled oil was 15.32% while in infested oil it was 16.11% and the percentage of Erucic acid in controlled oil was 53.71% while in infested oil it was 54.2%.

TABLE 3

Table showing variation in the fatty acid composition ofMustard Var. . (KALASONA) in percent age.

Fattyacid	Control	Infested
Palmitic Acid	2.92	3.7
Oleic Acid	14.28	14.37
Linoleic Acid	17.3	17.79
Erucic Acid	50.2	52.7

TABLE 4

Table showing variation in the fattyacid composition of Mustard Var. (KAVERI AK-47) in percentage.

Fattyacid	Control	Infested
Palmitic Acid	1.94	2.42
Oleic Acid	12.27	13.2
Linoleic Acid	15.32	16.11
Erucic Acid	53.71	54.2

TABLE 5
Table showing variation in the fatty acid composition of Mustard Var. (BASANTI) in percentage.

Fattyacid	Control	Infested
Palmitic Acid	2.82	2.97
Oleic Acid	7.5	8.2
Linoleic Acid	18.2	19.9
Erucic Acid	37.11	38.1

DISCUSSION

The oil content of the mustard seeds treated with various seed borne fungi was assessed and expressed as percentage of dry weight of the seeds. All the four species decreased the oil content significantly to the check (uninoculated). The effect of seed borne mycoflora on the quality of mustard was carried out to study its various parameters like Sugar content, Oil Content and percentage of fatty acids. The results given above for all the three varieties of mustard Basanti, Kalasona and Kaveri Ak-47 are mostly expressed by taking their mean values into consideration.

Sugar content

The maximum sugar content among control samples was recorded in Kaveri Ak-47, followed by Basanti and Kalasona respectively. While in case of the infested mustard seed samples the maximum sugar content was recorded in case of Kaveri Ak -47 followed by Basanti and Kalasona respectively. Results reveal that there was slight reduction in the sugar content of all the three varieties of mustard which were infested by the culture filtrate of *Aspergillus flavus* for 48 hours. According to our study the reduction of sugar might be due utilization of sugar by fungi as substrate for its growth. Rajendral *et al* (2011) investigated reduction of sugar in case of sunflower due to *Fusarium equiseti, Fusarium oxysporium* and *Curvularia lunata.* Catherine et al (1987) demonstrated decrease in sugar content with the infection of *Aspergillus flavus, Fusarium oxysporium* in seeds of some autoclaved oil palm kernals. Govindaswamy (1968) while working on paddy observed accumulation of reducing sugars due to fungal invasion which directly or indirectly affected the lipolytic activity of seed borne fungi.

OIL CONTENT

The maximum oil content among control samples was recorded in Kalasona followed by Kaveri Ak-47 and Basanti respectively. While in case of the infested samples the maximum oil content was recorded in case of Basanti followed by Kalasona and Kaveri Ak-47 respectively. The reduction in oil content of mustard may be due to lipolytic activity of seed

born fungi. Lalithakumeri *et al (*1971) reported that *Aspergillus flavus, Botryodiplodia* and *Cladosporium herbarum* also reduced oil content of groundnut. Rai and Saxena (1980) reported that *Aspergillus flavus* was more effective in reducing oil content of Indian mustard. Dubey et al (1985) observed that *Alternaria alternate, Aspergillus flavus, Aspergillus repens* and *Tornula allii* reduced oil content of linseed oil while *Cladosporium herbarum* slightly increased it. Tamoor *et al* (2005) also analysed that the weight and oil content of mustard seeds that were infected by different species of fungi such as *Aspergillus flavus, Alternaria brassicae,Fusarium oxysporium* was decreased as compared to that were used in control . Gehlot *et al* (2000) also observed decrease in oil content in seeds of mustard and tamarina inflicted by *Aspergillus flavus , Alternaria alternate, Fusarium moniliform* and infected oil emitted mouldy odour. The colour and odour of infested oil of three varieties of mustard also changed, in case of Basanti the colour in controlled conditions was light brown while in infested oil it was light yellow, in Kalasona it was yellow in controlled condition while its colour in infested oil was slightly yellow, however in Kaveri AK-47 the colour changed from brown to red. The changes observed in the colour of oil due to fungal infestation under present studies are in agreement with the findings of Ward and Diener (1961) and Lalithakumari *et al* (1971) working on groundnut. Ward and Diener (1961) suggested that the changes in colour may be due to pigments synthesized by the invading fungus. Rai and Saxena (1980) reported that *Aspergillus flavus* was responsible for changing the mustard oil colour to green. Thus it is evident that metabolities of invading fungi also inflict unpleasant odour in these oils.

Fatty acid composition

In Variety Basanti the percentage of Palmitic acid in controlled oil was 2.82% while in infested oil it was 2.97%. Oleic acid in controlled oil was 7.5% while in infested oil it was 8.2%. On the other hand percentage of Linoleic acid in controlled oil was 18.2% while in infested oil it was 19.9% and the percentage of Erucic acid in controlled oil was 37.1% while in infested oil It was 38.1%. In Variety Kalasona the percentage of Palmitic acid in controlled oil was 2.92% while in infested oil it was 3.7%. Oleic acid in controlled oil was 14.28% while in infested oil it was 14.37%, on the other hand percentage of Linoleic acid in controlled oil was 17.3% while in infested oil it was 17.79% and the percentage of Erucic acid in controlled oil was 50.2% while in infested oil it was 52.7%. In Variety Kaveri AK-47 the percentage of Palmitic acid in controlled oil was 1.94% while in infested oil It was 2.42%. Oleic acid in controlled oil was 12.27% while in infested oil it was 13.2%. On the other hand percentage of Linoleic acid in controlled oil was 15.32% while in infested oil it was 16.11% and the percentage of Erucic acid in controlled oil was 53.71% while in infested oil it was 54.2%. GLC analytical data indicated that major fatty acid composition of three varieties of mustard /advanced lines of mustard oil induced unsaturated fatty acids while only a minor amount of saturated fatty acids from the present data it might be suggested that all brassica oil seeds are suitable for edible purpose as they contain highest amount of unsaturated fatty acids. The prevailing results are quite in agreement with those of rapseed as reported by Ullah *et al* (1997), Mazzoncini *et al* (1993), Niraz *et al* (2001), Appelqvist (1980) and Rollet *et al* (1995). Chowdhury *et al* (2003) while working on the different varieties and advanced lines of mustard and rapeseed also found increase in fatty acid composition of infested oil as compared to that of control. Gehlot *et al* (2000) while working on seeds of mustard and tamarina oil also found increase in fatty acid content of infested oil as compared to that of control. Rai and Saxena (1980) while working on brassica seeds infested with *Aspergillus flavus, Alternaria brassicae* found

general increase in fatty acid composition of oil as compared to their control. Ashraf *et al* (1986) reported that there was increase in free fatty acid composition of brassica seeds infested with some seed born fungi such as *Fusarium oxysporium, F. moniliforme.* Actually the lipids present in seed are primary seed infecting fungi (Roberts 1987). Mckevith (2005) stated that after processing, oxidation is the main problem to affect oil and lipid leading to aldehyde production that may result in high fatty acid content. In the present investigation the increase in fatty acids in oil by fungal lipase shows nonconformity with earlier reports (Joshi 1987). The increase in free fatty acid content of groundnut and soybean seeds associated with temperature could have stimulated lipase activity.

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