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Impact of Aflatoxin Produced by *Aspergillus flavus* on the Biochemical Profile of Seeds of *Oryza sativa*. Linn

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A B S T R A C T

Aflatoxins are a group of secondary metabolites produced by certain toxigenic strains of *Aspergillus flavus* on various food and feed commodities. These group of mycotoxins are highly oxygenated heterocyclic compounds. Aflatoxins occupy the most important position among mycotoxins in view of their potent carcinogenic nature and high frequency of occurrence under natural conditions. Rice (*Oryza sativa* L.) is consumed by millions of people all over the world in different forms. Thus, any form of contamination may prove to be deleterious to health. *Aspergillus flavus and A. parasiticus* were reported to contaminate cereal grains with their toxic secondary metabolites, the Aflatoxins. Seventeen varieties of paddy seeds were infested with a highly toxigenic strain of *Aspergillus flavus* under laboratory conditions. None of the varieties was totally resistant to aflatoxin production but they facilitated aflatoxin production at varying levels. Four rice varieties viz. Turanta IET- 7991, Vaidehi, Dhanlaxmi and Mansuri produced aflatoxin B1 in very low amount i. e. 51.35, 65.52, 62.53 and 67.25µg/Kg and may be considered as highly resistant varieties. Shakuntala IET-11183, Gautam IET- 13439, Birsa Dhan- 202, Sita, Kanak, Jaishree, Pusa 2- 21 produced aglatoxin B1 in the range of 115.45 to 173µg/Kg after infestation with *Aspergillus flavus* and therefore, may be regarded as moderately resistant varieties. Pusa- 33, Basmati- 370, Katarni, Sugandha, BR- 9 and Kamini produced relatively high aflatoxin B1 after *A. flavus* infestation in the range of 218.25 to 370µg/Kg and may be considered as susceptible varieties. The kernel of different paddy varieties contained different concentration of amylase (in the range of 121mg/g to 168mg/g) after infestation with *A. flavus*. Obviously amylopectin content of total starch of different kernel varieties showed negative correlation with amylose content. In contrast to amylose content, the amylopectine level was found to be the highest, 571.8mg/g and 570.3mg/g in respectively in BR- 9 and Kamini variety and the lowest (438.4 mg/g) in the Gautam IET- 13439 variety. Significant reduction in protein content was observed during fungal infestation, the maximum being in Turanta IET- 7991 (17.39%) and Vaidehi- 625 (17.74%). After infestation with *A. flavus* all the paddy varieties showed an increase in total sugar content. Analysis of protein contents in the kernels shows that higher levels of protein in kernels somewhat inhibit the production of aflatoxin B1. Thus, the varieties containing higher amount of proteins were found to resist the production of aflatoxinB1 production. Significant reduction in protein content was observed in those varieties that facilitated higher amount of aflatoxin B1. Correlation coefficient (r) was calculated to be -0.59, which shows moderate negative correlation between the amount of protein and levels of aflatoxin production. Analysis of sugar contents shows that higher levels of both reducing and non- reducing sugar have an inhibitory effect on the production of aflatoxin B1 production. Correlation coefficient between sugar levels and aflatoxin B1 production was calculated to be -97, which shows almost perfect negative correlation.

Introduction

Aflatoxins are a group of secondary metabolites produced by certain toxigenic strains of *Aspergillus flavus* on various food and feed commodities. The discovery of these toxins is comparatively of recent origin. In 1960, about 100,000 Turkey poults died in England within few months. The cause of death was unknown, so the disease was tentatively termed as "Turkey-X-disease". Subsequently it was realized that the peanut meal fed to those birds was heavily contaminated with *Aspergillus flavus* and the toxic principle isolated from such peanut meal was given the name aflatoxin.

Since the initial implication of *A, flavus* in Turkey-X-disease, aflatoxin production by the organism as well as by *A. parasiticus* has been well documented. However, spme other fungi viz., *A. niger, A. oryzae, A. ochraceus, A. ostianus, Penicillium citrinum, p. puberulum* have also been reported to produce aflatoxins. These group of mycotoxins are highly oxygenated heterocyclic compounds. They contain a coumarin nucleus fused to a bifuran and are intensely fluorescent under ultraviolet light. Four closely related aflatoxins are designated as B1, B2, G1 and G2, in which the distinguishing letters refers to the color of fluorescence (B= blue and G=green) and the suffixes to their respective positions on TLC plate. In some mammals, aflatoxin B1, and B2, are partially metabolitezed to give hydroxylated derivatives, which have been called aflatoxins M1 and M2 or milk toxins. Two other hydroxyl aflatoxins i.e., B2 and G2, have also been identified as the secondary metabolites of *A.flavus*; while all the naturally occurring aflatoxins are highly toxic, there is, however appreciable variation so far as the extent of toxicity is concerned. Aflatoxins B1 is the most potent one with M1 and G1 almost equally toxic. This is because the presence of 2, 3 vinyl-ether double bond in all those aflatoxins.

Aflatoxins occupy the most important position among mycotoxins in view of their potent carcinogenic nature and high frequency of occurrence under natural conditions. A wide range of agricultural as well as industrial commodities get contaminated with these natural poisons. Traditionally this was regarded as the storage problem for cereals. However, their occurrence under field conditions is not uncommon. These group of fungal metabolites are toxic to a number of micro-organisms and even to higher plants. Evidence suggest that they are responsible for liver cancer in laboratory animals and in human beings. In recent years, control of aflatoxin contaminations has attracted worldwide attention and various physical, chemical and biological methods have been proposed for removal and detoxification of aflatoxins from various substrates.

High incidence of aflatoxin has been reported in cereals in Bihar (Bilgrami and Sinha, 1984). Amongst the cereals rice is a moderately susceptible commodity to aflatoxin (Bars and Bars, 1992). *A. flavus* can infect rice grains only when its moisture content is more than 12% (Reddy and Raghavender, 2006). Generally the infection resides on the surface of rice grains. Milling of rice has been found to be beneficial so far as minimization of aflatoxin is concerned.

Jayaraman and Kalyansundaram (1990) observed that milling of husked rice contaminated with aflatoxin yielded grain having 60-80% less aflatoxin present in original husked rice. This could be one of the physical methods to remove aflatoxin though in the process some vital nutrients may be lost. Bran obtained during polishing needs to be suitably diluted before they are usedas cattle feed (Sales and Yoshizawa, 2005a).

Aflatoxins have been found in a variety of agricultural commodities, but the most pronounced contamination has been encountered in maize, peanuts, cottonseed, and tree nuts. An extensive review of the amounts of aflatoxins in commodities in North America, South America, Europe, Asia and Africa was included in *IARC Monograph* (IARC, 1993; IARC, 2002).

Surveys of selected foods for the presence of aflatoxins in many countries have continued to detect some level of contamination; the amounts are highly variable, ranging from < 0.1 µg/kg to hundreds of µg/kg depending on source, food type, climate, storage conditions, and other factors (IARC, 2002). The fraction of samples with detectable levels of aflatoxin B1 or total aflatoxins (B1, B2, G1 and G2) can range from a few percent (e.g. 6.9% of imported peanuts in Japan, 1999–2000; Okano *et al.,* 2003) to as much as 30% or more (e.g. maize in some parts of Latin America and Asia (IARC, 2002).

Several recent studies have addressed the early detection, prevention and control of aflatoxins in the food and feed chain around the world (Williams *et al.,* 2004; Kabak *et al.,* 2006; Magan, 2006; Strosnider *et al.,* 2006; Bryden, 2007; Kendra & Dyer, 2007; Magan & Aldred, 2007; Wagacha & Muthomi, 2008). These publications described pre- and post- harvest strategies (such as field management, use of biological and chemical agents, improved drying and storage conditions, irradiation, moisture control, biocompetitiveness and biotechnology (e.g.transgenic expression of maize-specific genes) and early detection methods (such as molecular imprinted polymers, lateral-flow devices, and molecular-based technology). Among various aflatoxins, aflatoxinB1 is considered to be very potent hepatotoxin as well hepatocarcinogen for human beings and animals.

Warm and humid climatic conditions along with unscientific storage practices prevalent in Bihar provide the most favourable condition for the growth of Aspergillus flavus and subsequent production of aflatoxins in food grains (Sinha and Sinha, 1990; Sinha, 1991; Sinha and Kumar, 2010).

Rice (*Oryza sativa* L.) is consumed by millions of people all over the world in different forms. Thus, any form of contamination may prove to be deleterious to health. *Aspergillus flavus and A. parasiticus* were reported to contaminate cereal grains viz. wheat, rice, oat, maize, barley with their toxic secondary metabolites, the Aflatoxins (Furlong *et al.,* 1995; Curtui *et al.,* 1998).

Since rice is consumed by all the sections of our society, an attempt has been made in this investigation to estimate the levels of Aflatoxin B1 production in seeds of some selected varieties of paddy and find out their relation with total starch, amylose, amylopectin, total sugar, reducing sugar, non-reducing sugar and protein contents.

Materials and Methods

Paddy seeds of seventeen varieties (usually grown in Bihar) were collected from different authentic sources *viz.* Bihar Agriculture College Sabour, Bihar, Directorate of Rice Research, IARI, New Delhi, Rajendra Agriculture University, Pusa, Samastipur and from local market viz. Turanta IET- 7991, Birsa Dhan- 202, Vaidehi, Shakuntala IET-11183, Gautam IET- 13439, Dhanlaxmi, Sita, Kanak, Mansuri, Jaishree, Pusa- 33, Pusa- 2-21, Basmati 360, Katarni, Sugandha, B R- 9 and Kamini.

About 50g kernels of each of these varieties were taken in 250 mL conical flask. At first, these seeds were soaked in sterilized distilled water for 2 h. Extra water was discarded and

these were infested with 1 mL spore (6 x 106 spores/ mL) suspension of a highly toxigenic strain of *A. flavus* isolated from wheat seeds after extensive screening and incubated at 30 ± 2^0C for ten days. After incubation period, these samples were then kept in an oven at 55 ± 2^0 C for three days. Properly dried seeds were ground for the chemical extraction of aflatoxin as well as for the estimation of various biochemical parameters.

The aflatoxin from the rice seeds infested with the toxigenic strain of *A. flavus* was extracted by methods as suggested by Thomas *et al.,* (1975). Qualitative estimation of aflatoxin was done by Thin Layer Chromatographic (TLC) technique. 50 µL of chloroform extract was spotted on TLC plate along with the standard of aflatoxins. The spotted chromatoplate was developed in TLC tank containing toluene: iso-amyl alcohol: methanol (90:32:3, v/v/v).

Developed plates were dried and observed under long wave ultraviolet light. Initial identification of aflatoxin was made on visual basis by color and intensity of fluorescence of the sample and standard spots. The quantity of aflatoxin was estimated by spectrophotometer.

The method starch was estimated following the methods as suggested by Snell *et al.,* (1961). 100 mg of powdered sample was homogenized with2 mL of distilled water, 1 mL of 10% ZnSO4 and 1 mL 0.5 N NaOH. After 1 h the sediment was then mixed with 2 mL 52% perchloric acid for acid hydrolysis of storage polysaccharides into sugar molecules. The volume was ultimately dilute 100 times. To 2 mL of this stock solution, 8 mL of 0.1% anthrone reagent was added and placed in water bath. Extract was heated on water bath for 10 min at 100^oC and then cooled rapidly in running water to room temperature. The optical density was recorded at 625 nm in Vis-UV 117 Systronics Spectrophotometer against the blank prepared in distilled water. For amylose estimation method of Juliano (1971) was followed. 100 mg of fine powdered sample was poured in 1 mL of 70% ethanol and left for 30 min. To this solution, 10 mL of 1 N NaOH was added and left overnight. The volume was subsequently raised to 100 mL with distilled water. 2.5 mL of the extract was then mixed with 20 mL distilled water and three drops of 0.1% phenolphthalein after which the solution became pink. In order to neutralize the solution few drops of 0.1 N HCI were added until the pink color got disappeared. 1 mL iodine reagent (1 g iodine and 10 g KI in 50 mL distilled water) was then poured in experimental solution and the volume was raised up to 50 mL with distilled water. Optical density of this solution was recorded at 590 nm in Vis-UV 117 Systronics Spectrophotometer against the blank prepared in distilled water. Amylopectin content of different varieties of rice was calculated by subtracting the value of amylose content from total starch content.

Quantitative estimation of protein was done by the methods of Lowery *et al.,* (1951). 100mg seed was crushed in 5ml acetate buffer (pH 4.8). 0.5ml of this homogenate was mixed with 1ml of 15% cold trichloroacetic acid and then centrifuged at 6000rpm for 30 minutes. The supernatant was decanted and the precipitate was dissolved in 5ml 0.1N NaOH. The dissolved solution was then diluted ten times. This 5ml of alkaline reagent was mixed thoroughly and was allowed to stand at room temperature for 10 minutes. 0.5 ml folin ciocalteaue reagent (1: 1) in distilled water was then added to it. After 10 minutes the optical density was recorded at 750nm with blank prepared by similar process in distilled water. The standard curve of egg albumin was prepared via similar method and correlated.

Total sugar of the rice seed was estimated according to the method of Dubois *et al.*, (1956). Reducing sugar content of the sample was evaluated by the method of Somogyi-Nelson (Plummer, 1971). Non- reducing sugar was estimated by subtracting the value of reducing sugar from the total sugar.

For establishing correlation between different variables, Karl Pearson's coefficient of correlation (r) was used. Experiments were performed in replicates of three, and the data were statically analyzed by mean ± S.E. The results obtained have been in Table- 1- 6.

Results and Discussion

Table 1 shows that none of the paddy varieties was found totally resistant to aflatoxin production but they facilitated aflatoxin production at varying levels. From the results it is evident that four rice varieties viz. Turanta IET- 7991, Vaidehi, Dhanlaxmi and Mansuri produced aflatoxin B1 in very low amount i. e. 51.35, 65.52, 62.53 and 67.25µg/Kg and may be considered as highly resistant varieties. Shakuntala IET-11183, Gautam IET- 13439, Birsa Dhan- 202, Sita, Kanak, Jaishree, Pusa 2- 21 produced aglatoxin B1 in the range of 115.45 to 173µg/Kg after infestation with *Aspergillus flavus*. Pusa- 33, Basmati- 370, Katarni, Sugandha, BR- 9 and Kamini produced relatively high aflatoxin B1 after *A. flavus* infestation in the range of 218.25 to 370µg/Kg. On the basis of aflatoxinB1 production the screened paddy variety were grouped into three categories, i.g., highly resistant (< 100 µg/kg), moderately resistant (100-200 µg/kg) and susceptible (> 200 µg/kg). The Turanta, Vaidehi, Dhanlaxmi and Mansury varieties were considered highly resistant while seven varieties comprising Shakuntala IET- 11183, Gautam- IET- 13439, Birsa Dhan- 202, Sita, Kanak, Jaishreew and Pusa 2- 21were considered as moderately resistant. The remaining six varieties, viz., Pusa- 33, Basmati- 370, Katarni, Sugandha, BR- 9 and Kamini were placed in susceptible category. The present findings gain support from the work of Sinha and Singh (2013) who also found a more or less similar result in Aflatoxin B1 production in different rice varieties.

Total starch level of different paddy varieties varied to a great extant (Table 2). Maximum amount (713.4 mg/g) of starch was estimated in Jaishree (721mg/g), Pusa- 33 (712mg/g), Pusa 2- 21 (718mg/g), BR- 9 (716mg/g), Kamini and Kanak (715mg/g), Sita (713mg/g), Birsa Dhan (705mg/g) and Dhanlaxmi (706mg/g) varieties whereas minimum amount (613- 657mg/g) was found in rest of the varieties. The kernel of different paddy varieties contained different concentration of amylase (in the range of 121mg/g to 168mg/g) after infestation with *A. flavus*. Obviously amylopectin content of total starch of different kernel varieties showed negative correlation with amylose content. In contrast to amylose content, the amylopectine level was found to be the highest, 571.8mg/g and 570.3mg/g in respectively in BR- 9 and Kamini variety and the lowest (438.4 mg/g) in the Gautam IET- 13439 variety.

However, there is slight variation in amylopectin level of highly and moderately resistant varieties. Though the total starch, amylopectin and amylose level of different paddy varieties showed substantial variation, there is positive correlation between aflatoxin B1 elaboration and total starch and amylopectin content of the paddy varieties. The present findings are in agreement with the work of Sinha and Singh (2013).

Table- 3 shows the protein contents of kernel of different paddy varieties under healthy and infested conditions. The total protein content in the healthy kernel ranged in between

120.15mg/g (Basmati- 370) to 130.45mg/g (Kanak). Significant reduction in protein content was observed during fungal infestation, the maximum being in Turanta IET- 7991 (17.39%) and Vaidehi- 625 (17.74%) (Table- 3: Fig- 1).

Table.1 Production of aflatoxin in µg/Kg by *Aspergillus flavus* in the kernela of different Varieties of Paddy under laboratory conditions

Paddy variety	Aflatoxin B1 in µg/Kg	Resistant/ Susceptible group
Turanta IET7991	51.35±1.22	Highly resistant
Vaidehi	65.52±1.43	Highly resistant
Shakuntala IET- 11183	118.65±1.35	Moderately resistant
Gautam IET- 13439	121.75±1.71	Moderately resistant
Birsa Dhan 202	134.75±1.65	Moderately resistant
Dhanlaxmi	62.53±1.16	Highly resistant
Sita	165.25±1.32	Moderately resistant
Kanak	173.35±1.25	Moderately resistant
Mansuri	67.25±1.63	Highly resistant
Jaishree	135.35±1.34	Moderately resistant
Pusa- 33	218.25±1.75	Susceptible
Pusa 2- 21	115.45±1.71	Moderately resistant
Basmati 370	295.73±1.35	Susceptible
Katarni	325.35±1.41	Susceptible
Sugandha	370.65±1.23	Susceptible
BR- 9	290.35±1.16	Susceptible
Kamini	377.35±1.75	Susceptible

Table.2 Total starch, Amylose and Amylopectin contents in the kernel of different paddy Varieties infested with *Aspergillus flavus*

Paddy variety	Total starch mg/g±SE_m	Amylose content mg/g±SE_m	Amylopectin contentmg/g±SE_m
Turanta IET7991	613±0.45	150±0.21	450.6±0.56
Vaidehi	625±0.47	161±0.23	152.7±0.51
Shakuntala IET- 11183	655±0.36	165±0.16	451.5±0.56
Gautam IET- 13439	657±0.51	167±0.18	438.4±0.43
Birsa Dhan 202	705±0.61	167±0.15	442.5±1.05
Dhanlaxmi	706±1.12	168±0.22	445.6±1.07
Sita	713±1.07	121±0.21	535.7±0.51
Kanak	715±1.25	123±0.21	538.6±0.76
Mansuri	618±0.71	171±0.23	472.5±0.65
Jaishree	721±1.12	134±0.24	570.6±0.35
Pusa- 33	712±1.25	138±0.23	573.5±0.42
Pusa 2- 21	718±1.31	142±0.21	551.8±0.51
Basmati 370	627±1.15	157±0.11	523.6±0.76
Katarni	635±1.21	154±0.16	518.7±0.46
Sugandha	637±1.20	151±0.13	512.5±0.53
BR- 9	716±1.13	136±0.21	571.8±0.42
Kamini	715±1.15	138±0.14	570.3±0.41

Table.3 Protein content in the kernel of different paddy varieties under healthy and infested Condition with *Aspergillus flavus*

Paddy variety	Protein content in mg/g±SE_m		
	Healthy Kernel	Infested Kernel	% Change
Turanta IET7991	126.50±1.45	104.50±1.21	17.39
Vaidehi	125.65±1.47	103.35±1.23	17.74
Shakuntala IET- 11183	124.75±1.36	107.23±1.16	14.04
Gautam IET- 13439	127.75±1.51	112.25±1.18	12.13
Birsa Dhan 202	122.65±1.61	115.35±1.15	5.90
Dhanlaxmi	121.35±1.12	116.25±1.20	4.20
Sita	127.35±1.17	113.23±1.21	11.08
Kanak	230.45±1.15	116.35±1.18	10.80
Mansuri	128.65±0.61	115.75±1.13	10.02
Jaishree	123.25±1.13	113.45±1.14	7.95
Pusa- 33	122.35±1.15	109.25±1.15	10.70
Pusa 2- 21	125.55±1.30	112.21±1.21	10.62
Basmati 370	120.15±1.15	113.70±1.01	5.36
Katarni	121.25±1.21	109.35±1.16	9.81
Sugandha	122.55±1.21	107.25±1.03	12.38
BR- 9	127.35±1.13	114.37±1.12	10.19
Kamini	129.36±1.14	115.35±1.10	10.83

Table.4 Level of total sugar in the kernel of different paddy varieties under healthy and infested Condition with *Aspergillus flavus*

Paddy variety	Total sugar in mg/g±SE_m		
	Healthy Kernel	Infested Kernel	% Change
Turanta IET7991	17.75±0.45	23.55±0.21	32.67
Vaidehi	15.35±0.47	24.35±0.23	58.63
Shakuntala IET- 11183	14.55±0.36	23.75±0.16	63.23
Gautam IET- 13439	16.75±0.51	22.65±1.12	35.22
Birsa Dhan 202	16.25±0.61	23.85±1.13	46.76
Dhanlaxmi	16.34±0.17	24.25±1.10	48.40
Sita	17.66±0.16	24.35±0.21	37.88
Kanak	17.26±0.15	22.35±0.18	29.49
Mansuri	17.25±0.61	22.45±0.13	30.14
Jaishree	17.75±0.14	22.55±1.12	27.04
Pusa- 33	16.65±0.15	23.34±1.14	40.18
Pusa 2- 21	16.85±0.34	23.57±0.21	33.94
Basmati 370	18.85±0.16	23.73±1.01	25.88
Katarni	18.75±0.21	23.65±1.13	26.13
Sugandha	18.65±0.23	23.26±1.12	24.71
BR- 9	15.75±0.14	21.35±1.15	35.55
Kamini	17.67±0.15	22.75±1.12	28.74

Table.5 Level of reducing sugar in the kernel of different paddy varieties under healthy and infested condition with *Aspergillus flavus*

Paddy variety	Total sugar in mg/g±SE_m		
	Healthy Kernel	Infested Kernel	% Change
Turanta IET7991	4.25±0.41	6.23±0.21	46.58
Vaidehi	4.22±0.42	6.21±0.22	47.15
Shakuntala IET- 11183	4.12±0.32	6.22±0.16	50.97
Gautam IET- 13439	4.14±0.50	6.25±0.12	50.96
Birsa Dhan 202	5.50±0.61	7.16±0.13	30.18
Dhanlaxmi	5.45±0.15	7.17±0.11	31.37
Sita	5.25±0.16	7.20±0.21	37.14
Kanak	4.75±0.17	6.35±0.13	33.68
Mansuri	4.35±0.21	6.32±0.15	45.28
Jaishree	4.45±0.16	6.27±0.12	40.89
Pusa- 33	4.81±0.15	6.25±0.14	49.52
Pusa 2- 21	4.85±0.36	6.45±0.21	32.98
Basmati 370	5.65±0.26	7.37±0.11	30.44
Katarni	5.75±0.24	7.42±0.13	29.04
Sugandha	5.70±0.25	7.45±0.12	30.70
BR- 9	4.35±0.15	6.30±0.15	44.82
Kamini	4.67±0.25	6.21±0.12	32.97

Table.6 Level of non- reducing sugar in the kernel of different paddy varieties under healthy and infested condition with *Aspergillus flavus*

Paddy variety	Total sugar in mg/g±SE_m		
	Healthy Kernel	Infested Kernel	% Change
Turanta IET7991	13.50±0.42	19.35±0.25	32.67
Vaidehi	11.13±0.41	18.45±0.21	65.76
Shakuntala IET- 11183	10.43±0.32	18.35±0.16	75.93
Gautam IET- 13439	12.61±0.53	19.75±0.18	55.62
Birsa Dhan 202	10.75±0.61	18.45±0.17	71.62
Dhanlaxmi	10.89±0.15	18.57±0.11	70.52
Sita	12.41±0.16	19.76±0.25	59.22
Kanak	12.51±0.15	20.55±0.13	74.80
Mansuri	12.90±0.23	20.25±0.15	52.25
Jaishree	13.30±0.16	20.35±0.12	58.48
Pusa- 33	12.84±0.15	20.27±0.16	55.92
Pusa 2- 21	13.00±0.32	20.57±0.21	55.83
Basmati 370	13.20±0.26	20.65±0.14	59.45
Katarni	13.00±0.24	18.75±0.13	64.47
Sugandha	12.95±0.25	20.75±0.15	59.61
BR- 9	11.40±0.14	18.75±0.17	64.47
Kamini	13.00±0.26	18.65±0.12	43.46

Fig.1 Change in protein content in different paddy varieties after infestation with *A. flavus*

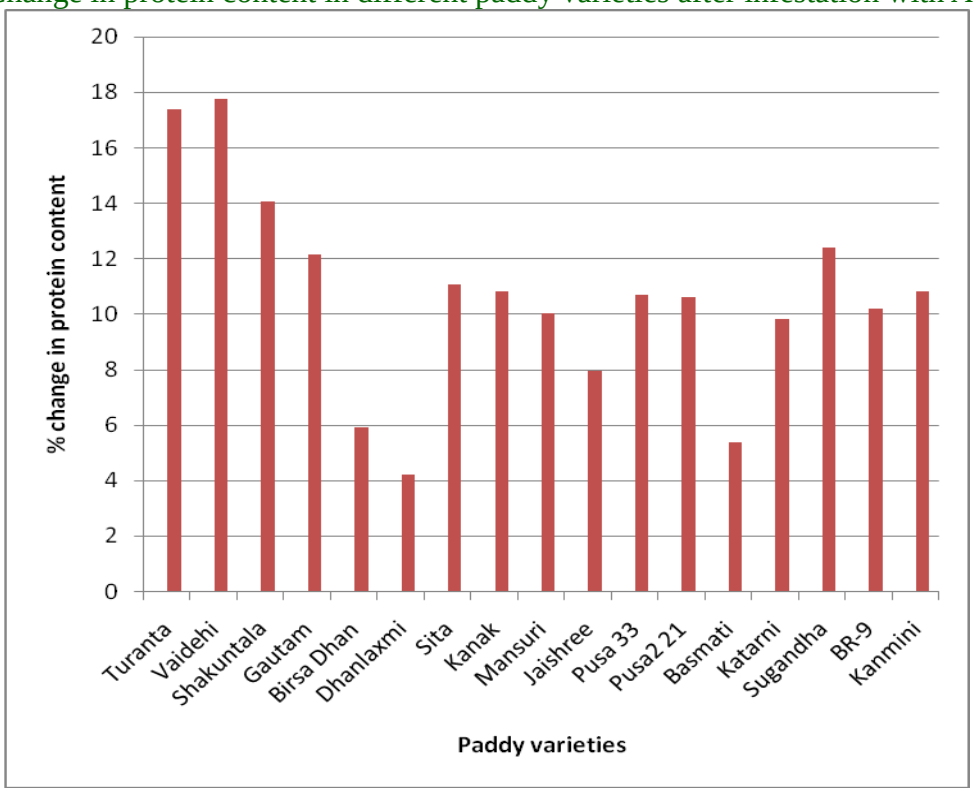

Fig.2 Change in total sugar in different paddy varieties after infestation with *A. flavus*

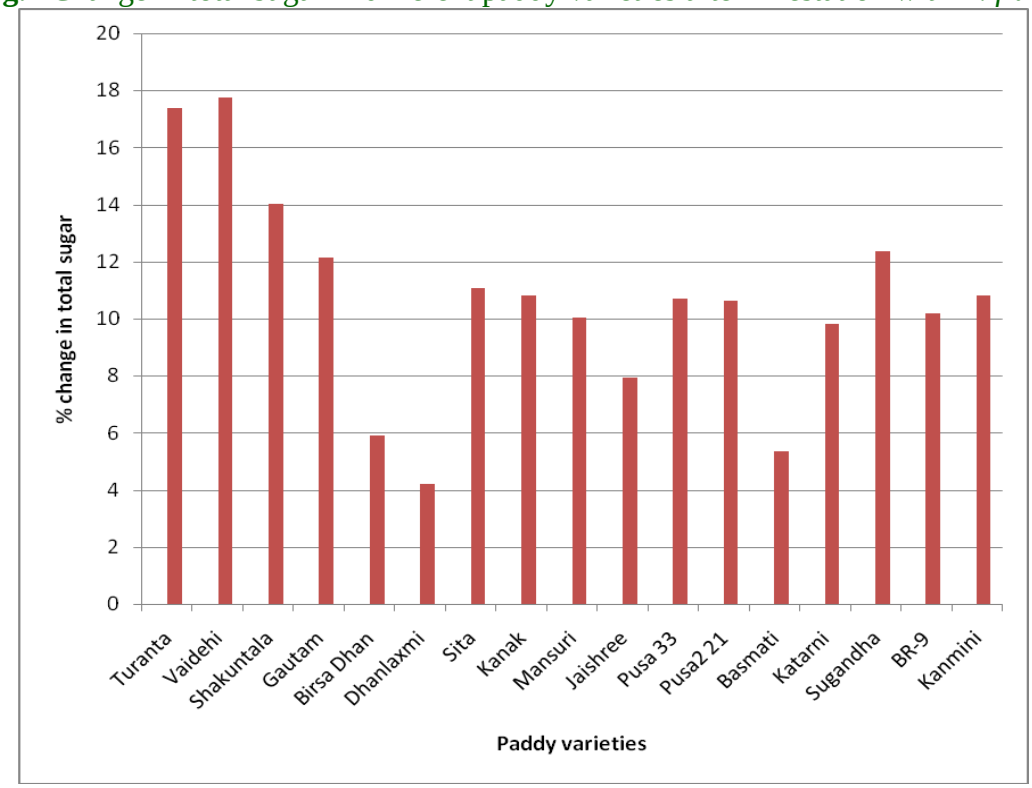

Fig.3 Change in reducing sugar in different paddy varieties after infestation with *A. flavus*

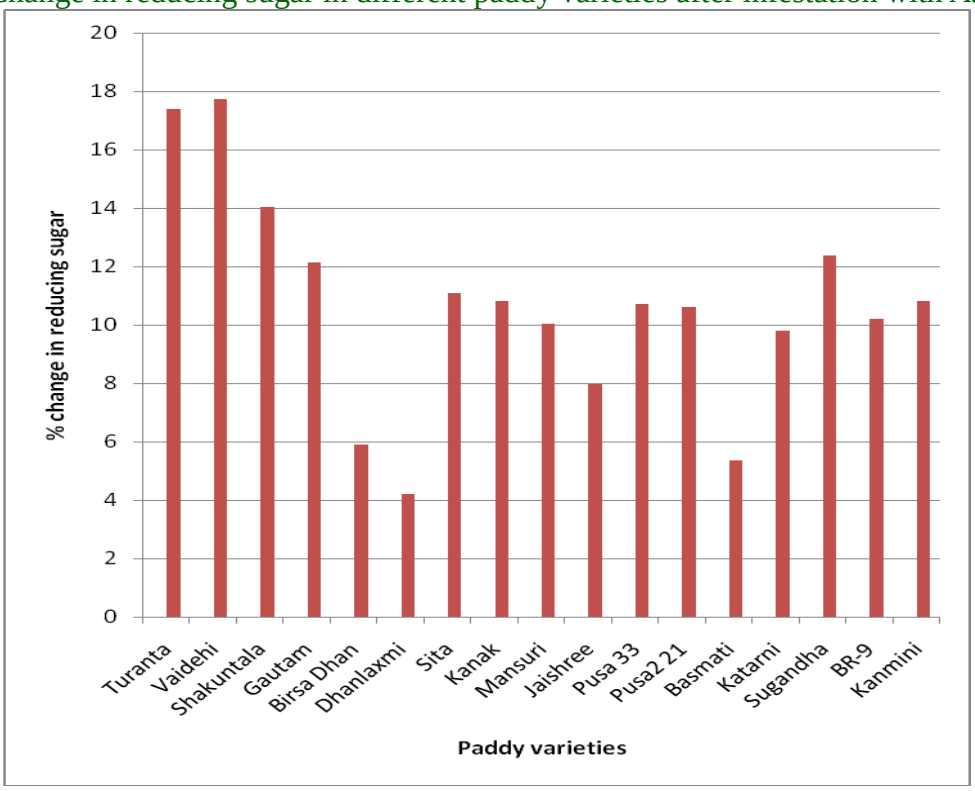

Fig.4 Change in non- reducing sugar in different paddy varieties after infestation with *A. flavus*

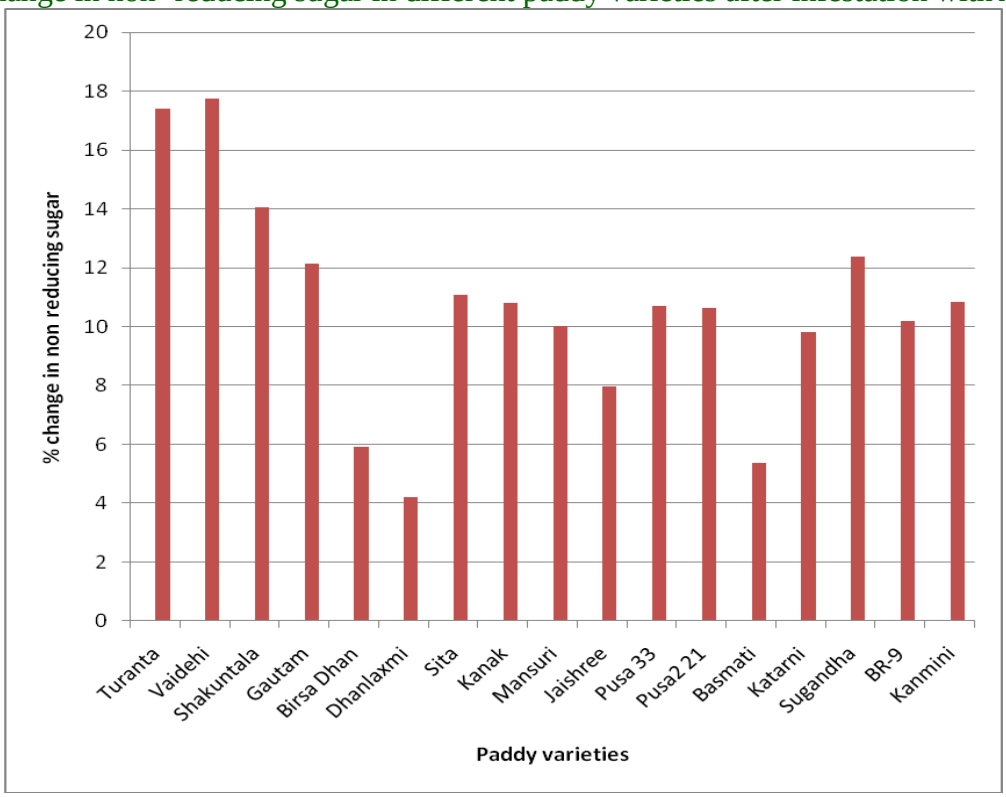

Table- 4 depicts the levels of total sugar. In the healthy kernel the total sugar content was maximum in paddy varieties Basmati- 370, Katarni (18.85mg/g) and Sugandha (18.65mg/g) and minimum in Vaidehi (15.35mg/g) and BR- 9 (15.75). After infestation with *A. flavus* all the paddy varieties showed an increase in total sugar content. The varieties Turanta, Gautam, Kanak, Mansuri, Jaishree Pusa 2- 21, Basmati- 370, Katarni, Sugandha and Kamini showed an increase in total sugar content to 32.67%, 35.22%, 29.49%, 30.14%, 27.04%, 33.94%, 25.88%,26.13%, 24.71% and 28.74% respectively. The other paddy varieties showed more than 40% increase in total sugar content (Table- 4: Fig- 2). The reducing sugar in the healthy kernels of paddy varieties ranged between 4.12mg/g to 5.75mg/g, but after infestation with *A. flavus* the reducing sugar content showed an increasing trend. After fungal infestation the increase in reducing sugar level was in the range of 30.18% (Birsa Dhan) to 50.97% (Shakuntala) (table- 5, Fig- 3). Similarly Table- 6 depicts the level of non- reducing sugar in the kernels of healthy and infested paddy varieties. The concentration of non-reducing sugar in healthy kernel was minimum in Vaidehy (11.13mg/g), Shakuntala (10.43mg/g), Birsa Dhan 202 (10.75mg/g), Dhanlaxmi (10.89mg/g), and BR- 9 (11.40mg/g) and maximum Turanta (13.50mg/g), Jaishree (13.30), Pusa 2- 21 (13.00, Basmati- 370 (13.20) and Katarni and Kamini (13.00mg/g). After infestation with *A. flavus* all the paddy varieties showed an increase in the level of non- reducing sugar. The level of non- reducing sugar in kernel of Shakuntala, Birsa Dhan, Dhanlaxmi and Kanak was increased to 75.93%, 71.62%, 70.52% and 74.80% respectively after fungal infestation (Table- 6; Fig- 4). Turanta, a highly resistant variety showed only 32.67% increase in the level of non- reducing sugar after fungal infestation.

Analysis of protein contents in the kernels of paddy varieties selected for present investigation shows that higher levels of protein in kernels somewhat inhibit the production of aflatoxin B1. Thus, the varieties containing higher amount of proteins were found to resist the production of aflatoxinB1 production. Significant reduction in protein content was observed in those varieties that facilitated higher amount of aflatoxin B1. Correlation coefficient ® was calculated to be -0.59, which shows moderate negative correlation between the amount of protein and levels of aflatoxin production.

Analysis of sugar contents shows that higher levels of reducing and non- reducing sugar have an inhibitory effect on the production of aflatoxin B1 production. Correlation coefficient between sugar levels and aflatoxin B1 production was calculated to be -97, which shows almost perfect negative correlation. The present findings are in conformity with the Sinha and Kumar (2010), Sinha (1991) and Maharaj (2000) who also recorded a more or less similar trend in different wheat varieties.

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