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Screening of maize cultivars and effect of their protein extracts on fungal growth

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Among the naturally occurring toxins namely aflatoxin B1(AFB1) is the most potent toxin found in food and feed. Their toxicity causes severe health and economic problems worldwide. The prevalence of AFB1 in a variety of foods and feeds destined for human consumption is a major concern in crops like corn, groundnut etc.AFB1 is most carcinogenic and toxic, thus, causing severe economic losses for farmers with affected grain. Therefore, eliminating AFB1 contamination is a research priority nowadays. Host plant resistance has been considered a pre-eminently logical and economical strategy for controlling aflatoxin contamination. This strategy has gained even greater prominence due to recent discoveries of natural resistance in corn that can be exploited in plant breeding procedures (Brown *et al.* 2010; Williams *et al.* 2006).

Identification of corn resistant genotypes and incorporation of resistance from these sources into commercial hybrids requires identification and characterization of factors associated with the resistance to aflatoxin biosynthesis and accumulation. This would help in saving time and money utilized in the varietal screening procedures (Brown *et al.* 2010). Aflatoxin levels in corn could be controlled by differences in the ability to resist (i)invasion of fungus into the kernel, (ii) minimize the amount of fungal growth within the kernel,(iii)Inhibit aflatoxin biosynthesis. There have been reports that the accumulation of certain proteins lead to inhibition of fungal growth or aflatoxin production (Chen *et al*. 2006; Huang *et al.* 1997).

Thus, in these study 22 maize cultivars already screened as resistant, moderately resistant and susceptible categories were taken. These cultivars were divided on the basis of AFB1 produced when infected by *A. parasiticus.* Antifungal proteins synthesized in the kernels during seed development protect or reduce fungal infection of the kernels during storage and germination. To ascertain this inhibitory effect of proteins extracted from differently categorized maize was observed on the growth of *A. parasiticus*.

Fungal culture of *Apergillus parasiticus* SRRC148 was obtained from Dr. Deepak Bhatnagar, Commodity Safety Research Laboratory, USDA, USA. Seeds of 22 maize cultivars were collected from Department of Genetics & Plant Breeding, GBPUA&T, Pantnagar. Fungal culture was routinely revived on PDA slants at monthly intervals.

Antifungal bioassay was performed with 0.05 ml aliquot of spore suspension (10^6 spores/ml) of *A. parasiticus.* The aliquot (0.05 ml) was placed in the petri plate. PDA was then poured and allowed to solidify in the petri plate. Sterile paper discs were then placed over the PDA with the help of sterile forcep.25µl of protein extracts of different maize cultivars were then incubated at 37°c for 3-5 days.

Table 1: The categorization of different maize varieties on the basis of AFB1 production into Resistant(R), Moderately Resistant (M) & Susceptible (S) cultivars

Sl. No.	Resistant (R)	Moderately Resistant (M)	Susceptible (S)
1.	Pant 97K1038	Hyd 97R 112	Hyd 97R 113
2.	Pant 97 K1041	Hyd 97R 116	Pant 97K1040
3.	Pant 97 K1043	Pant 97K1044	Pant97K 1097x1096
4.	Pant97K1058x1059	Pant 97 k1045	Pant 97K 1123x1124
5.	Pant97K1087x1086	Pant 97 K1046	Pant 97K 1109x1108
6.	Pant 97K1091x1090	Pant97K1120x1121	Pant 98K 1010x1011
7.	Pant97K 1115x1114	Pant98K1016x1017	
8.		Pant97K1083x1082	
9.		Pant98K1014x1015	

This study was performed on 21 maize cultivars already characterized into Resistant (R), Moderately resistant (M) and Susceptible (S) on the basis of AFB1 produced on autoclaved maize seeds by infecting with *A. parasiticus* (Table1). Here antifungal bioassay was done to ascertain the crude protein effect on the above stated maize cultivars on the growth of the fungus *A. parasiticus* (Fig.1&2).

Here, it was observed that the protein extracts of resistant cultivars (depicted by no.2 (Pant 97K 1041), 3 (Pant 97K 1087 × 1086), 4 (Pant 97K 1043) & 8 (Pant 97 K 1115 × 1114) in Fig.1 and 3' (Pant 97K 1038) and 2" (Pant 97K 1091× 1090), 3" (Pant 97K 1041) in fig. 2) inhibited the growth of the fungus. Moderately

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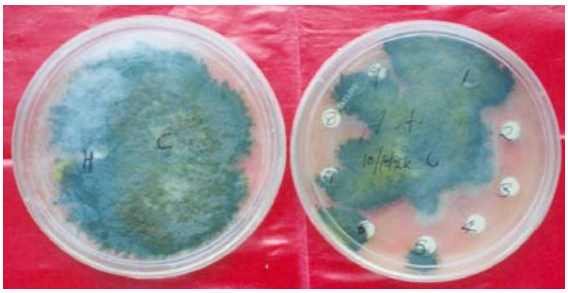
resistant cultivars inhibited the growth of fungus to a limited extent while susceptible cultivars showed no inhibition of fungal growth [indicated by 1 (Pant 97K 1097 × 1096), 1' (Pant 97K 1123 × 1124), 4' (Pant 97K 1040), 5'(Pant 97K 1109 × 1108) in fig.1 and 1" (Pant 98K 1016 × 1017), 4" (HYD 97R 113), 7" (Pant 98K 1010 ×1011) in Fig.2].

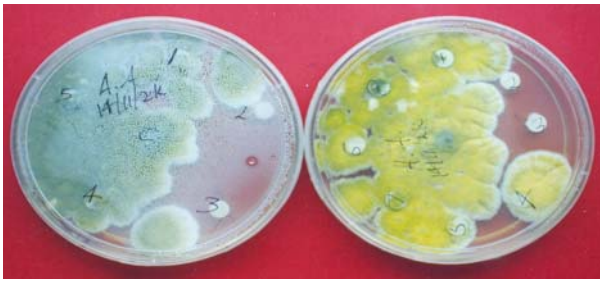
The inhibitory effect of crude protein extracts from seeds of resistant cultivars was observed as in case of Pant 97K 1038, 1041, 1043& (1087×1086) etc. while moderately resistant cultivars inhibited the growth of fungus to a limited extent. Susceptible cultivars like Pant 97K (1123×1124) and (1097×1096) etc. showed no inhibition of fungal growth as seen in fig.1&2.

This indicates that crude protein extract from seeds of resistant cultivars have some proteins which act synergistically with other proteins and some none protein factors to prevent the aflatoxin accumulation. These results are in accordance with Guo *et al.* 1998 and Chen *et al.* 2007, where resistant kernel protein extracts showed remarkable antifungal activity against *A. Flavus* than did susceptible kernel protein extracts. This suggests a role for kernel proteins in resistance to fungus infection and aflatoxin accumulation in resistant corn cultivars.



Left - 1. Control with water
Right - 1. Pant 97K 1097X1096(S)
2. Pant 97K 1041 (R)
3. Pant 97K 1087X1086 (R)
4. Pant 97K 1043 (R)
5. Pant 97K 1120X1121 (M)
6. Pant 97K 1045 (M)
7. Pant 97K 1046(M)
8. Pant 97K 1115X1114 (R)

Fig. 1. Antifungal bioassay of different categorized corn kernels against *Aspergillus parasiticus*



Left- 1. Pant 97K 1123X1124 (S)
2. Pant 97K 1083X1082 (M)
3. Pant 97K 1038(R)
4. Pant 97K 1040 (S)
5. Pant 97K 1109X1108 (S)
Right- 1. Pant 98K 1016X1017 (S)
2. Pant 97K 1091X1090 (R)
3. Pant 97K 1041 (R)
4. Hyd 97R 113 (S)
5. Hyd 97R 112 (M)

Fig. 2. Antifungal bioassay of different categorized corn kernels against *Aspergillus parasiticus*

Antifungal bioassay revealed limited restriction on the growth of fungus in case of moderately resistant cultivars. This may be attributed to the presence of limited or less amount of proteins and other factors contributing to resistance for fungal growth. No inhibition was observed in fungal growth with control (water) and by protein extracts from susceptible cultivars in antifungal bioassay which may be assumed due to the absence of proteins and other factors contributing to resistance for fungal growth. These results are in accordance with Chen *et al.* (2007); Huang *et al.* (1997) in maize.

The above study indicates an important role for kernel proteins in fungal growth resistance and therefore, resisting aflatoxin biosynthesis and its accumulation in maize. Currently experiments are being going on in our lab to identify the factors like proteins conferring resistance to aflatoxin producing fungus in Resistant maize cultivars.

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