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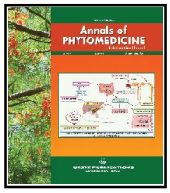

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Studies on aflatoxin resistance in chilli (*Capsicum annuum* L.) germplasm

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Abstract

Chilli is rich in vital nutrients with numerous health benefits. Chilli fruits are commonly contaminated by *Aspergillus* species, which produces aflatoxins. Aflatoxins are highly toxic and carcinogenic mycotoxins. Identification of aflatoxin contamination-resistant sources of chillies is highly preferred to breed new genotypes for cultivation. Hence, 35 chilli genotypes were evaluated at the Mycotoxicology Laboratory of the International Crops Research Institute for the Semi-Arid Tropics, Patancheru during 2019-20 to estimate their resistance to aflatoxin production. Chilli fruits were artificially inoculated and aflatoxin content was estimated by ELISA technique. A significant amount of genetic variability concerning aflatoxin among the tested genotypes was noticed. Results revealed that the samples were contaminated with aflatoxin B1 to the extent of 40655.55 µg/kg. Estimates of average aflatoxin from 35 genotypes indicated a high quantity of aflatoxin of 11754.68 µg/kg. The genotype IC-334383 showed less aflatoxin content (125.25 µg/kg), which indicates resistance to aflatoxin production, while the genotype EC-402113 showed very high aflatoxin content, *i.e.*, 40655.55 µg/kg, which indicates susceptibility to aflatoxin production. The *A. flavus* resistant germplasm identified in this investigation has the potential for chilli aflatoxin resistance breeding programs.

1. Introduction

Chilli is widely used as both vegetable and spice. The consumption of fresh chilli whose fruits are rich in vitamin C is an ideal means of contributing to the problems of food insecurity and malnutrition (Saisupriya *et al.*, 2021). Capsaicin is the compound which contributes to the pungency in chilli. Capsaicin has been demonstrated to help diabetic patients (Jyoti *et al.*, 2023). Chilli is rich in phytochemicals and antioxidants; hence, consumption of chilli increases health benefits and lowers the chances of developing chronic diseases. Chilli is an important spice cum vegetable crop from a global perspective and there is a continuous rise in the demand for chilli pepper. In the world, India holds the first rank in the production and consumption of chilli.

Chilli is one of the major sufferers of a significant number of field and post-harvest diseases, but the infection of chilli due to aflatoxin fungi, *A. flavus* is specific during and after harvest. Storage fungi especially *A. flavus* and *A. niger* were found to be prevalent in stored chillies (Giridhar and Reddy, 1999). Post-harvest the red chillies are spread out on the ground to dry in the open air where the climatic conditions are ideal for the growth of moulds which produce mycotoxins. *Aspergillus* sp. is commonly found on chilli fruits stored in humid regions. Especially the stored fruits get contaminated with aflatoxin and are subjected to deterioration leading to quality spoilage. Consequently, produce becomes unfit for human consumption. Iqbal *et al.* (2010) explained the main reasons for the buildup of fungal biomass, which leads to aflatoxin production in chilli. Reddy *et al.* (2001) reported that nearly 9% of the dry chilli powders available in supermarkets sampled with non-permissible levels of aflatoxin and those samples accessed from cold storage reported alarming levels of aflatoxin B1 (5.5 µg per kg) (Ravi Kiran *et al.*, 2005), whereas, about 11% of the 57 samples tested were detected with higher concentration of aflatoxin B1 as noted by Babu *et al.* (2012).

Contreras *et al.* (2016) quantified aflatoxins in 3 types of chilli peppers, “Ancho”, “Guajillo” and “Piquín” and the result of this study indicated that the chilli pepper is almost always contaminated with all aflatoxins. Hossain *et al.* (2018) studies revealed that most of the dry red chillis available in Bangladesh are contaminated with *Aspergillus* sp. containing a considerable amount of aflatoxin, which is a lethal mycotoxin. Naik *et al.* (2018) reported the use of ELISA for rapid and large-scale screening of aflatoxin-producing strains with accurate measurements of aflatoxin produced by *A. flavus*. Chilli varieties with *A. flavus infection* followed by aflatoxin production were reported (Desai *et al.*, 1991), however, to date; any of such variety for resistance to aflatoxin has been commercially released for farming. Keeping the above in view, an experiment was designed to evaluate 35 germplasm of chilli for screening for aflatoxin resistance through artificial inoculation and their correlation with fruit quality traits.

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2. Materials and Methods

2.1 Materials

The 35 genotypes (Table 1) experimented in the present investigation were sourced from NBPGR regional station (32 germplasm) Rajendranagar, Hyderabad, Lam farm (2 germplasm), Guntur, Andhra Pradesh and IARI, New Delhi (1 germplasm).

Table 1: List of chilli genotypes used for evaluation along with their sources and important traits

Genotype	Source	Trait
IC-347044	ICAR-NBPGR RS, R'nagar, Hyderabad	High yielder
IC-363918	ICAR-NBPGR RS, R'nagar, Hyderabad	Erect fruits and highest number of fruits per plant
IC-363993	ICAR-NBPGR RS, R'nagar, Hyderabad	Highly pungent fruits
IC-561676	ICAR-NBPGR RS, R'nagar, Hyderabad	High capsanthin content
IC-561622	ICAR-NBPGR RS, R'nagar, Hyderabad	High capsanthin content
IC-610381	ICAR-NBPGR RS, R'nagar, Hyderabad	Erect fruits
IC-505237	ICAR-NBPGR RS, R'nagar, Hyderabad	High yielder
IC-447018	ICAR-NBPGR RS, R'nagar, Hyderabad	Semi pendant fruits
IC-572459	ICAR-NBPGR RS, R'nagar, Hyderabad	Pungent fruits
IC-610383	ICAR-NBPGR RS, R'nagar, Hyderabad	High capsanthin content
IC-214965	ICAR-NBPGR RS, R'nagar, Hyderabad	High yielder
EC-402113	ICAR-NBPGR RS, R'nagar, Hyderabad	High chlorophyll content
IC-410423	ICAR-NBPGR RS, R'nagar, Hyderabad	Erect and oil-painted fruits
IC-526448	ICAR-NBPGR RS, R'nagar, Hyderabad	High capsanthin content and highly pungent fruits
EC-399567	ICAR-NBPGR RS, R'nagar, Hyderabad	Pungent fruits
IC-561655	ICAR-NBPGR RS, R'nagar, Hyderabad	Mild fruit pungency
EC-390030	ICAR-NBPGR RS, R'nagar, Hyderabad	Low yielder
IC-528433	ICAR-NBPGR RS, R'nagar, Hyderabad	Low ascorbic acid content
IC-528442	ICAR-NBPGR RS, R'nagar, Hyderabad	Erect fruits and high yielding
EC-399535	ICAR-NBPGR RS, R'nagar, Hyderabad	High chlorophyll content
EC-378632	ICAR-NBPGR RS, R'nagar, Hyderabad	High capsanthin content
IC-215012	ICAR-NBPGR RS, R'nagar, Hyderabad	High ascorbic acid content
EC-378688	ICAR-NBPGR RS, R'nagar, Hyderabad	Low yielder
IC-214966	ICAR-NBPGR RS, R'nagar, Hyderabad	Low yielder
IC-319335	ICAR-NBPGR RS, R'nagar, Hyderabad	Erect fruits and highly pungent fruits
IC-394819	ICAR-NBPGR RS, R'nagar, Hyderabad	Low yielder
IC-572498	ICAR-NBPGR RS, R'nagar, Hyderabad	Low yielder
EC-399581	ICAR-NBPGR RS, R'nagar, Hyderabad	High ascorbic acid content
IC-526737	ICAR-NBPGR RS, R'nagar, Hyderabad	Erect fruits
IC-570408	ICAR-NBPGR RS, R'nagar, Hyderabad	High yielding and highly pungent fruits
IC-561648	ICAR-NBPGR RS, R'nagar, Hyderabad	Pungent fruits
IC-334383	ICAR-NBPGR RS, R'nagar, Hyderabad	Pungent fruits
Sindhur (C)	HRS, Lam, Guntur, Andhra Pradesh	Large fruits
LCA-625 (C)	HRS, Lam, Guntur, Andhra Pradesh	High yielder
Pusa Jwala (C)	IARI, New Delhi	High Ascorbic acid content

ICAR-NBPGR RS- Indian Council of Agricultural Research-National Bureau of Plant Genetic Resources Regional Station; HRS- Horticultural Research Station; IARI- Indian Agriculture Research Institute; C - Check variety.

2.2 Methods

The experiment was carried out at the ICRISAT, Asia Center, Patancheru, Telangana during 2019-20. Chilli fruits were harvested at red red-ripened stage. The pin-prick method of artificial inoculation (Naik and Rawal, 2002) was used for chilli fruit infection. The per cent infection of *A. flavus* was recorded by Ajithkumar and Naik (2006). A suitable control was maintained by dipping the chilli fruits in sterile water, followed by pricking the fruits without inoculation by the pathogen. The experiment was repeated twice. The per cent disease incidence of chilli was calculated by visual observation. The disease was scored by using a 0-5 scale. The aflatoxin content (µg/kg) of all the samples was estimated by using the ELISA method. Aflatoxin in the sample was assessed following the formula of Reddy *et al*. (2001) as given below:

Aflatoxin concentration

$$= \frac{\text{AfB1}\,(\mu g / ml) \times \alpha\,\text{dilution}}{\text{Sample weight}} \times \text{Extract solvent (ml)}$$

Further, the correlation of aflatoxin content with yield, ascorbic acid, chlorophyll content, capsanthin and capsaicin was estimated using the Pearson correlation coefficient (2008).

3. Results

Among the 35 genotypes screened under laboratory conditions by pinprick method, IC-347044, IC-572459, IC-410423, EC-399567, EC-378632 and IC-334383 revealed resistant reactions. However, none of the genotypes were found to be immune (Table 2). There were three moderately resistant genotypes, *viz.,* IC-447018, IC-214965 and IC-394819; six moderately susceptible genotypes, *viz.,* IC-505237, EC-390030, IC-215012, IC-214966, IC-570408 and Sindhur. Ten genotypes, *viz.,* IC-363918, IC-363993, IC-561676, IC-610381, IC-528442, IC-319335, EC-399581, IC-526737, LCA-625 and Pusa jwala showed susceptible reaction, while another ten germplasm, *i.e.,* IC-561622, IC-610383, EC-402113, IC-526448, IC-561655, IC-528433, EC-399535, EC-378688, IC-572498, and IC-561648 were highly susceptible. The resistant genotypes identified can be utilised in breeding resistant varieties for aflatoxin for commercial exploitation.

Table 2: Reaction of chilli genotypes against *A. flavus* using artificial inoculation

Grade	Per cent fruits infected	Reaction	Genotypes
0	0	Immune	None
1	1-10	Resistant	IC-347044, IC-572459, IC-410423, EC-399567, EC-378632, IC-334383
2	11-25	Moderately resistant	IC-447018, IC-214965, IC-394819
3	26-50	Moderately susceptible	IC-505237, EC-390030, IC-215012, IC-214966, IC-570408, Sindhur
4	51-75	Susceptible	IC-363918, IC-363993, IC-561676, IC-610381, IC-528442, IC-319335, EC-399581, IC-526737, LCA-625, Pusajwala
5	More than 75	Highly susceptible	IC-561622, IC-610383, EC-402113, IC-526448, IC-561655, IC-528433, EC-399535, EC-378688, IC-572498, IC-561648

The 35 chilli fruit samples artificially inoculated were estimated for aflatoxin B1 by ELISA technique (Table 3). Significant variability concerning aflatoxin among the genotypes of chilli was reported. ELISA analysis revealed that they are contaminated with aflatoxin B1 to the tune of 40655.55 µg/kg. The average aflatoxin of 35 genotypes was reported with a very high quantity of aflatoxin (11754.68 µg/kg). Similar to the present investigation results, high levels of aflatoxin production in chilli samples are reported by Reddy *et al.* (2001) and Ravikiran *et al.* (2005).

Table 3: Fruit yield per plant and aflatoxin content of 35 genotypes

Genotypes	Fruit yield per plant		Aflatoxin content (µg/kg)						Pooled aflatoxin content (µg/kg)
			Experiment 1			Experiment 2			
	R_1	R_2	R_1	R_2	Mean	R_1	R_2	Mean	
IC-347044	0.81	0.91	161.2	175.6	168.4	167.8	180.8	174.3	171.35
IC-363918	0.69	0.48	10288.9	10227.9	10258.4	10314.4	10280.8	10297.6	10278
IC-363993	0.09	0.16	14879.3	14938.1	14908.7	14819.6	14849.5	14834.55	14871.63
IC-561676	0.19	0.15	4622.4	4535.8	4579.1	4606.2	4587.6	4596.9	4588
IC-561622	0.45	0.45	13755.6	13978.8	13867.2	13766.4	13810.6	13788.5	13827.85
IC-610381	0.28	0.35	3658.2	3567.8	3613	3649.4	3620.8	3635.1	3624.05
IC-505237	0.43	0.42	9688.4	9770.2	9729.3	9726.8	9770.8	9748.8	9739.05
IC-447018	0.28	0.31	8572	8444	8508	8488.1	8466.3	8477.2	8492.6

IC-572459	0.25	0.22	5254.2	5332	5293.1	5305.4	5324	5314.7	5303.9
IC-610383	0.22	0.25	14589.9	14535.3	14562.6	14519.6	14477.6	14498.6	14530.6
IC-214965	0.63	0.53	16524.6	16746.8	16635.7	16658.7	16719.9	16689.3	16662.5
EC-402113	0.25	0.34	40636.5	40580.9	40608.7	40731.2	40673.6	40702.4	40655.55
IC-410423	0.42	0.3	992.7	1189.1	1090.9	1074.1	1092.3	1083.2	1087.05
IC-526448	0.16	0.24	842.5	683.7	763.1	763.4	754.2	758.8	760.95
EC-399567	0.43	0.3	6392.6	6619.4	6506	6509.5	6529.3	6519.4	6512.7
IC-561655	0.12	0.15	762.2	728.4	745.3	758.8	746.9	752.85	749.075
EC-390030	0.16	0.11	15638.1	15735.9	15687	15673.2	15739	15706.1	15696.55
IC-528433	0.14	0.2	9092.3	9079.7	9086	9076.9	9051.3	9064.1	9075.05
IC-528442	0.64	0.46	38426.4	38508.2	38467.3	38316.3	38395.5	38355.9	38411.6
EC-399535	0.11	0.18	10832.8	10797.4	10815.1	10784.6	10740.4	10762.5	10788.8
EC-378632	0.33	0.3	21138.7	21188.5	21163.6	21199.7	21231.9	21215.8	21189.7
IC-215012	0.29	0.3	1698.2	1673.6	1685.9	1679.1	1657.5	1668.3	1677.1
EC-378688	0.1	0.11	26137.2	26184	26160.6	26013.2	26146	26079.6	26120.1
IC-214966	0.21	0.21	17562.2	17501.4	17531.8	17482.5	17455.5	17469	17500.4
IC-319335	0.2	0.17	15516.6	15551.8	15534.2	15534.4	15628	15581.2	15557.7
IC-394819	0.14	0.17	1472.3	1438.7	1455.5	1436.8	1422.8	1429.8	1442.65
IC-572498	0.1	0.12	372.9	414.3	393.6	384.2	395.6	389.9	391.75
EC-399581	0.21	0.24	13911.2	13877.6	13894.4	13832.6	13791.8	13812.2	13853.3
IC-526737	0.14	0.14	11863.4	11897.6	11880.5	11895.1	11925.5	11910.3	11895.4
IC-570408	0.63	0.62	17192.1	17137.7	17164.9	17124.8	17080.4	17102.6	17133.75
IC-561648	0.17	0.2	3892.4	3922.4	3907.4	3863	3910.8	3886.9	3897.15
IC-334383	0.13	0.17	129.1	119.5	124.3	128.3	124.1	126.2	125.25
Sindhur	0.44	0.49	6509.7	6543.5	6526.6	6491.7	6512.5	6502.1	6514.35
LCA-625	0.82	0.69	26449.2	26423	26436.1	26493.8	26481.6	26487.7	26461.9
Pusa jwala	0.4	0.36	21829.9	21863.3	21846.6	21788.5	21824.3	21806.4	21826.5

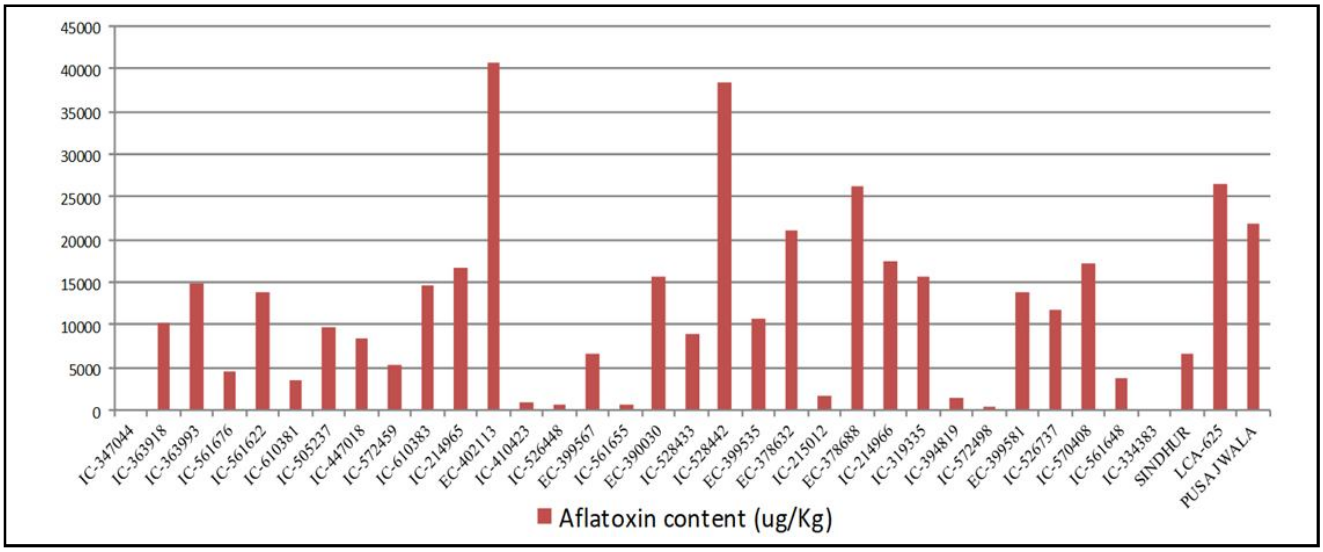

Figure 1: Pooled aflatoxin content of 35 chilli genotypes.

The genotype IC-334383 showed less aflatoxin content of 125.25 µg/kg, followed by genotype IC-347044 with an aflatoxin content of 171.35 µg/kg (Figure 2), while the genotype EC-402113 showed the highest aflatoxin content of 40655.55 µg/kg. The variation between the genotypes was due to the resistant mechanism offered by the genotype (Guruprasad *et al.,* 2018). It indicates that the genotypes with less aflatoxin content, *i.e.,* IC-334383 and IC-347044 exhibited resistance to aflatoxin production while the genotypes showing high aflatoxin content (Figure 3), which showed susceptible reaction against *A. flavus* under artificial inoculation.

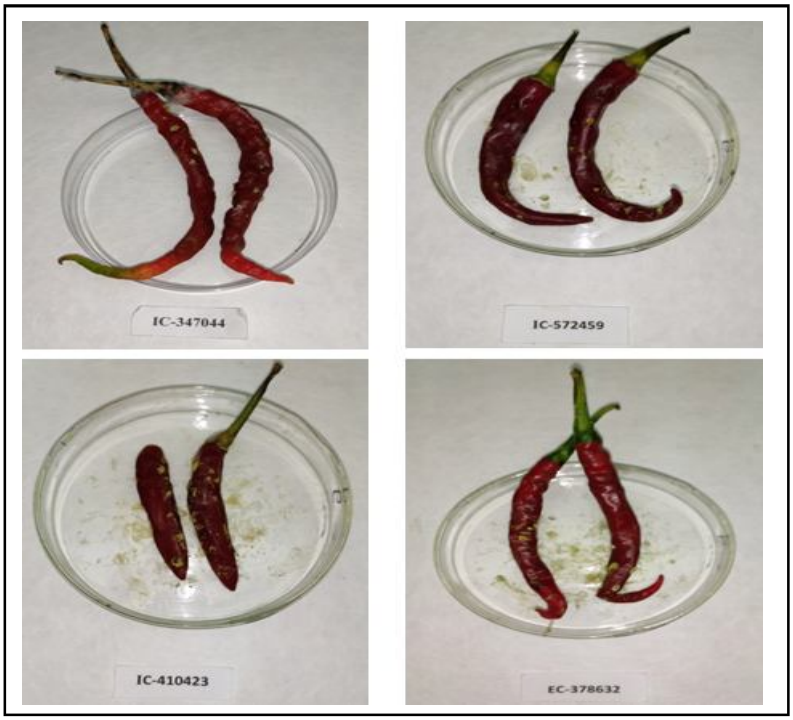

Figure 2: Chilli genotypes showing resistance reaction to *Aspergillus flavus.*

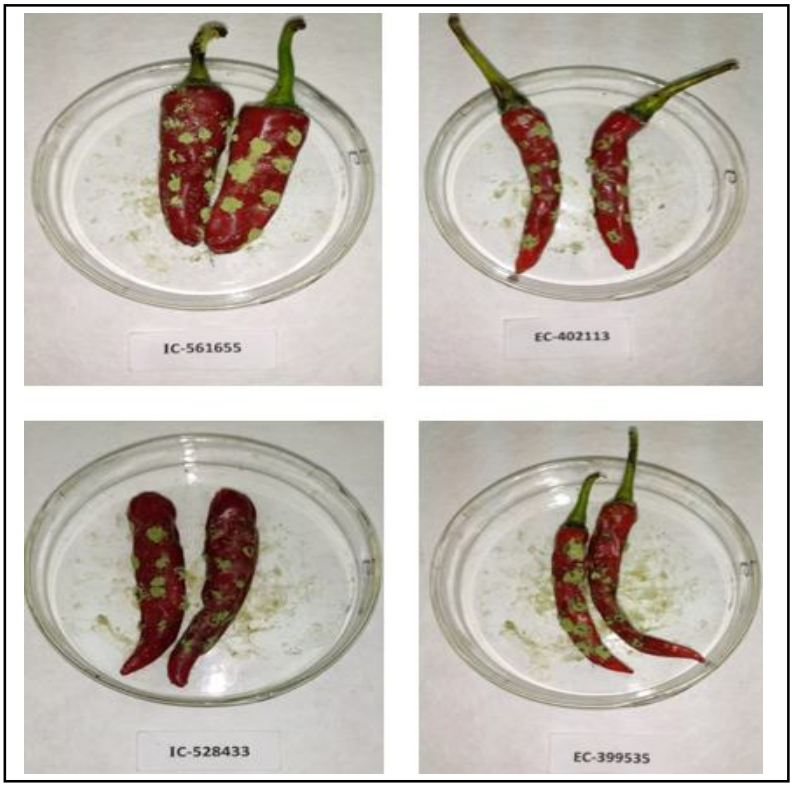

Figure 3: Chilli genotypes showing susceptible reaction to *A. flavus.*

Table 4: Correlation coefficients among aflatoxin content and other traits in 35 chilli genotypes

Trait	Fruit yield per plant	Ascorbic acid	Chlorophyll	Capsanthin	Capsaicin	Aflatoxin content
Fruit yield per plant	1	0.181	-0.240	0.038	0.069	0.214
Ascorbic acid		1	-0.169	-0.080	-0.316	0.225
Chlorophyll			1	0.105	-0.208	-0.019
Capsanthin				1	0.219	-0.083
Capsaicin					1	-0.024
Aflatoxin content						1

Correlation is used to measure the association between two or more traits. Understanding the correlation between traits is useful in the development of dependent traits by utilizing the positively correlated traits. Hence correlation between aflatoxin content and five traits namely fruit yield per plant, ascorbic acid, chlorophyll, capsanthin and capsaicin was estimated. A correlation study indicates the interrelationship between the traits and creates ample scope for improvement of a particular character by improving the highly correlated traits. Results revealed that the traits fruit yield per plant (0.214) and ascorbic acid (0.225) showed positive correlation with aflatoxin content, while the other three traits chlorophyll (-0.019), capsanthin (-0.083) and capsaicin content (-0.024) showed negative correlation with aflatoxin content. Hence, the selection of genotypes based on the negatively correlated traits, *i.e.*, chlorophyll, capsanthin and capsaicin content is useful in the development of resistant varieties, which develop low levels of aflatoxin content.

4. Discussion

A. flavus is a common fungus that is known to produce potent mycotoxins called aflatoxins. Chilli is an important crop globally and there is a continuous increase in its demand. India is the leading producer and consumer of chilli. *A. flavus* and *A. niger* are major threats to stored dry chillies, as reported by Giridhar and Reddy (1999). Aflatoxin is a global problem that restricts quality chilli production in chilli-growing countries, as stated by Paterson in 2007. Many countries that import chilli have strict regulations regarding aflatoxin contamination, which leads to the dumping of infected produce at local markets, resulting in higher toxin concentrations (Bhat, 1988). It is estimated that approximately 5 billion people, especially in developing countries, are at risk of chronic aflatoxin exposure through eating infected food (Strosnider *et al*., 2006). It is crucial to detect aflatoxin in chillies and chilli powder suspected to be contaminated. Therefore, its management should be ensured at the earliest (Sridhara Babu *et al*., 2012).

A proper strategy for effective management of aflatoxin content in dry chillies is the need of the hour (Sudha *et al.*, 2013). One of such options is to use resistant varieties or hybrid varieties by the farmers that resist the aflatoxin contamination. Aflatoxins are the metabolites that are toxic produced by *A. flavus* or, and *A. parasiticus*. Moreover, aflatoxins are carcinogenic (Peskta and Bonday, 1990). Hence, the breeding and development of aflatoxin-resistant chilli cultivars is an urgent activity to be addressed to manage the health issues caused by them.

In the present study, two genotypes, IC-334383 and IC-347044 were found to be resistant to *A. flavus* which can be used in breeding programs aimed at developing resistant varieties and also as donors to develop aflatoxin-resistant cultivars. Earlier Supriya *et al.* (2015) studied 17 chilli genotypes through artificial screening for resistance to *A. flavus* under laboratory conditions, among them, six genotypes including LCA 334 were resistant. Sudha *et al.* (2009) reported that one genotype BK-21-SPS-06 showed a resistant reaction to *A. flavus* infection. Many other researchers have explored the occurrence of aflatoxins and the presence of *A. flavus* in various red chilli cultivars. A total of 69 samples from 6 different chilli cultivars were analyzed. The study aimed to identify resistant and susceptible germplasm based on aflatoxin contamination and *A. flavus* presence. Detection methods included thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) for aflatoxins, as well as isolation and identification techniques for *A. flavus*. The findings revealed that 67% of samples were contaminated with aflatoxin B1 (AFB1). About 75% of samples were positive for *A. flavus*. The cultivar 'Nagina' was highly susceptible, with significant aflatoxin contamination. In contrast, cultivars 'Kunri' and 'Drooping Type' exhibited resistance, showing low levels of aflatoxins and fungal counts. This research highlights the importance of identifying and promoting resistant chilli cultivars to enhance chilli production (Akhund *et al*., 2017).

Though, there are high dry chilli yielders but majority are susceptible to the attack of *Aspergillus flavus* which is damaging and harmful to consumers who take them. Therefore, the selection of genotypes which are rich in chlorophyll, capsanthin and capsaicin content and showed a negative correlation with aflatoxin content will reward safe and healthy dry chilli pods with low aflatoxin in them. Those genotypes would be useful in the development of resistant varieties, which develop low levels of aflatoxin content. Resistant breeding is an important strategy to avoid the hazardous effects of aflatoxins. Hence, the two resistant genotypes, *i.e.*, IC-334383 and IC-347044, identified in the present experiment are important sources in the resistant breeding program. Molecular characterization of the identified resistant sources can be useful in recognizing the aflatoxin-resistant genes, which may be further useful in the development of varieties with broad-spectrum resistance in future. The most effective way to prevent aflatoxin contamination is using genetic resistance. Therefore, the resistant genotypes recognized, thus are the sources of genetic resistance, further useful in chilli aflatoxin resistance breeding.

5. Conclusion

The present investigation resulted in the identification of two resistant (IC-334383 and IC-347044) germplasm sources and a few moderately resistant sources to aflatoxin. The identified source of resistance would be of immense use in breeding programmes for the development of aflatoxin-resistant varieties in chilli. Further, they

can also be used for mapping resistant genes and studying the inheritance pattern of genes conferring resistance to aflatoxin. This concluded information will be a useful directive for breeders of chilli cultivars in developing aflatoxin-resistant varieties for immediate use.

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Conflict of interest

The authors declare no conflict of interest relevant to this article.

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