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Molecular Characterization and Management of *Aspergillus flavus* Link Ex Fries in Groundnut

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Abstract

Thirty one isolates collected from different parts of major groundnut growing areas of Karnataka and one from ICRISAT were identified as *Aspergillus flavus* by molecular technique using species specific primers. Later a field experiment was conducted at two locations under artificial inoculation and natural infection conditions for the pre-harvest management of *A. flavus* incidence and thereby aflatoxin. The results indicated that all the treatments are effective in reducing the *A. flavus* incidence and there by aflatoxin level in the produce. Among all the treatments, T-7 was most effective under both the situations. Under artificial inoculation conditions T-7 recorded minimum incidence of *A. flavus* (1.00 per cent) and aflatoxin content (2.40 µg/kg) with higher benefit to cost ratio (2.86) as compared to control (25.90%, 19.90 µg/kg). In natural conditions also T-7 recorded lowest *A. flavus* incidence (1.40 per cent) and aflatoxin content (0.0 µg/kg) with highest benefit to cost ratio (2.77). Among all the treatments, T-5 (foliar spray with neem oil @ 5 ml/l) was the least effective under both the conditions. The integrated approach was found best under both the situations.

Keywords

A. flavus; Aflatoxin; Characterization; Integrated management

Introduction

Groundnut (*Arachis hypogea* L.) is one of the premier oilseed crops of the world. It is cultivated in the tropical and subtropical regions (40° N to 40° S) of the world. It is the thirteenth most important food crop of the world and third most important oil seed crop used for vegetable oil production. Groundnut suffers from many major diseases *viz.,* leaf spots, rust, stem rot, root rot, collar rot, bud necrosis and many others. Of late aflatoxin contamination caused by *Aspergillus flavus* Link Ex Fries and *Aspergillus parasiticus* Speare has become a serious problem in groundnut since it affects the quality of produce [1]. Of the different types of aflatoxin analogues aflatoxin B_1 is the most toxic leading to teratogenicity and liver cancer in human beings. Due to food safety, problems posed by aflatoxins, importing countries have prescribed the standards for groundnut. Maximum of 20 ppb of aflatoxin in exporting material is permissible in international trade (WTO). Groundnut producers in both developing and developed countries with advanced agriculture have found it almost impossible to meet above regulations as *Aspergillus* spp. can infect and produce aflatoxin at various stages of the cropping period including pre-harvest, post-harvest, and storage [2]. Hence, it necessitates taking the precautionary measures before sowing until it reaches the end users. Aid of molecular technique for accurate identification and exploring the integrated approach will be a best solution to address this problem. In this context, species specific primers used for accurate identification and the different components of integrated management (chemicals, botanicals and biocontrol agents) were screened under *in vitro* against *A. flavus* and most effective ones were further tested in the field by applying each component as individually as well as integrated manner. The present paper will through the light on the molecular characterization and management of *Aspergillus flavus* producing aflatoxin in groundnut.

Materials and Methods

Molecular identification of isolates of *Aspergillus* sp.

DNA extraction: Potato Dextrose Broth (PDB) was used for mycelial growth of fungus from which DNA was extracted. One hundred fifty ml of broth was dispensed in 250 ml conical flasks and sterilized at 121.6 °C at 1.1 kg/cm^2 pressure for 15 min. Each flask containing PDB was inoculated with spore suspension (100 µl) of different isolates. The inoculated flasks were incubated for 48 hrs at 27 ± 1°C. After incubation, the mycelial mats were harvested by filtering through sterilized Whatman No.1 filter paper. The harvested mycelial mats were freeze-dried and DNA extraction was performed using CTAB (Cetyl Trimethyl Ammonium Bromide) method [3]. The DNA pellet was rehydrated in 100 µl TE buffer and allowed to re-suspend at 4°C overnight.

Polymerase chain reaction (PCR)

The Fungal DNA (rDNA) was amplified with the specific primers ASPITSF2 (5' –GCCCGCCATTCATGG-3') and ASPITSR3 (5'-CCTACAGAGCGGGTGACAAA-3') [4]. Primers for amplification were custom synthesized at Bangalore Genie Pvt Ltd, Bangalore and supplied as lyophilised products of desalted oligos. Amplification reaction mixture was prepared in 0.2 ml thin walled PCR tubes containing the 1.0 µl Template DNA (25 ng/µl), 1.0 µl of each Primer (5PM/µl), 1.0 µl dNTPs mix (2.5 mM each), 2.0 µl of 10 x assay buffer with 15 mM $MgCl_2$ and 0.5 µl *Taq* DNA polymerase (6.0U µl^{-1}). Except template the master mix was distributed to PCR tubes (19 µl/tube) and later 1 µl of template DNA from the respective isolates was added making the final volume of 20 µl. The PCR was carried out in thermo cycler as follows: Initial denaturation 94 for 5 min, 35 cycles of denaturation at 94°C for 1 min, primer annealing at 55°C for 1 min, and extension at 72°C for 2 min and a final extension at 72°C for 10 min.

Separation of amplified products by agarose gel electrophoresis

The PCR products were resolved using 1.2 per cent agarose in 1X TBE (Tris Borate EDTA) buffer, 0.5 mg ml^{-1} of Ethidium Bromide and loading buffer (0.25% Bromophenol Blue in 40% sucrose).

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Electrophoresis was carried at 70 V for 1.0 hr. The gel was observed under UV light and documented using gel documentation unit.

Pre-harvest management of *A. flavus* incidence

A field experiment was conducted during *kharif,* 2013 on the pre-harvest management of *A. flavus* incidence and there by aflatoxin in Groundnut at Main Agricultural Research Station (MARS), Dharwad, Karnataka and in the farmer's fields located at Murgod, Soundatti taluk, Belagavi district of Karnataka. The highly susceptible variety GPBD-4 was taken for the experiment (Tables 1 and 2).

Mass multiplication of pathogen and inoculation

A. flavus culture (AF-11-4 from ICRISAT) was inoculated to 1000 ml conical flask containing 500 ml Potato Dextrose Broth and incubated at 27 ± 1^0 C for 10 days. The culture was filtered through Whatman No. 40 filter paper and conidia were collected by washing the mycelial mat with water. The concentration of conidia was adjusted (1x10^6 conidia/ml) and spore suspension was applied through spraying at flowering and pod development stage.

Observations

***Aspergillus flavus* incidence:** The harvested kernels were analysed for *A. flavus* colonization by following rolled towel method and disease incidence in the field was assessed with the formula [5].

$$Percent\ A.flavus\ incidence = \frac{No\ of\ seeds\ colonized\ with\ A.flavus}{Total\ no.of\ seeds\ incubated} * 100$$

Aflatoxin quantification: The efficacy of different treatments were analysed by estimating the aflatoxin in the harvested crop by following indirect competitive ELISA [6].

Results and Discussion

Molecular characterization of *Aspergillus* sp.

All the 32 isolates of *Aspergillus* sp. were amplified by using *A. flavus* specific primers ASPITSF2 and ASPITSR3 and expected PCR product size of 397 bp was obtained in all the 32 isolates of *Aspergillus* sp. including standard isolate from ICRISAT (*AF-11-4*). Primer ASPITSF2 targeting within the ITS 1 region is specific at species level (*A. flavus*) and primer ASPITSR3 targeting within the ITS 2 region is specific at genus level. Midorikawa et al. [4] designed the *Aspergillus flavus*-specific PCR primers, ASPITSF2 and ASPITSR3 from ribosomal DNA internal transcribed spacers (ITS 1 and 2) and identified that all the strains isolated from Brazil nut and cashew were *Aspergillus flavus.* The present results are in line with the Midorikawa et al. [4] report (Plate 1).

Pre-harvest management of *A. flavus* incidence

Under artificial inoculation at MARS, Dharwad

***A. flavus* incidence:** Field experiment conducted during kharif, 2013 for pre-harvest management of *A. flavus* incidence at MARS, Dharwad indicated that incidence of *A. flavus* was lower in all the treatments compared to control. Among the different treatments, T-7 (Seed treatment with carbendazim 25% + mancozeb 50% @ 3.0 g/ kg of seeds + soil application of *Trichoderma harzianum* @ 1 kg/ 50 kg of FYM + foliar spray with carbendazim 12% + mancozeb 63% @ 0.2 % at pegging stage) recorded minimum *A. flavus* incidence (1.00 %) as compared to control (25.93 %) followed by T-6. Among the various treatments foliar spray with neem oil @ 5 ml /l (T-5) was least effective (19.44 % incidence) (Table 3). Kumar et al. [7] evaluated an integrated package at ICRISAT, Patancheru, Andhra Pradesh, India during the rainy season in 2001 to demonstrate the effectiveness of improved package vis-a-vis farmers' practice. In Integrated package the seed infection by *A. flavus* was very less (2.0 %) compared to farmers' practice (10.0 %). The results are in comparison with Kumar et al. [7], as integrated management practice was more promisable than individual approach.

Table 1: Details of Experiment.

Year: *kharif,* 2013	Location: MARS, Dharwad
Variety: GPBD-4	Design: RCBD
No. of treatments : 12	Replications: 3
Plot size: 2 X 2.7 m (9 lines)	Spacing: 30 X 10 cm
Soil type: Black clay	Observations: *Aspergillus flavus* incidence, Aflatoxin quantification

Table 2: Treatment details.

Year: *kharif,* 2013	Location: Farmer's field (Village: Murgod)
Variety: GPBD-4	Design: RCBD
No. of treatments : 12	Replications: 3
Plot size: 2 X 2.7 m (9 lines)	Spacing: 30 X 10 cm
Soil type: Black clay	Observations: *Aspergillus flavus* incidence and aflatoxin quantification

Aflatoxin contamination: Aflatoxin contamination in the kernels obtained from various treatments ranged from 2.40 to 19.90 µg/kg. Among the different treatments, T-7 (Seed treatment with carbendazim 25% + mancozeb 50% @ 3.0 g/kg of seeds + soil application of *Trichoderma harzianum* @ 1 kg/ 50 kg of FYM + foliar spray with carbendazim 12% + mancozeb 63% @ 0.2 % at pegging stage) and T-6 (seed treatment with tebuconazole @1 g/kg of seeds + soil application of *Trichoderma harzianum* @ 1 kg/ 50 kg of FYM + foliar spray with tebuconazole @0.1 % at pegging stage) recorded the least aflatoxin contamination (2.40 µg/kg) and they differed significantly from other treatments. The highest aflatoxin was recorded in control (19.90 µg/kg) (Table 3).

Yield and benefit: Cost ratio (B:C): The result revealed that, all the treatments recorded higher yields compared to control. Among different treatments, highest yield was recorded in T-7 (27.20 q/ha) with a benefit cost ratio of 2.86 followed by T-10 (25.20 q/ha) with B:C. ratio of 2.69 while lowest was recorded in T-5 (17.70 q/ha) with a B:C ratio of 1.97 (Table 3a)

Under natural conditions in farmer's field

***A. flavus* incidence:** Field experiment conducted during *kharif* 2013 for Pre- harvest management of *A. flavus* incidence in the farmer's field revealed that the *A. flavus* incidence ranged from 15.74 per cent (Control T-12) to 1.4 per cent (T-7). All the treatments were effective in reducing the *A. flavus* incidence. Among all the treatments, T-7 was found most effective with lower *A. flavus* incidence (1.40 per cent) while T-5 was least effective (Table 4).

Aflatoxin contamination: Aflatoxin contamination in the kernels obtained from various treatments ranged from 0.0 to 11.10 µg/kg. All the treatments were effective in reducing the aflatoxin content compared to control. Among the different treatments, T-7, T-8, T-9, T-10 and T-11 showed nil aflatoxin contamination The highest aflatoxin was recorded in control (Table 4).

Yield and benefit: cost ratio (B:C): The result showed that highest yield (26.30 q/ha) was obtained in T-7 (carbendazim 25% + mancozeb 50% @ 3.0 g/kg of seeds + soil application of *Trichoderma harzianum* @ 1 kg/50 kg of FYM + foliar spray with carbendazim 12%

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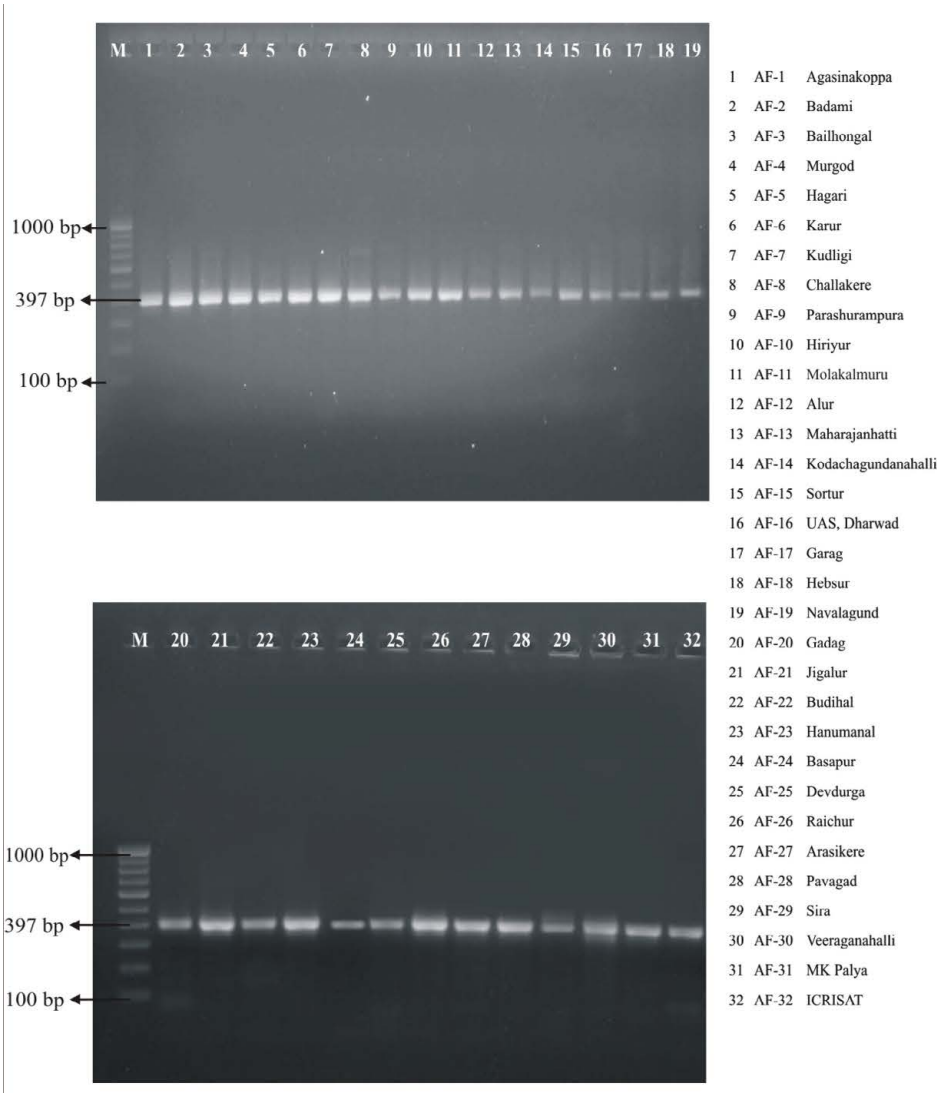

Plate 1: Identification of *A. flavus* isolates by amplification with specific primers at ITS Region

Table 3: Pre-harvest management of *A. flavus* incidence in artificial inoculation conditions at MARS, Dharwad.

Treatment	Treatment details
T_1	Seed treatment with tebuconazole @ 1 g/kg of seeds + Foliar spray with Tebuconazole @ 0.1 % at pegging stage
T_2	Seed treatment with carbendazim 25 % + mancozeb 50 % @ 3.0 g/kg of seeds + foliar spray with carbendazim 12% + mancozeb 63% @ 0.2% at pegging stage
T_3	Seed treatment with iprodione 25% + carbendazim 25% @ 2 g/kg of seeds + foliar spray with iprodione 25% + carbendazim 25% @ 0.2% at pegging stage
T_4	Seed treatment with *Trichoderma harzianum* @ 10 g/kg of seeds + soil application of *Trichoderma harzianum* @ 1 kg/ 50 kg of FYM
T_5	Foliar spray with neem oil @ 5 ml/l at pegging stage
T_6	Seed treatment with tebuconazole @ 1 g/kg of seeds + soil application of *Trichoderma harzianum* @ 1 kg/ 50 kg of FYM + foliar spray with tebuconazole @0.1 % at pegging stage
T_7	Seed treatment with carbendazim 25% + mancozeb 50% @ 3.0 g/kg of seeds + soil application of *Trichoderma harzianum* @ 1 kg/ 50 kg of FYM + foliar spray with carbendazim 12% + mancozeb 63% @ 0.2 % at pegging stage
T_8	Seed treatment with iprodione 25% + carbendazim 25% @ 2 g/kg of seeds + soil application of *Trichoderma harzianum* @ 1 kg/ 50 kg of FYM + foliar spray with iprodione 25% + carbendazim 25% @ 0.2 % at pegging stage
T_9	Seed treatment with tebuconazole @ 1 g/kg of seeds + soil application of *Trichoderma harzianum* @ 1 kg/ 50 kg of FYM + Foliar spray with neem oil @ 5ml/l at pegging stage
T_{10}	Seed treatment with carbendazim 25% + mancozeb 50% @ 3.0 g/kg of seeds + + soil application of *Trichoderma harzianum* @ 1 kg/ 50 kg of FYM + Foliar spray with neem oil @ 5 ml/l at pegging stage
T_{11}	Seed treatment with iprodione 25% + carbendazim 25% @ 2 g/kg of seeds + soil application of *Trichoderma harzianum* @ 1 kg/ 50 kg of FYM + Foliar spray with neem oil @ 5 ml/l at pegging stage
T_{12}	Untreated control

Table 3a: Economics of pre-harvest management of *A. flavus* incidence in groundnut at MARS Dharwad

Treatments	Treatment details	*A. flavus* incidence (%)	Reduction of *A. flavus* incidence over control (%)	Aflatoxin (µg/kg)	Yield (q/ha)
T_1	Seed treatment with tebuconazole @1g/kg of seeds + Foliar spray with tebuconazole @0.1% at pegging stage	12.50 (*3.60)	51.64	8.10	22.00
T_2	Seed treatment with carbendazim 25% + mancozeb 50% @ 3.0 g/kg of seeds + foliar spray with carbendazim 12% + mancozeb 63% @ 0.2% at pegging stage	9.26 (3.10)	64.10	6.10	24.00
T_3	Seed treatment with iprodione 25% + carbendazim 25% @ 2g/kg of seeds + foliar spray with iprodione 25% + carbendazim 25% @ 0.2% at pegging stage	11.11 (3.40)	56.98	7.40	23.50
T_4	Seed treatment with *Trichoderma harzianum* @10g/kg of seeds + soil application of *Trichoderma harzianum* @ 1kg/ 50kg of FYM	14.81 (3.93)	42.74	9.90	19.50
T_5	Foliar spray with neemoil @ 5ml/l at pegging stage	19.44 (4.46)	24.93	14.10	17.70
T_6	Seed treatment with tebuconazole @1g/kg of seeds + soil application of *Trichoderma harzianum* @ 1kg/ 50kg of FYM + foliar spray with tebuconazole @0.1% at pegging stage	1.85 (1.47)	92.59	2.40	25.10
T_7	Seed treatment with carbendazim 25% + mancozeb 50% @ 3.0 g/kg of seeds + soil application of *Trichoderma harzianum* @ 1kg/ 50kg of FYM + foliar spray with carbendazim 12% + mancozeb 63% @ 0.2% at pegging stage	1.00 (1.13)	95.80	2.40	27.20
T_8	Seed treatment with iprodione 25% + carbendazim 25% @ 2g/kg of seeds + soil application of *Trichoderma harzianum* @ 1kg/ 50kg of FYM + foliar spray with iprodione 25% + carbendazim 25% @ 0.2% at pegging stage	3.24 (1.80)	87.25	3.50	24.10
T_9	Seed treatment with tebuconazole @1g/kg of seeds + soil application of *Trichoderma harzianum* @ 1kg/ 50kg of FYM + Foliar spray with neem oil @ 5ml/l at pegging stage	5.56 (2.47)	78.35	4.10	25.00
T_{10}	Seed treatment with carbendazim 25% + mancozeb 50% @ 3.0 g/kg of seeds + + soil application of *Trichoderma harzianum* @ 1kg/ 50kg of FYM + Foliar spray with neem oil @ 5ml/l at pegging stage	4.17 (2.13)	83.69	3.70	25.20
T_{11}	Seed treatment with iprodione 25% + carbendazim 25% @ 2g/kg of seeds + soil application of *Trichoderma harzianum* @ 1kg/ 50kg of FYM + Foliar spray with neem oil @ 5ml/l at pegging stage	7.41 (2.77)	71.23	4.20	24.20
T_{12}	Untreated control	25.93 (5.12)	-	19.90	16.10
	S. Em. ±	0.10		0.05	0.51
	CD (P=0.05)	0.29		0.17	1.52

*square root transformed

Table 4: Pre-harvest management of *A. flavus* incidence in natural conditions at Farmer's field, Murgod.

Treatments	Treatment details	Yield (q/ha)	Total cost of cultivation (Rs/ha)	Gross returns (Rs/ha)	Net returns (Rs/ha)	B:C ratio
T_1	Seed treatment with tebuconazole @1g/kg of seeds + Foliar spray with tebuconazole @0.1% at pegging stage	22.00	35793.00	85800.0	50007.00	2.40
T_2	Seed treatment with carbendazim 25% + mancozeb 50% @ 3.0 g/kg of seeds + foliar spray with carbendazim 12% + mancozeb 63% @ 0.2% at pegging stage	24.00	36449.00	93600.0	57151.00	2.57
T_3	Seed treatment with iprodione 25% + carbendazim 25% @ 2g/kg of seeds + foliar spray with iprodione 25% + carbendazim 25% @ 0.2% at pegging stage	23.50	36626.00	89700.0	53074.00	2.45
T_4	Seed treatment with *Trichoderma harzianum* @10g/kg of seeds + soil application of *Trichoderma harzianum* @ 1kg/ 50kg of FYM	19.50	34630.00	76050.0	41420.00	2.20
T_5	Foliar spray with neemoil @ 5ml/l at pegging stage	17.70	34984.00	69030.0	34046.00	1.97
T_6	Seed treatment with tebuconazole @1g/kg of seeds + soil application of *Trichoderma harzianum* @ 1kg/ 50kg of FYM + foliar spray with tebuconazole @0.1% at pegging stage	25.10	36423.00	97890.0	61467.00	2.69
T_7	Seed treatment with carbendazim 25% + mancozeb 50% @ 3.0 g/kg of seeds + soil application of *Trichoderma harzianum* @ 1kg/ 50kg of FYM + foliar spray with carbendazim 12% + mancozeb 63% @ 0.2% at pegging stage	27.20	37079.00	106080.0	69001.00	2.86
T_8	Seed treatment with iprodione 25% + carbendazim 25% @ 2g/kg of seeds + soil application of *Trichoderma harzianum* @ 1kg/ 50kg of FYM + foliar spray with iprodione 25% + carbendazim 25% @ 0.2% at pegging stage	24.10	37256.00	93990.0	56734.00	2.52
T_9	Seed treatment with tebuconazole @1g/kg of seeds + soil application of *Trichoderma harzianum* @ 1kg/ 50kg of FYM + Foliar spray with neem oil @ 5ml/l at pegging stage	25.00	37407.00	97500.0	60093.00	2.61
T_{10}	Seed treatment with carbendazim 25% + mancozeb 50% @ 3.0 g/kg of seeds + + soil application of *Trichoderma harzianum* @ 1kg/ 50kg of FYM + Foliar spray with neem oil @ 5ml/l at pegging stage	25.20	38063.00	98280.0	60217.00	2.58
T_{11}	Seed treatment with iprodione 25% + carbendazim 25% @ 2g/kg of seeds + soil application of *Trichoderma harzianum* @ 1kg/ 50kg of FYM + Foliar spray with neem oil @ 5ml/l at pegging stage	24.20	38240.00	94380.0	56140.00	2.47
T_{12}	Untreated control	16.10	34000.00	62400.0	28400.00	
	S. Em.±	0.51				
	CD (p = 0.05)	1.52				

Table 4a: Economics of pre-harvest management of *A. flavus* incidence in groundnut at Farmer's field, Murgod.

Treatments	Treatment details	Yield (q/ha)	Total cost of cultivation (Rs/ha)	Gross returns (Rs/ha)	Net returns (Rs/ha)	B:C ratio
T_1	Seed treatment with tebuconazole @1g/kg of seeds + Foliar spray with tebuconazole @0.1% at pegging stage	21.30	35793.00	83200.0	47407.00	2.32
T_2	Seed treatment with carbendazim 25% + mancozeb 50% @ 3.0 g/kg of seeds + foliar spray with carbendazim 12% + mancozeb 63% @ 0.2% at pegging stage	23.70	36449.00	92300.0	55851.00	2.53
T_3	Seed treatment with iprodione 25% + carbendazim 25% @ 2g/kg of seeds + foliar spray with iprodione 25% + carbendazim 25% @ 0.2% at pegging stage	22.30	36626.00	87100.0	50474.00	2.38
T_4	Seed treatment with *Trichoderma harzianum* @10g/kg of seeds + soil application of *Trichoderma harzianum* @ 1kg/ 50kg of FYM	18.30	34630.00	71500.0	36870.00	2.06
T_5	Foliar spray with neemoil @ 5ml/l at pegging stage	17.10	34984.00	66690.0	31706.00	1.91
T_6	Seed treatment with tebuconazole @1g/kg of seeds + soil application of *Trichoderma harzianum* @ 1kg/ 50kg of FYM + foliar spray with tebuconazole @0.1% at pegging stage	24.50	36423.00	95550.0	59637.00	2.66
T_7	Seed treatment with carbendazim 25% + mancozeb 50% @ 3.0 g/kg of seeds + soil application of *Trichoderma harzianum* @ 1kg/ 50kg of FYM + foliar spray with carbendazim 12% + mancozeb 63% @ 0.2% at pegging stage	26.30	37079.00	102570.0	65491.00	2.77
T_8	Seed treatment with iprodione 25% + carbendazim 25% @ 2g/kg of seeds + soil application of *Trichoderma harzianum* @ 1kg/ 50kg of FYM + foliar spray with iprodione 25% + carbendazim 25% @ 0.2% at pegging stage	24.20	37256.00	94380.0	57124.00	2.53
T_9	Seed treatment with tebuconazole @1g/kg of seeds + soil application of *Trichoderma harzianum* @ 1kg/ 50kg of FYM + Foliar spray with neem oil @ 5ml/l at pegging stage	23.00	37407.00	89700.0	52293.00	2.40
T_{10}	Seed treatment with carbendazim 25% + mancozeb 50% @ 3.0 g/kg of seeds + + soil application of *Trichoderma harzianum* @ 1kg/ 50kg of FYM + Foliar spray with neem oil @ 5ml/l at pegging stage	24.10	38063.00	93990.0	55927.00	2.47
T_{11}	Seed treatment with iprodione 25% + carbendazim 25% @ 2g/kg of seeds + soil application of *Trichoderma harzianum* @ 1kg/ 50kg of FYM + Foliar spray with neem oil @ 5ml/l at pegging stage	23.00	38240.00	89700.0	51460.00	2.35
T_{12}	Untreated control	15.80	34000.00	61620.0	27620.00	
	S. Em.±	0.61				
	CD (p = 0.05)	1.82				

+ mancozeb 63% @ 0.2 % at pegging stage) with a benefit: cost ratio of 2.77. The lowest yield (15.80 q/ha) was recorded in T-12 (control) (Table 4a).

In both the natural and artificial inoculation conditions the *A.flavus* colonization was lower in treatments compared to control. Among the treatments, the integrated ones showed lower *A.flavus* incidence compared to individual component application. The treatments in the natural conditions showed lower aflatoxin content compared to artificial inoculation condition. This may be due to lack of drought situation at the fag end of the crop and may also due to lack of sufficient inoculum in the soil which are prerequisite for infection and aflatoxin production. Bruce [8] reported that there is a direct relationship between soil density of *Aspergillus flavus* and the incidence of groundnut colonization. Arunyanark et al. [9] reported that drought in combination with higher levels of *A. flavus* inoculum load in the soil resulted in increased kernel colonization (6 to 68%) and subsequent aflatoxin contamination (4 to183 µg/kg). From the present study it was concluded that use of molecular technique is a quick, time saving and aid in accurate identification of *A.flavus* and integrated approach is best in addressing the pre-harvest management of *A.flavus* incidence and aflatoxin content in groundnut.

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