International Journal of Ecology and Environmental Sciences
www.ecologyjournal.in
ISSN: 2664-7133
Received: 20-09-2026, Accepted: 20-10-2026, Published: 04-11-2026
Volume 8, Issue 4, 2026, Page No. 221-227

Cross infection potential of *Aspergillus flavus* isolates across different host plants

Prajwal Piraddi[1*], Raghavendra B T[1], Ajithkumar K[2], Mallikarjun Kenganal[3], Rajanna B[4]
[1] Department of Plant Pathology, College of Agriculture, University of Agricultural Sciences, Raichur, Karnataka, India
[2] ICAR-AICRP on Linseed, MARS, Raichur, University of Agricultural Sciences, Raichur, Karnataka, India
[3] ICAR-AICRP on Kharif Pulses, ZARS, Kalaburagi, University of Agricultural Sciences, Raichur, Karnataka, India
[4] Department of Genetics and Plant Breeding, College of Agriculture, Raichur, University of Agricultural Sciences, Raichur, Karnataka, India
Corresponding Author: Prajwal Piraddi
DOI: https://doi.org/10.66856/ijees.2026.8.4.8130

Abstract
The cross-infection potential of *Aspergillus flavus* isolates obtained from groundnut, chilli, maize and pistachio was evaluated on these four host materials. The experiment was conducted during 2024-25 and 2025-26 using four isolates and four host materials in a completely randomised design with five replications. The time required for the first visible mycelial growth and sporulation along with host colonisation was recorded. First mycelial growth was observed within 1-4 days across replications, while first sporulation occurred within 2-6 days. Mean host colonisation ranged from 65.00 to 100.00 per cent across isolate host combinations. The groundnut, chilli and maize isolates recorded 100 per cent colonisation on groundnut, chilli and maize, while the pistachio isolate recorded 100 per cent colonisation on pistachio. The effects of isolate source, host material and their interaction on colonisation were significant at the one per cent probability level. The results demonstrated the ability of *A. flavus* isolates to colonise multiple host materials, although the extent and timing of colonisation varied among isolate host combinations.

Keywords: *Aspergillus flavus*, cross infection, host colonisation, groundnut, pistachio

Introduction
Aspergillus flavus is a widely distributed fungus associated with several agricultural commodities including groundnut, chilli, maize and pistachio. Its occurrence in these commodities is of concern because certain strains produce aflatoxins that affect the safety and quality of agricultural produce (Klich, 2007) [2].
The fungus can colonise diverse host materials and is not necessarily restricted to a single crop. A chilli associated isolate of *A. flavus* has the ability to colonise several agricultural and horticultural crops including groundnut, maize, sesame, onion and fig (Sudha *et al*., 2009) [6]. This broad host range highlights the importance of cross infection potential in understanding the spread and colonisation of *A. flavus* across different agricultural commodities.
Host materials differ in their suitability for fungal colonisation, sporulation and aflatoxin production. Chilli associated populations of *A. flavus* can colonise maize kernels, while aflatoxin production varies among fungal haplotypes (Singh *et al*., 2022) [5]. Groundnut supports greater conidial production than maize, whereas maize supports higher aflatoxin production, indicating differences in fungal sporulation and toxin production between these host substrates (Chinganda *et al*., 2023) [1].
In the present study, the cross-infection potential of *A. flavus* isolates obtained from groundnut, chilli, maize and pistachio was evaluated on these four host materials. The time required for the appearance of initial mycelial growth and sporulation along with the percentage of host colonisation was assessed to compare fungal development across different isolate host combinations.

Material and methods
Collection of isolates and host materials
The experiment was conducted during 2024-25 and 2025-26 at the Department of Plant Pathology, College of Agriculture, University of Agricultural Sciences, Raichur. Three isolates of *Aspergillus flavus* designated AFG13 (groundnut), AfC22 (chilli) and AfV16 (maize) were obtained from the Department of Plant Pathology, UAS, Raichur. A fourth isolate, designated AfP was obtained from infected pistachio nuts collected from a local market and purified on Potato Dextrose Agar (PDA).

Molecular identification and detection of aflatoxin-biosynthesis genes
The pistachio isolate of *Aspergillus flavus* was subjected to molecular identification by amplification and sequencing of the internal transcribed spacer (ITS) region of ribosomal DNA using the universal fungal primers ITS-1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS-4 (5′-TCCTCCGCTTATTGATATGC-3′). The amplified product, approximately 500-600 bp in size, was purified and submitted to Medauxin Labs Pvt. Ltd., Bengaluru, for Sanger sequencing. The forward end sequence was edited and analysed using the Basic Local Alignment Search Tool (BLAST) against sequences available in the National Center for Biotechnology Information (NCBI) database. The sequence was submitted to GenBank under accession number QB102452.
PCR amplification targeting the aflatoxin-biosynthesis genes *aflE* and *aflM* was also performed using gene-specific primers. The primer sequences used are presented below.

Primers used for amplification of aflatoxin-biosynthesis genes

Target gene	Primer	Sequence (5′–3′)
aflE	*aflE*-1	CCTTATGCCTGGGAACGAT
aflE	*aflE*-2	TTCGCATCACTTCCTCACA
aflM	*aflM*-1	ACCACCGTTTAGATGGCAAA
aflM	*aflM*-2	AGAGCTGGTCAGGATAATCCG

The PCR products were assessed to determine the amplification of the targeted genes. The ITS sequence was used for molecular identification, whereas *aflE* and *aflM* were targeted to investigate the presence of selected genes associated with aflatoxin biosynthesis.

Preparation of inoculum and cross inoculation

Healthy, untreated and surface sterilised groundnut kernels, maize cobs, chilli fruits and pistachio nuts were used as host materials. The host materials were surface sterilised using mercuric chloride (1:1000) rinsed three times with sterile distilled water and dried aseptically. Spore suspensions of the four isolates were prepared in sterile saline containing 0.05 per cent Tween 20 and adjusted to 1×10^6 ml^{-1} spores using a haemocytometer. The experiment comprised four isolate sources, four host materials and five replications with each isolate host combination maintained in a separate tray. Each tray contained 10 units each of groundnut kernels, chilli fruits and pistachio nuts and four maize cobs. Individual host units within a tray were treated as subsamples, while the tray constituted the experimental unit. Each host material was inoculated separately with each isolate. The inoculated materials were placed in trays lined with moist blotter paper and humidity was maintained using sterile distilled water moistened cotton swabs. The trays were incubated at room temperature for seven days and observations were recorded at 24 hours interval.

Assessment of mycelial growth, sporulation and host colonisation

The incubation period to observe first visible mycelial growth and sporulation were recorded for each isolate host combination. Host colonisation was assessed by recording the number of inoculated host units showing visible colonisation and expressed as a percentage of the total number of units inoculated.

Statistical analysis

The experiment was conducted using a Completely Randomised Design (CRD). The effects of isolate source, host material and their interaction were assessed. Colonisation percentages were subjected to arcsine square root transformation before statistical analysis. Treatment differences were evaluated at the one per cent probability level.

Results and Discussion

Molecular identification of the pistachio isolate

The pistachio isolate was identified as *Aspergillus flavus* based on ITS sequence analysis. BLAST analysis against the NCBI database showed 99.81 per cent sequence identity, 100 per cent query coverage and an E-value of 0.0. The sequence was submitted to GenBank under accession number QB102452 (Table 1). Phylogenetic analysis placed the isolate in association with the reference *A. flavus* sequence MG659657.1, with 97 per cent bootstrap support (Fig. 1). These findings supported the molecular identification of the pistachio isolate as *A. flavus*.

PCR amplification of the aflatoxin biosynthesis genes *aflE* and *aflM* was successfully obtained in the pistachio isolate of *A. flavus* (Plate 1). The amplification of these genes indicated their presence in the isolate, suggesting its genetic potential for aflatoxin biosynthesis.

Effect of host and isolate source on the incubation period to observe first mycelial growth and first sporulation

The time required for the appearance of first mycelial growth and first sporulation of *A. flavus* varied among the different isolate host combinations (Tables 2 and 3). First visible mycelial growth occurred within 1-4 days after inoculation, with the earliest growth, at 1 day, recorded when the groundnut, chilli, maize and pistachio isolates were inoculated onto their respective source hosts. Across host materials, the mean time to first mycelial growth ranged from 1.75 days on groundnut to 2.25 days on pistachio. First visible sporulation occurred within 2-6 days after inoculation. The mean time to first sporulation was lowest on groundnut and chilli at 3.00 days each and highest on pistachio, at 3.75 days. At the individual treatment level, the pistachio isolate sporulated within 2 days on pistachio, whereas the chilli isolate required 5 days to sporulate on pistachio.

The main effects of isolate source and host material on the time to first mycelial growth was non significant, whereas their interaction was significant at the 1 per cent probability level. For the time to first sporulation, the main effect of isolate source was non-significant, while the effects of host material and the isolate source × host interaction were significant at the 1 per cent probability level. These results indicate that the timing of initial fungal establishment and sporulation varied according to the particular isolate host combination.

Leger *et al.* (2000) [3] found that *A. flavus* isolates from different sources caused symptoms in injured bean leaves and corn kernels, with no evidence of strict host specialisation. Similarly, in the present study, isolates from groundnut, chilli, maize and pistachio initiated mycelial growth on their respective source hosts within 1 day and were also capable of establishing growth on alternative hosts, with first visible growth occurring within 1-4 days. These findings indicate that the tested isolates were not restricted to their original hosts, although the time required for initial growth varied among isolate host combinations.

Mehl and Cotty (2013) [4] found that maize, sorghum, cotton and soybean supported different levels of *A. flavus* sporulation and that host species influenced competitive success during host invasion and sporulation. Similarly, in the present study, the mean time to first sporulation ranged from 3.00 days on groundnut and chilli to 3.75 days on pistachio, while the significant isolate source × host interaction indicated that sporulation timing depended on the specific isolate host combination.

Chinganda *et al.* (2023) [1] found greater conidial production by *Aspergillus* section *Flavi* on groundnut than on maize, whereas aflatoxin production was greater on maize. In the present study, first sporulation occurred within 2-6 days, with the mean time to first sporulation being lower on groundnut (3.00 days) than on pistachio (3.75 days). Although the present results similarly show variation in

fungal development among host materials, conidial production and aflatoxin accumulation are different response variables from the time required for first visible sporulation and therefore cannot be directly compared.

The variation in the time to first mycelial growth and sporulation may be associated with differences in the physical structure, nutrient composition and moisture status of the host materials. Following inoculation, fungal establishment involves conidial germination, germ tube emergence and hyphal extension, while sporulation requires the subsequent development of reproductive structures. Differences in the availability of nutrients and moisture, as well as the suitability of host tissues for fungal establishment, may therefore affect the time required for these developmental stages. The significant isolate source × host interaction indicates that these responses depended on the compatibility of each isolate with the host material rather than on isolate source alone.

Overall, isolates of *A. flavus* obtained from groundnut, chilli, maize and pistachio were capable of initiating mycelial growth and producing spores on the tested host materials. First mycelial growth occurred within 1-4 days and first sporulation within 2-6 days. The significant isolate source × host interaction for both parameters indicates that the timing of fungal establishment and sporulation varied among isolate host combinations, demonstrating cross infection potential with differential developmental responses across hosts.

Effect of host and isolate source on the incubation period for colonisation of different hosts

The percentage colonisation of host materials by *A. flavus* ranged from 65.00 to 100.00 per cent across the different isolate host combinations (Table 4, Fig. 2 and Plates 2, 3, 4 and 5). The groundnut, chilli and maize isolates each recorded 100.00 per cent colonisation on their respective source hosts. On pistachio, colonisation was 90.00, 92.00 and 84.00 per cent for the groundnut, chilli and maize isolates, respectively. The pistachio isolate recorded 100.00 per cent colonisation on pistachio, compared with 76.00, 80.00 and 65.00 per cent on groundnut, chilli and maize, respectively.

The mean colonisation was highest for the chilli isolate (98.00 per cent), followed by the groundnut isolate (97.50 per cent) and the maize isolate (96.00 per cent), whereas the pistachio isolate recorded the lowest mean (80.25 per cent). The effects of isolate source, host material and their interaction were significant at the 1 per cent probability level, indicating that colonisation varied according to the specific isolate host combination.

Leger *et al.* (2000) [3] found that *A. flavus* isolates from different sources caused symptoms in injured bean leaves and corn kernels with no evidence of strict host specialisation. Similarly, all isolates in the present study colonised hosts beyond their original sources, although the extent of colonisation varied among combinations.

Mehl and Cotty (2013) [4] found that host species, including maize, sorghum, cotton and soybean, influenced the success of *A. flavus* during host tissue invasion and sporulation. This is consistent with the present findings, in which mean colonisation ranged from 80.25 per cent for the pistachio isolate to 98.00 per cent for the chilli isolate, and the significant isolate source × host interaction indicated that colonisation depended on the host isolate combination.

Chinganda *et al.* (2023) [1] found host dependent differences in the outcomes of competition between *Aspergillus* section *Flavi* species on maize and groundnut, including differences in conidial production and aflatoxin accumulation. Although these responses differ from colonisation percentage, the findings support the influence of host material on fungal performance.

The comparatively lower mean colonisation of the pistachio isolate was associated with reduced colonisation of alternative hosts, particularly maize (65.00 per cent), compared with its source host, pistachio (100.00 per cent). Differences in host tissue structure, nutrient availability and the suitability of the host environment may contribute to variation in colonisation, however, these factors were not measured directly in the present study.

Overall, the tested *A. flavus* isolates exhibited cross infection potential across groundnut, chilli, maize and pistachio with colonisation ranging from 65.00 to 100.00 per cent. The incubation periods for initial growth and sporulation, as well as the percentage colonisation, varied with the isolate source and host material. These results indicate that cross infection was possible across the tested hosts, but the speed of fungal development and the extent of colonisation depended on the specific isolate host combination.

Table 1: Homology (BLAST) results of the ITS sequenced sample with the reference strain deposited in NCBI GenBank, Mary Land, USA

Sl. No.	Isolate name	Scientific name	Accession no.	Identity (%)
1	AfP	*Aspergillus flavus*	QB102452	99.81

Table 2: Effect of isolate source and host on the incubation period to observe first mycelial growth of *Aspergillus flavus*

Days to first mycelial growth*						
Isolate source	Host source				Isolate mean	
	Groundnut	Chilli	Maize	Pistachio		
Groundnut	1.00	2.00	2.00	2.00	1.75	
Chilli	2.00	1.00	2.00	3.00	2.00	
Maize	2.00	2.00	1.00	3.00	2.00	
Pistachio	2.00	3.00	3.00	1.00	2.25	
Host mean	1.75	2.00	2.00	2.25	2.00	
Statistical parameters						
S.Em. ±	Isolate source	0.137	Host	0.137	Isolate source × host	0.274
C.D. at 1 %		0.51		0.51		1.03

*Mean of five replications.

Table 3: Effect of isolate source and host on the incubation period to observe first sporulation of *Aspergillus flavus*

Days to first sporulation*						
Isolate source	**Host source**				**Isolate mean**	
	Groundnut	**Chilli**	**Maize**	**Pistachio**		
Groundnut	3.00	3.00	3.00	4.00	3.25	
Chilli	3.00	3.00	3.00	5.00	3.50	
Maize	3.00	3.00	3.00	4.00	3.25	
Pistachio	3.00	3.00	4.00	2.00	3.00	
Host mean	3.00	3.00	3.25	3.75	3.25	
Statistical parameters						
S.Em. ±	Isolate source	0.153	Host	0.153	Isolate source × Host	0.306
C.D. at 1 %		0.57		0.57		1.15

* Mean of five replications.

Table 4: Effect of isolate source and host on colonisation of different hosts by *Aspergillus flavus*

Colonisation of different hosts (%) *						
Isolate source	**Host source**				**Isolate mean**	
	Groundnut	**Chilli**	**Maize**	**Pistachio**		
Groundnut	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	90.00 (71.57)	97.50 (85.39)	
Chilli	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	92.00 (73.57)	98.00 (85.89)	
Maize	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	84.00 (66.42)	96.00 (84.11)	
Pistachio	76.00 (60.67)	80.00 (63.43)	65.00 (53.73)	100.00 (90.00)	80.25 (66.96)	
Host mean	94.00 (82.67)	95.00 (83.36)	91.50 (80.93)	91.50 (75.39)	92.94 (80.59)	
Statistical parameters						
S.Em. ±	Isolate source	0.73	Host	0.73	Isolate source × Host	1.45
C.D. at 1 %		2.72		2.72		5.45

* Mean of five replications.

Values within parentheses represent arcsine square-root transformed values in degrees used for statistical analysis.

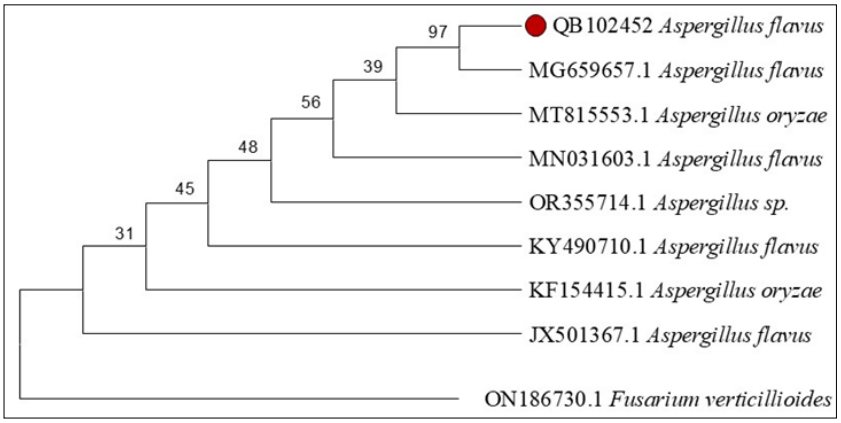


Fig. 1: Phylogenetic analysis of *Aspergillus flavus* isolate of pistachio for ITS-1 and ITS-4 using neighbourhood joining method

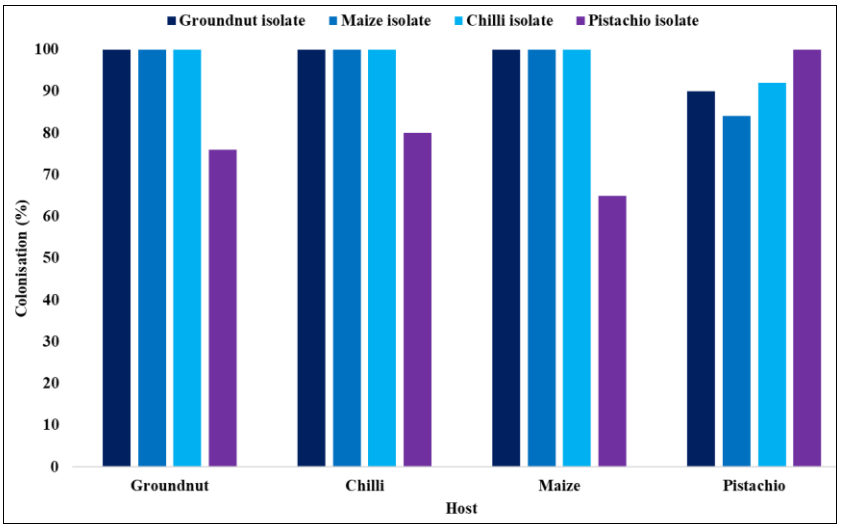
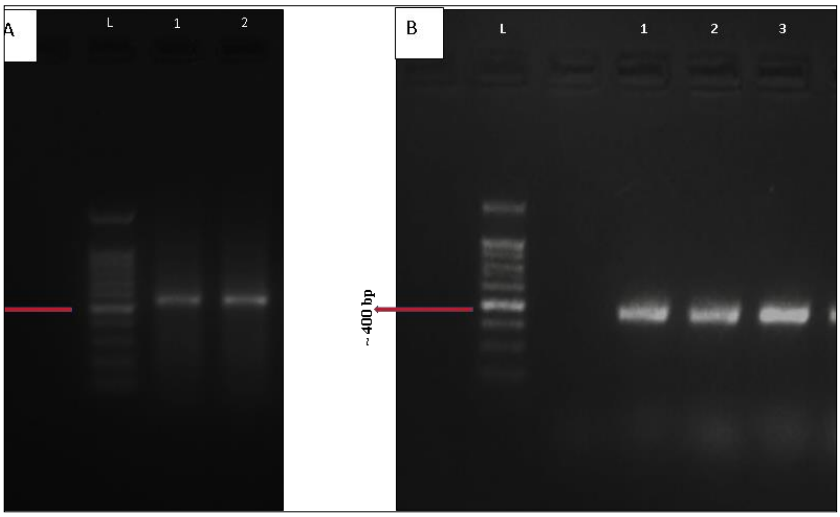


Fig. 2: Variation in host colonisation by *Aspergillus flavus* isolates from different sources



(A) Amplification using ITS1 and ITS4 primers; (B) Amplification using *aflE*-1, *aflE*-2, *aflM*-1 and *aflM*-2 primers. L: Ladder (100 bp)

Plate 1: PCR amplification of ITS and aflatoxin biosynthesis gene regions in *Aspergillus flavus*

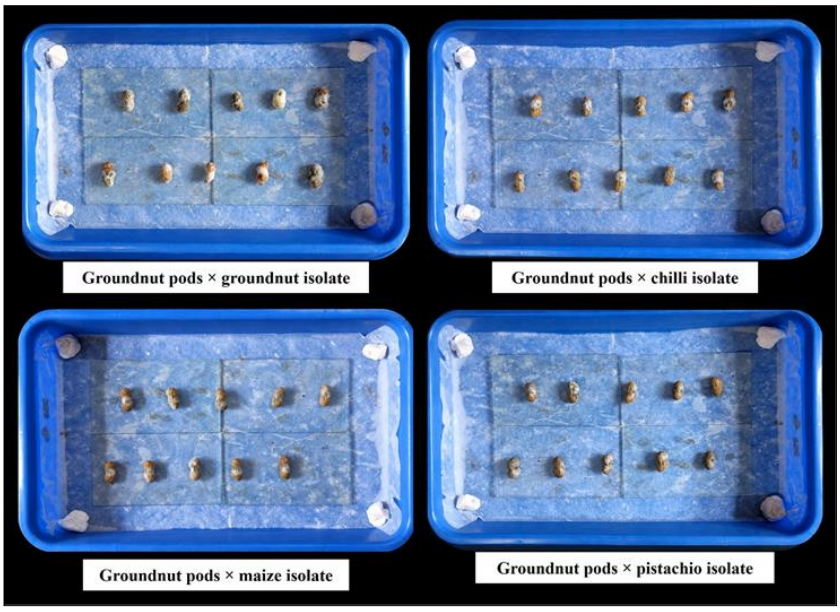


Plate 2: Colonisation of groundnut pods by *Aspergillus flavus* isolates from different host sources



Plate 3. Colonisation of chilli fruits by *Aspergillus flavus* isolates from different host sources

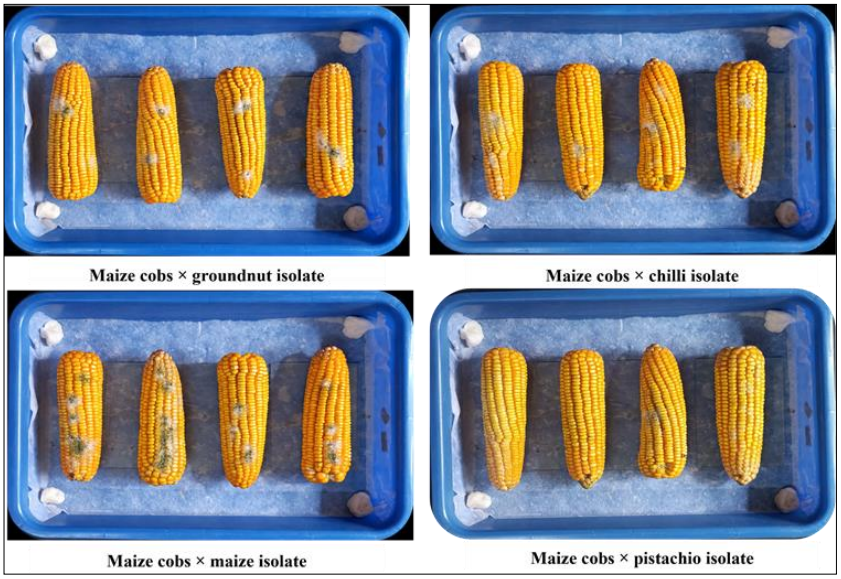


Plate 4: Colonisation of maize cobs by *Aspergillus flavus* isolates from different host sources

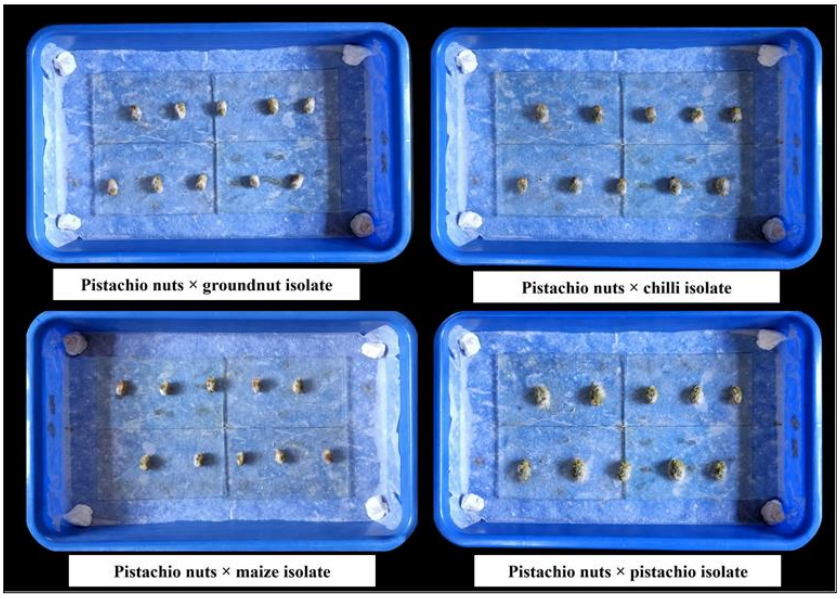


Plate 5: Colonisation of pistachio nuts by Aspergillus flavus isolates from different host source

Conclusion

The study established the cross-infection potential of *Aspergillus flavus* isolates obtained from groundnut, chilli, maize and pistachio across the four host materials. All isolates colonised hosts beyond their original sources, with colonisation ranging from 65.00 to 100.00 per cent. The groundnut, chilli and maize isolates exhibited high mean colonisation of 97.50, 98.00 and 96.00 per cent, respectively, whereas the pistachio isolate recorded a comparatively lower mean of 80.25 per cent, despite complete colonisation of its source host. The significant effects of isolate source, host material and their interaction indicated that the extent of colonisation depended on the specific isolate host combination. These findings highlight the broad host compatibility of *A. flavus* and the variation in its colonisation potential across different host materials.

Acknowledgement

The authors are grateful to the Department of Plant Pathology, College of Agriculture, University of Agricultural Sciences, Raichur, for providing the facilities and support required to conduct this study.

References

1. Chinganda C, Bandyopadhyay R, Callicott KA, Orbach MJ, Cotty PJ, Mehl HL. Crop host influences on outcomes of competition between *Aspergillus* section Flavi species coinfecting maize and groundnuts. PhytoFrontiers,2023:3(2):369-381.
2. Klich MA. *Aspergillus flavus*: the major producer of aflatoxin. Mol. Plant Pathol.,2007:8(6):713-722.
3. Leger SRJ, Screen SE, Shams-Pirzadeh B. Lack of host specialization in *Aspergillus flavus*. Appl. Environ. Microbiol.,2000:66(1):320-324.
4. Mehl HL, Cotty PJ. Influence of plant host species on intraspecific competition during infection by *Aspergillus flavus*. Plant Pathol.,2013:62(6):1310-1318.
5. Singh P, Mehl HL, Orbach MJ, Callicott KA, Cotty PJ. Genetic diversity of *Aspergillus flavus* associated with chili in Nigeria and identification of haplotypes with

potential in aflatoxin mitigation. Plant Dis.,2022:106(7):1818-1825.
6. Sudha S, Naik MK, Hosmani RM. Screening of chilli genotypes and host range studies against aflatoxin contamination caused by *Aspergillus flavus* (Link.) Fers. Veg. Sci.,2009:36(2):155-158.