LR-5578
[1-7]

RESEARCH ARTICLE
Legume Research- An International Journal

Jasmonic and Salicylic Acid-mediated Defence Enhancement in Maize under Soybean Intercropping for Aflatoxin Suppression

M. Siyon Kumari[1], Rajeev[1], Rajeev Kumar Gupta[1], Jeevana Sai[1]
Jitendra Singh[2], Rohit Kumar[3], Atin Kumar[4]

10.18805/LR-5578

ABSTRACT

Background: Aflatoxin contamination in maize, mainly caused by *Aspergillus flavus*, poses a serious threat to food safety and crop value in tropical regions. Conventional control measures are increasingly inadequate under changing climatic conditions This study evaluated the integrated effects of soybean intercropping and foliar application of jasmonic acid (JA) and salicylic acid (SA) on biochemical, molecular and ecological defence responses in maize.

Methods: A two-year field experiment (2022-2023 and 2023-2024) was conducted at Punjab, India, in a randomized complete block design (RCBD) with four representative treatment combinations: T_1 - Sole maize (control), T_2 - Sole maize + JA (100 µM), T_3 - Maize + soybean intercropping (2:2 row) and T_4 - Maize + soybean + JA + SA (1 mM). Treatments were assessed for aflatoxin B_1 and B_2 content, endogenous hormone levels (JA and SA), antioxidant enzymes (POD, SOD, CAT), PR protein (β-1,3-glucanase), gene expression (PR1, PR5), rhizospheric microbial diversity, soil moisture and temperature.

Results: Significant differences were observed among treatments. Aflatoxin content was highest in T_1 (38.6 µg kg^{-1}) and lowest in T_4 (16.9 µg kg^{-1}), which was below the FSSAI threshold of 20 µg kg^{-1}. T_4 also recorded the maximum JA (320.6 ng g^{-1}) and SA (310.9 ng g^{-1}) levels, the highest antioxidant enzyme activities (POD: 2.85, SOD: 3.02, CAT: 2.77 U mg^{-1} protein) and the strongest β-1,3-glucanase activity (3.54 µg glucose eq. mg^{-1} protein). Defence-related genes PR1 and PR5 were upregulated 3.6- and 3.3-fold, respectively, in T_4 compared with the baseline in T. Rhizospheric microbial diversity increased from 6.1 log CFU g^{-1} (T_1) to 8.2 (T_4), while soil moisture improved from 14.8% to 20.4% and soil temperature decreased from 32.4°C to 29.8°C. These findings demonstrate the potential of combining legume intercropping with phytohormonal priming as a biologically intensive and climate-resilient strategy for aflatoxin mitigation in maize.

Key words: Aflatoxin B_1 and B_2, Jasmonic acid, Maize-soybean intercropping, PR gene expression, Rhizosphere microbiome, Salicylic acid.

INTRODUCTION

Aflatoxin contamination of food and feed crops poses a major global threat to health and food safety. Surveillance studies report incidence rates ranging from 37% to 92%, with mean concentrations between 1.15 and 107.9 µg kg^{-1} across countries (Kabak *et al*., 2006). In Europe, 80% of samples contain detectable mycotoxins and about 20% exceed legal limits (EFSA, 2020). In India, contamination is equally concerning: maize from Bihar showed 47% infection during *kharif* and 43% in storage, often surpassing the 20 µg kg^{-1} safety threshold (Reddy and Raghavender, 2007). National surveys report that 50-74% of maize exceeds permissible limits (FSSAI, 2020). Acute outbreaks, such as the 1974 Gujarat incident that caused over 200 deaths (Krishnamachari *et al*., 1975), highlight the public health risks. With projected temperature rises of 2-5°C, aflatoxin prevalence is expected to intensify in tropical and subtropical regions (Sarmah *et al*., 2023), emphasizing the need for integrated field-level mitigation.

Maize (*Zea mays* L.) is one of the most important cereals, contributing 42% of global grain production (>1.2 billion tonnes annually) (FAOSTAT, 2022). In India, maize covers ~10 million ha and yields over 31 million tonnes, ranking third after rice and wheat (DACandFW, 2023). However, its quality is threatened by *Aspergillus flavus* and *A. parasiticus*, which produce aflatoxins under warm, humid conditions (Mishra *et al*., 2019). Aflatoxin B (AFB), classified

---

[1]Department of Agronomy, School of Agriculture, Lovely professional University, Phagwara-144 401, Punjab, India.
[2]Department of Soil Science and Agricultural Chemistry, Veer Kunwar Singh College of Agriculture Dumraon, Bihar Agriculture University, Sabour-802119, Bihar, India.
[3]Faculty of Agricultural Sciences, GLA University, Mathura-281 406, Uttar Pradesh, India.
[4]School of Agriculture, Uttaranchal University, Dehradun-248 007, Uttarakhand, India.

Corresponding Author: Rajeev, Department of Agronomy, School of Agriculture, Lovely Professional University, Phagwara-144 401, Punjab, India. Email: .rajeev.26421@lpu.co.in
ORCID: 0009-0000-4252-9357

How to cite this article: Kumari, M.S., Rajeev, Gupta, R.K., Sai, J., Singh, J., Kumar, R. and Kumar, A. (2026). Jasmonic and Salicylic Acid-mediated Defence Enhancement in Maize under Soybean Intercropping for Aflatoxin Suppression. *Legume Research.* 1-7. doi: 10.18805/LR-5578.

Submitted: 23-09-2025 **Accepted:** 17-01-2026 **Online:** 30-01-2026

as a Group 1 carcinogen (IARC, 2012), contributes to food safety, trade and health risks worldwide. More than 25% of food crops are affected by mycotoxins annually, leading to losses exceeding USD 1 billion (WHO, 2018). In India, poor post-harvest infrastructure exacerbates the problem, with surveys reporting up to 74% of market maize samples exceeding 20 μg kg^{-1} (FSSAI, 2020). Traditional approaches such as drying, storage improvement, fungicides and resistant varieties have limited success under climate variability (Paterson and Lima, 2010).

Attention has shifted towards activating plant innate immunity. Jasmonic acid (JA) and salicylic acid (SA) are central defence regulators: JA mediates induced systemic resistance (ISR) against necrotrophic pathogens, while SA governs systemic acquired resistance (SAR), effective against biotrophic fungi (Kuchlan *et al*., 2023). Exogenous JA and SA prime defence pathways, enhancing PR proteins (β-1,3-glucanase, POD, SOD, CAT) that degrade fungal cell walls and detoxify ROS (Wasternack and Hause, 2013).

At the same time, intercropping with legumes such as soybean improves soil nitrogen, microbial diversity and microclimate, thereby reducing fungal infestation (Vandermeer, 1989; Singh *et al*., 2019). Maize-soybean systems lower canopy temperature, vapor pressure deficit and fungal colonization risk (Zhou *et al*., 2020). Soybean root isoflavonoids may also trigger maize defence responses via root-root or microbe-mediated signalling (Moraes *et al*., 2021). Despite evidence for individual success, little is known about the synergistic effects of combining intercropping with JA and SA priming on aflatoxin suppression.

Therefore, this study was undertaken to assess the efficacy of an integrated approach under field conditions. The objectives were to: (i) evaluate the impact of soybean intercropping and hormonal priming on aflatoxin B_1 and B_2 levels in maize; (ii) quantify endogenous JA and SA accumulation and antioxidant enzyme activities (POD, SOD, CAT, β-1,3-glucanase); (iii) examine the expression of defence genes (PR1, PR5) and (iv) analyse rhizospheric microbial diversity and soil parameters. This research provides insights into host-pathogen interactions and offers a biologically intensive strategy for aflatoxin management within climate-resilient maize production systems in South Asia and beyond.

MATERIALS AND METHODS

A field experiment was conducted during the *kharif* seasons of 2022 and 2023 at the Agronomy Research Farm of Lovely Professional University, Punjab, India (31.25°N latitude, 75.58°E longitude, 234 m above mean sea level). The site falls under the subtropical humid agroclimatic zone, characterized by hot summers and moderately cool winters. The study aimed to evaluate the effects of soybean intercropping and foliar application of defence-inducing phytohormones on aflatoxin contamination and defence responses in maize.

The experiment was laid out in a randomized complete block design (RCBD) with three replications. For field feasibility, four representative treatment combinations were evaluated: T_1 - Sole maize (control), T_2 - Sole maize + jasmonic acid (JA, 100 μM), T_3 - Maize + soybean intercropping (2:2 row arrangement) and T_4 - Maize + soybean intercropping + JA (100 μM) + salicylic acid (SA, 1 mM). Before sowing, land preparation involved one deep ploughing followed by two harrowing and levelling to obtain a uniform seedbed. The maize hybrid variety *NMH 589* and the soybean variety *JS-9560* were used as test crops. In sole cropping, maize was sown at 40 cm × 25 cm spacing, while soybean was spaced at 30 cm × 5 cm. In the intercropping system, maize and soybean were planted in a 2:2 paired row arrangement, maintaining the respective intra-row spacings. Each net plot measured 4 m × 5 m (20 m^2).

The recommended dose of fertilizer (RDF) was applied to maize (80:60:20 NPK kg ha^{-1}) and soybean (25:60:20 NPK kg ha^{-1}). Nitrogen for maize was applied in three splits: 25% as basal, 50% at 30 days after sowing (DAS) and the remaining 25% at 60 DAS. Phytohormones were applied as foliar sprays at two crop growth stages: 45 DAS (vegetative) and 60 DAS (early reproductive). Jasmonic acid (JA, 100 μM) and salicylic acid (SA, 1 mM) were freshly prepared in distilled water with 0.1% Tween-20 as surfactant. Treatments T_2 and T_4 received JA sprays, whereas only T_4 received both JA and SA. Foliar applications were carried out using a hand-held knapsack sprayer in the early morning to ensure maximum absorption and to minimize photooxidative degradation.

At harvest maturity, maize cobs were manually harvested, sun-dried and shelled. Grain samples were stored under standardized low-moisture conditions before analysis. Aflatoxin B_1 and B_2 concentrations were determined using competitive ELISA kits (Romer Labs, Austria; Catalogue No. COKAQ1). Grain extracts were prepared by homogenizing 20 g of maize grain in 100 mL of 80% methanol, shaking for 30 min at room temperature and centrifuging at 4000 g for 10 min. The supernatant was diluted according to the manufacturer's protocol and absorbance was measured at 450 nm using a microplate reader. The detection limit of the assay was 1.0 μg kg^{-1} and results were expressed as μg kg^{-1}.

For hormonal profiling, leaf tissues sampled at 45 and 60 DAS were frozen in liquid nitrogen and stored at -80°C until analysis. JA and SA were quantified using high-performance liquid chromatography (HPLC, Shimadzu, Japan) equipped with a C18 column (250 × 4.6 mm, 5 μm). The mobile phase consisted of methanol: water (80:20 v/v) with 0.1% formic acid at a flow rate of 1.0 mL min^{-1} and detection was performed at 230 nm. Standard curves were prepared using authentic JA and SA standards (Sigma-Aldrich, USA) and concentrations were expressed as ng g^{-1} fresh weight.

Biochemical assays of antioxidant enzymes were conducted using spectrophotometric methods. POD activity was assayed using 20 mM guaiacol and 10 mM H_2O_2 as substrates, recording absorbance at 470 nm (extinction coefficient = 26.6 mM^{-1} cm^{-1}). CAT activity was determined by monitoring the decomposition of 10 mM H_2O_2 at 240 nm (extinction coefficient = 39.4 mM^{-1} cm^{-1}). SOD activity was measured by inhibition of NBT (50 µM) reduction at 560 nm, using riboflavin–methionine–NBT assay. β-1,3-glucanase activity was estimated from the release of glucose equivalents from 4 mg mL^{-1} laminarin substrate incubated at 37°C, with absorbance measured at 540 nm. Each assay was performed with three biological replicates, analysed in triplicate.

For molecular analysis, leaf samples collected at 60 DAS were used for RNA extraction with TRIzol reagent (Invitrogen, USA). Following cDNA synthesis, quantitative real-time PCR (qRT-PCR) was conducted using SYBR Green chemistry on a Bio-Rad CFX96 system. Primer sequences for PR1, PR5 and the internal reference gene *ZmAct1* are provided (Table 1). The PCR cycling program was 95°C for 3 min; 40 cycles of 95°C for 15 s and 60°C for 30 s. Relative fold change was calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). Each treatment was represented by three biological replicates, with three technical replicates per sample. Rhizospheric soil samples were collected from all plots at 60 DAS. Microbial populations were quantified by serial dilution up to 10^{-6}, with 0.1 mL aliquots plated on nutrient agar (for bacteria) and potato dextrose agar (for fungi). Plates were incubated at 28±2°C for 48-72 h and counts were expressed as log CFU g^{-1} soil.

Statistical analysis

Data were analysed using OPSTAT software (CCS HAU, Hisar). One-way analysis of variance (ANOVA) was performed on treatment means and comparisons were made using the least significant difference (LSD) test at $P \leq 0.05$. Results are presented as mean ± standard error (SE), n = 3 and different letters indicate significant differences among treatments. Graphs were generated using OriginPro 2024 software. ANOVA indicated significant treatment effects: aflatoxin $B_1 + B_2$ content differed significantly among treatments ($F_{3,8} = 12.45$, $p < 0.01$), endogenous JA ($F_{3,8} = 15.32$, $p < 0.01$) and SA ($F_{3,8} = 14.87$, $p<0.01$) levels also varied strongly and antioxidant enzyme activities (POD, SOD, CAT), β-1,3-glucanase and defence gene expression (PR1, PR5) all showed significant differences ($p<0.05$).

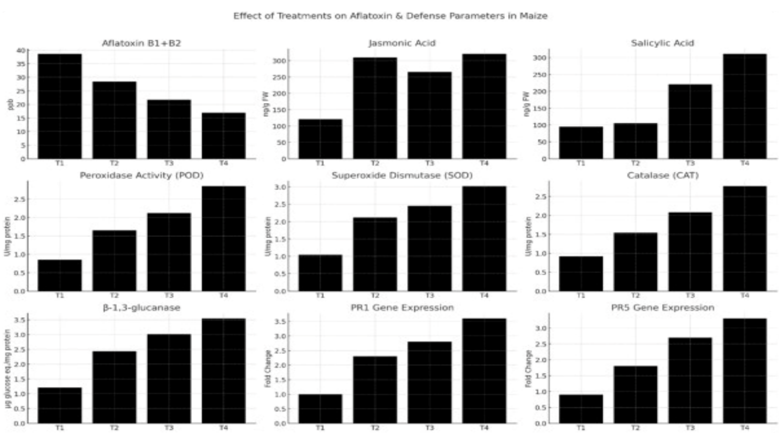
RESULTS AND DISCUSSION

Aflatoxin contamination in maize grains

Aflatoxin contamination, particularly by aflatoxin B_1 (AFB) and B_2, remains a critical food safety concern in maize-producing regions. In the present study, maize grains from different

Table 1: Primer sequences used for qRT-PCR analysis of maize defence-related genes.

Gene	Forward primer (5'→ 3')	Reverse primer (5'→3')	Reference
PR1	TGC TGA GGA GAT GGT TGT GA	CCT TGT GCA TTT GGT AGG TT	Glazebrook, 2005
PR5	ACA GAG GCT GAG GAA GAT GA	GGT CCT TGA TCT TCC TGA AC	Zhang *et al*., 2015
Actin (ZmAct1)	GCT GGA GAT GAT GCT CCT GA	GGA TCC ACA CGA GGA CTT CA	Livak and Schmittgen, 2001



Treatments included T_1 : sole maize (control), T_2 : sole maize + JA, T_3 : maize + soybean intercropping and T_4: maize + soybean + JA + SA. Parameters measured were aflatoxin $B_1 + B_2$ content, endogenous hormone levels (jasmonic acid and salicylic acid), antioxidant enzyme activities (POD, SOD, CAT), β-1,3-glucanase activity and expression of defence-related genes (PR1 and PR5). The integrated treatment (T_4) consistently exhibited the strongest defence activation, reflected in reduced aflatoxin levels and enhanced biochemical and molecular responses.

Fig 1: Effect of different treatment combinations on aflatoxin accumulation and defence-related parameters in maize. T_1- sole maize (control), T_2 - sole maize + JA, T_3 - maize + soybean intercropping and T_4 - maize + soybean + JA + SA.

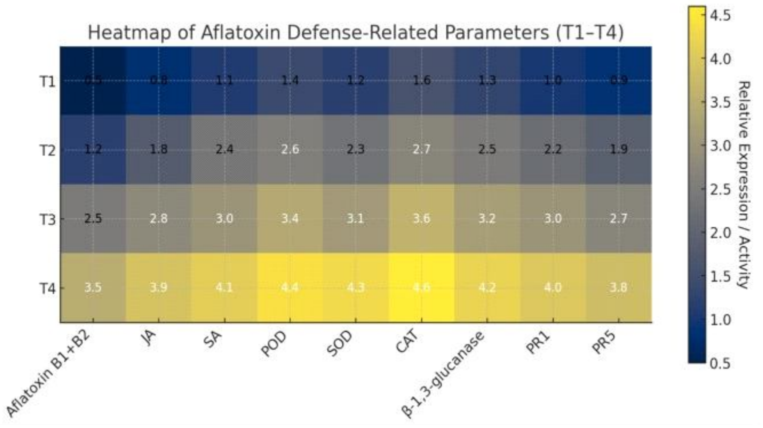
treatment combinations exhibited significant variation ($P<0.05$) in aflatoxin content at harvest. The untreated control (T_1: sole maize) recorded the highest mean aflatoxin concentration at 38.6 μg kg^{-1}, far exceeding the safety threshold of 20 μg kg^{-1} prescribed by the Food Safety and Standards Authority of India (FSSAI, 2020). This observation aligns with earlier reports indicating that maize monocultures, particularly under warm and humid conditions, are highly vulnerable to *Aspergillus flavus* colonization and subsequent mycotoxin accumulation (Kabak *et al.*, 2006; Reddy and Raghavender, 2007). The treatment involving sole maize with jasmonic acid foliar spray (T_2) showed a moderate reduction in aflatoxin levels (28.4 μg kg^{-1}), indicating partial induction of systemic resistance. A more pronounced decrease was recorded in T_3 (maize + soybean intercropping), where aflatoxin concentration declined to 21.7 μg kg^{-1}, possibly due to microclimate modification, increased canopy shading and allelopathic influences from soybean root exudates. The greatest reduction was achieved under the integrated treatment (T_4: maize + soybean intercropping + JA + SA), where aflatoxin levels dropped to 16.9 μg kg^{-1} (Fig 1). This reduction of more than 56% compared with the control highlights the effectiveness of combining intercropping with phytohormonal priming and underscores the role of synergistic JA-SA defence activation in suppressing aflatoxin biosynthesis, as also suggested by Paterson and Lima (2010).

Endogenous hormonal induction: JA and SA accumulation

Quantification of endogenous jasmonic acid (JA) and salicylic acid (SA) in leaf tissues revealed substantial variation among the treatment combinations. In the control (T_1: sole maize), basal levels of 120.5 ng g^{-1} (JA) and 95.3 ng g^{-1} (SA) were recorded. The treatment involving sole maize with JA foliar spray (T_2) increased JA content to 310.2 ng g^{-1}, while SA remained nearly unchanged, reflecting the specificity of exogenous JA application. The intercropping treatment (T_3 : maize + soybean) enhanced both JA (265.4 ng g^{-1}) and SA (220.7 ng g^{-1}), which may be attributed to soybean-maize root interactions and microbiome-mediated cross-activation. The integrated treatment (T_4: maize + soybean + JA + SA) recorded the highest hormonal levels-320.6 ng g^{-1} (JA) and 310.9 ng g^{-1} (SA)-indicating robust hormonal crosstalk and comprehensive defence activation (Fig 1). This hormonal profile supports the model of parallel JA and SA signalling in defence regulation, particularly in modulating host responses against necrotrophic pathogens such as *A. flavus* (Glazebrook, 2005; Zhang **et al.**, 2015).

Antioxidant defence enzymes

The oxidative burst, a hallmark of plant immunity, triggers rapid accumulation of reactive oxygen species (ROS), which are detoxified by antioxidant enzymes including peroxidase (POD), superoxide dismutase (SOD) and catalase (CAT). In the present study, the control treatment (T_1 : sole maize) exhibited the lowest activity levels: POD (0.85 U mg^{-1} protein), SOD (1.05 U mg^{-1} protein) and CAT (0.92 U mg^{-1} protein). The treatment with sole maize + JA foliar spray (T_2) increased enzyme activities moderately, whereas the intercropping treatment (T_3: maize + soybean) resulted in comparatively higher activities, likely due to rhizosphere-mediated biotic interactions. The integrated treatment (T_4: maize + soybean + JA + SA) recorded the maximum activities-POD (2.85 U mg^{-1} protein), SOD (3.02 U mg^{-1} protein) and CAT (2.77 U mg^{-1} protein)-demonstrating that the combination of intercropping and hormonal priming most effectively stimulated systemic antioxidant responses. These observations consistent with earlier findings (Aebi, 1984) and highlight that enhanced ROS



Parameters include aflatoxin B_1 + B_2 content, endogenous hormone accumulation (JA, SA), antioxidant enzyme activities (POD, SOD, CAT), PR protein (β-1,3-glucanase) and defence gene expression (PR1, PR5). Progressive intensification of colour values from T_1 to T_4 illustrates enhanced biochemical and molecular defence responses under integrated management, with T_4 showing the most pronounced activation.

Fig 2: Heatmap of aflatoxin defence-related parameters in maize under four treatment combinations.

detoxification capacity is central to restricting aflatoxin biosynthesis and fungal spread.

PR protein response: β-1,3-glucanase activity

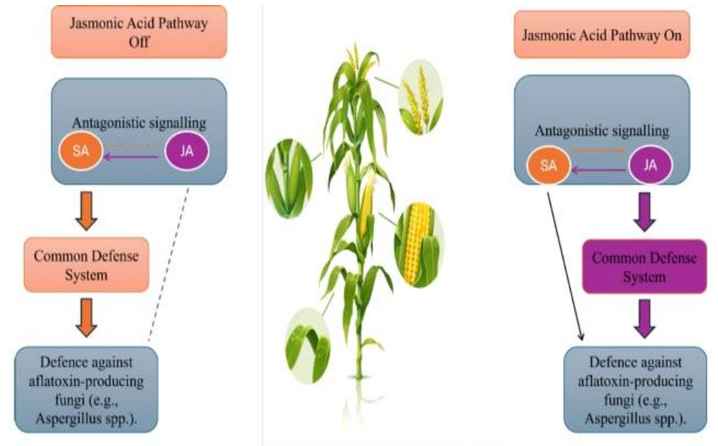
β-1,3-glucanase is a hallmark pathogenesis-related (PR) protein involved in fungal cell wall degradation. In the present study, its activity showed a progressive increase across the treatment combinations, with the lowest value recorded in the control (T_1: 1.21 µg glucose eq. mg^{-1} protein) and the highest in the integrated treatment (T_2: 3.54 µg glucose eq. mg^{-1} protein). Intermediate activities were observed in T_3 (sole maize + JA: 2.43 µg glucose eq. mg^{-1} protein) and T_4 (maize + soybean intercropping: 3.01 µg glucose eq. mg^{-1} protein) (Fig 2). These results indicate that while T_2 and T_3 each enhanced glucanase activity to a certain extent, the combined strategy in T_4 exerted the strongest induction, reflecting a synergistic effect of intercropping with hormonal priming. The role of β-1,3-glucanase in resistance to *A. flavus* has been well documented in oilseeds and cereals (Velazhahan and Vidhyasekaran, 2000), which supports the present findings.

Gene expression of PR1 and PR5

Defence gene expression analysis using qRT-PCR revealed significant upregulation of PR1 and PR5 genes in the treated maize plants. Expression was normalized using the actin gene (ZmA_cT_1) as an internal reference and relative fold change was calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). In the control (T_1: sole maize), baseline expression was maintained at 1.0 for both genes. The treatment with sole maize + JA foliar spray (T_2) increased expression to 2.3-fold for PR1 and 1.8-fold for PR5. The intercropping treatment (T_3: maize + soybean) further enhanced expression to 2.8-fold (PR1) and 2.7-fold (PR5). The maximum induction was observed under the integrated treatment (T_4 : maize + soybean + JA + SA), where PR1 and PR5 reached 3.6- and 3.3-fold, respectively (Fig 3). These results demonstrate that T_4 elicited the most robust transcriptional activation of defence-related genes. Since PR1 and PR5 are well-established markers of systemic acquired resistance (SAR) pathways (Glazebrook, 2005), these findings confirm the successful activation of systemic immunity under the integrated treatment. The mechanistic basis of this coordinated defence activation, driven by jasmonic acid-salicylic acid signalling crosstalk and its role in suppressing aflatoxin biosynthesis, is schematically illustrated (Fig 3).

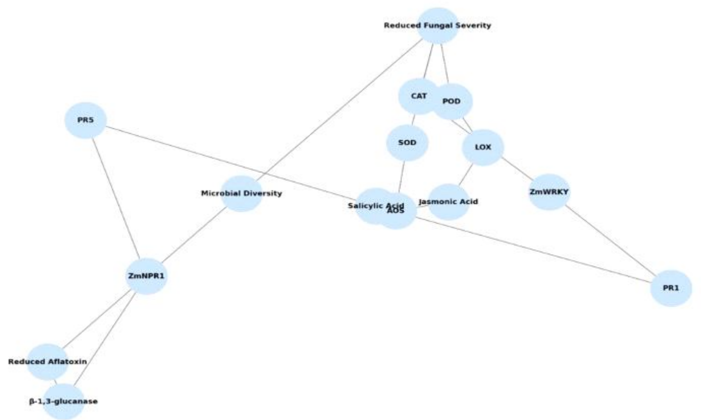
Rhizospheric microbial diversity, soil moisture and temperature effects

Soil microbiological and physicochemical attributes play a critical role in crop resilience and disease suppression under intercropping systems. In the present study, rhizospheric microbial diversity, soil moisture content and soil temperature were monitored across the four treatment combinations (T_1 -T_4) (Fig 4). A progressive enhancement in microbial diversity was observed, with the control (T_1: sole maize) recording the lowest value (6.1 log CFU g^{-1}) and T_2 (sole maize + JA) showing a slight increase to 6.5 log CFU g^{-1}. Higher microbial proliferation was noted in the intercropping treatment (T_3: maize + soybean, 7.4 log CFU g^{-1}), while the integrated treatment (T„ : maize + soybean + JA + SA) achieved the maximum value (8.2 log CFU g^{-1}). The enrichment of microbial diversity under T_4 may be attributed to increased rhizospheric root exudates and hormone-



When the JA pathway is activated (JA pathway on), antagonistic signalling is overridden, enabling a coordinated defence response against *Aspergillus* spp. and associated aflatoxin biosynthesis. The figure highlights the central role of JA-SA interactions in modulating systemic defence pathways.

Fig 3: Schematic representation of salicylic acid (SA) and jasmonic acid (JA) signalling in maize defence against aflatoxin-producing fungi. Under normal conditions (JA pathway off), antagonistic crosstalk between SA and JA limits activation of the common defence system.



The network highlights the roles of jasmonic acid (JA) and salicylic acid (SA) signalling pathways, associated PR gene expression (PR1, PR5), enzymatic activities (POD, SOD, CAT, β-1,3-glucanase) and rhizospheric microbial diversity in mitigating aflatoxin accumulation. The integrated treatment (T_4: maize + soybean intercropping + JA + SA) exhibited the most robust defence induction, linking hormonal priming, enzymatic responses and microbial interactions to reduced aflatoxin levels and fungal severity.

Fig 4: Gene-enzyme interaction network illustrating the coordinated activation of maize defence mechanisms under soybean. intercropping.

induced systemic resistance, which together promote the colonization of beneficial microbes (Mendes *et al*., 2013; Bhattacharyya and Jha, 2023). Soybean root exudates are known to support populations such as *Trichoderma spp*. and *Pseudomonas fluorescens*, potentially contributing to the suppression of aflatoxigenic fungi (Moraes *et al*., 2021).

Soil moisture also increased progressively among the treatments. T_1 maintained the lowest moisture level (14.8%), while T_2 and T_3 recorded 16.3% and 18.2%, respectively. The integrated treatment (T_4) displayed the highest soil moisture (20.4%), likely due to canopy shading from soybean, improved soil aggregation by microbial exudates and reduced evapotranspiration (Reddy, 2020). These findings align with reports that diversified cropping systems enhance water retention by increasing organic matter and improving root structure (Sharma *et al*., 2021). Conversely, soil temperature showed a declining trend across treatments. T_1 exhibited the highest mean temperature (32.4°C), followed by T_2 (31.9°C) and T_3 (30.7 °C), while the lowest value was recorded in T_4 (29.8°C). This reduction is likely attributable to greater canopy cover under intercropping, reduced soil albedo and improved moisture conservation, which buffer against temperature fluctuations and support microbial activity (Singh *et al*., 2022). Collectively, these results demonstrate that the integrated treatment (T_4) not only maximizes microbial community richness but also modifies the soil microenvironment in ways that strengthen plant defence and minimize aflatoxin accumulation.

CONCLUSION

The integrated treatment (T_4: maize + soybean intercropping + JA + SA) significantly reduced aflatoxin contamination to 16.9 µg kg^{-1}, below the FSSAI limit. It also achieved the highest JA (320.6 ng g^{-1}) and SA (310.9 ng g^{-1}) accumulation. Antioxidant enzyme activities (POD, SOD, CAT) and PR gene expression (PR1, PR5) were maximized in T_4. Soil microbial diversity, moisture content and microclimate conditions were most favourable under this treatment. These results highlight the potential of integrating intercropping with phytohormonal priming for aflatoxin suppression. Future research should focus on multi-location trials and full factorial designs to confirm scalability of this integrated approach under diverse agroclimatic conditions.

ACKNOWLEDGMENT

The author greatly acknowledges Lovely Professional University (LPU), Jalandhar, Punjab, for providing facilities to conduct research during Ph.D. programme at Division of Agronomy and School of Agriculture (SOA).

Disclaimers

The opinions and conclusion outlined in this article reflect those of the author and should not be considered as representing the perspectives of their affiliated institutions. While the authors have ensured the accuracy and completeness of the information presented, they disclaim any responsibility for direct or indirect damages that may result from the application of this content.

Ethical statement

This study did not involve human participants or vertebrate animals. Field experiments were conducted in accordance with institutional and national agricultural research guidelines.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

Aebi, H. (1984). Catalase *in vitro. Methods in Enzymology.* **105:** 121-126.

Bhattacharyya, P.N. and Jha, D.K. (2023). Plant growth-promoting rhizobacteria (PGPR): Emergence in agriculture. In *Advances in PGPR Research* Springer. (pp. 23-45).

Department of Agriculture, Cooperation and Farmers Welfare (DAC and FW). (2023). Agricultural Statistics at a Glance 2023. Ministry of Agriculture and Farmers Welfare, Government of India.

European Food Safety Authority (EFSA). (2020). Scientific opinion on the risks for human and animal health related to the presence of aflatoxins in food and feed. *EFSA Journal.* **18(3):** e06040.

Food and Agriculture Organization of the United Nations (FAOSTAT). (2022). Crop Production Statistics.

Food Safety and Standards Authority of India (FSSAI). (2020). Food safety and standards (Contaminants, Toxins and Residues) Regulations, 2011 - Consolidated notification. https://www.fssai.gov.in

Glazebrook, J. (2005). Contrasting mechanisms of defence against biotrophic and necrotrophic pathogens. *Annual Review of Phytopathology*. **43:** 205-227.

International Agency for Research on Cancer (IARC). (2012). Aflatoxins. In *IARC monographs on the evaluation of carcinogenic risks to humans* (Vol. 100F). World Health Organization.

Kabak, B., Dobson, A.D.W. and Var, I. (2006). Strategies to prevent mycotoxin contamination of food and animal feed: A review. *Critical Reviews in Food Science and Nutrition*. **46(8):** 593-619.

Krishnamachari, K.A.V.R., Bhat, R.V., Nagarajan, V. and Tilak, T.B.G. (1975). Hepatitis due to aflatoxicosis: An outbreak in Western India. *The Lancet.* **305(7905):** 1061-1063.

Kuchlan, P. and Kuchlan, M.K. (2023). Effect of salicylic acid on plant physiological and yield traits of soybean. *Legume Research.* **46(1):** 56-61. doi: 10.18805/LR-4527.

Livak, K.J. and Schmittgen, T.D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods*: ***25*(4):** 402-408.

Mendes, R., Garbeva, P. and Raaijmakers, J.M. (2013). The rhizosphere microbiome: Significance of plant beneficial, plant pathogenic and human pathogenic microorganisms. *FEMS Microbiology Reviews*. **37(5):** 634-663.

Mishra, J., Kumar, M., Sharan, K.A. and Kumar, J. (2019). Detoxification assessment of Aflatoxin in *Aspergillus flavus* under the effect of *Calendula officinalis* Linn and rsquo;s methanolic extract. *Agricultural Science Digest*. **39(1):** 21-25. doi: 10.18805/ag.D-4874.

Moraes, J.C., de Oliveira, M.G.A. and Borges, R.M. (2021). Isoflavonoids in soybean roots influence rhizosphere microbial community and pathogen defence. *Plant Physiology and Biochemistry*. **166:** 378-386.

Paterson, R.R.M. and Lima, N. (2010). How will climate change affect mycotoxins in food. *Food Research International.* **43(7):** 1902-1914.

Reddy, K.R.N. and Raghavender, C.R. (2007). Outbreaks of aflatoxicoses in India. *African Journal of Food, Agriculture, Nutrition and Development*. **7(5):** 1-15.

Reddy, P.P. (2020). Plant growth promoting rhizobacteria (PGPR) for sustainable agriculture. Springer.

Sarmah, R.S., Subudhi, P.K., Dutta, T., Roychoudhury, P., Mohan, J. and Singh, Y.D. (2023). Comparison of aflatoxin yield from peanut isolate of *aspergillus flavus* with other isolates. *Asian Journal of Dairy and Food Research.* 10.18805/ajdfr.DR-2028.

Sharma, S., Pramanik, A. and Agrawal, P.K. (2021). Role of biofertilizers in improving soil fertility and plant growth: An overview. *Current Agriculture Research Journal*. **9(1):** 50-60.

Singh, G., Yadav, R.S. and Kumar, A. (2019). Effect of legume intercropping on maize growth and rhizosphere microflora. *Legume Research.* **42(3):** 403-408.

Singh, S., Yadav, A. and Patel, M. (2022). Synergistic effects of bioinoculants and nitrogen fertilization on soil microbial diversity and crop productivity. *Soil Biology and Biochemistry*. **164:** 108506.

Vandermeer, J. (1989). The ecology of intercropping. Cambridge University Press. https://doi.org/10.1017/CBO9780511623523.

Velazhahan, R. and Vidhyasekaran, P. (2000). β-1,3-glucanase activity and induced resistance of groundnut against leaf spot caused by *Cercosporidium personatum*. *Journal of Plant Disease Protection*. **107(4):** 425-430.

Wasternack, C. and Hause, B. (2013). Jasmonates: Biosynthesis, perception, signal transduction and action in plant stress response, growth and development. *An update to the 2007 review in Annals of Botany.* **111(6):** 1021-1058.

World Health Organization (WHO). (2018). Mycotoxins - Key facts. https://www.who.int/news-room/fact-sheets/detail/mycotoxins.

Zhang, W., Qin, X. and Liu, Y. (2015). Hormonal crosstalk in plant resistance to fungal pathogens. *Frontiers in Plant Science.* **6:** 1008.

Zhou, M., Jin, Y. and Liu, J. (2020). Impact of maize-soybean intercropping on microclimate, disease suppression and yield advantage. *Field Crops Research*. **246:** 107682.