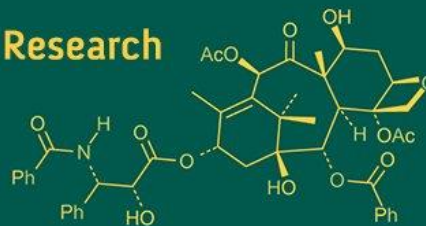


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Exploitation of biochemical basis of resistance against major insect pests in sorghum genotypes

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Abstract

A comprehensive field evaluation of twenty-six sorghum genotypes conducted during 2024–2025 identified substantial genotypic variability in resistance to the economically important insect pests, namely shoot fly (*Atherigona soccata*), stem borer (*Chilo partellus*), and fall armyworm (*Spodoptera frugiperda*). Genotypes AKR-150, NJ-2669, and NJ-2403 consistently exhibited superior levels of resistance, indicating their potential as stable sources of host plant resistance. Biochemical characterization demonstrated a significant positive association between insect resistance and elevated concentrations of total phenols (7.232 mg g⁻¹) and tannins (0.187%), whereas total soluble sugars (3.32%) showed a negative association with resistance. The enhanced accumulation of phenolic compounds and tannins is presumed to confer resistance through constitutive biochemical defence mechanisms, including antibiosis, antixenosis, and reduced tissue palatability, while lower soluble sugar levels decrease host nutritional suitability for insect establishment and larval development. These findings establish phenols and tannins as robust biochemical markers for resistance phenotyping and highlight their utility in marker-assisted and conventional breeding programmes aimed at developing broad-spectrum insect-resistant sorghum cultivars. The identified resistant genotypes constitute valuable donor resources for enhancing host plant resistance and promoting environmentally sustainable integrated pest management strategies.

Keywords: Genotypes, tolerance, susceptible, bio chemical factors, host plant resistance

Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is the fifth most important cereal crop worldwide after maize, rice, wheat and barley and constitutes a vital component of food, feed, fodder, fuel and industrial production systems across the semi-arid tropics. Globally, sorghum is cultivated on approximately 42–45 million hectares with an annual production exceeding 60 million tonnes, while India remains one of the major centres of cultivation, genetic diversity and utilization. Owing to its C4 photosynthetic pathway, exceptional water-use efficiency, deep root architecture and remarkable tolerance to drought, heat and poor soil fertility, sorghum has emerged as a climate-resilient crop capable of sustaining agricultural productivity under increasingly erratic climatic conditions. Consequently, sorghum has gained renewed global attention as a strategic crop for ensuring food and nutritional security, climate adaptation and sustainable agriculture in rainfed ecosystems. Recent advances in sorghum genomics and breeding have further strengthened its importance as a model crop for developing resilient cereal production systems.

Despite its inherent adaptability, sorghum productivity is severely constrained by a wide spectrum of insect pests occurring from seedling emergence to grain maturity. More than 150 insect species have been reported to infest sorghum worldwide, among which shoot fly (*Atherigona soccata* Rondani), stem borer (*Chilo partellus* Swinhoe), fall armyworm (*Spodoptera frugiperda* J.E. Smith), sorghum midge (*Contarinia sorghicola* Coquillett), corn leaf aphid (*Rhopalosiphum maidis* Fitch), sorghum aphid (*Melanaphis sorghi*), earhead bugs and armyworms are considered economically important. These pests collectively reduce grain yield, fodder quality and seed production through direct tissue destruction and indirect physiological impairment. Yield losses attributable to insect infestation may vary from 20% to more than 80% depending on pest species, genotype, crop stage and prevailing environmental conditions.

The recent global invasion of fall armyworm and the rapid spread of sorghum aphid have further intensified the need for durable host plant resistance in sorghum improvement programmes.

Conventional insect management in sorghum relies predominantly on repeated applications of synthetic insecticides. Although insecticides provide rapid suppression of pest populations, their indiscriminate use has resulted in numerous ecological and economic concerns including pesticide resistance, resurgence of secondary pests, destruction of natural enemies, environmental contamination, pesticide residues in food and increased production costs. Furthermore, chemical control is often ineffective against concealed feeders such as stem borers and offers limited protection under rainfed farming systems where resource-poor farmers have restricted access to crop protection inputs. Consequently, integrated pest management (IPM) strategies emphasizing host plant resistance have gained increasing prominence as environmentally safe, economically viable and sustainable alternatives.

Host plant resistance represents one of the most effective and durable approaches for managing insect pests because it provides season-long protection without additional cost to farmers and is fully compatible with biological control, cultural practices and precision agriculture. Resistant cultivars reduce pest colonization, retard insect development, suppress population build-up and minimize crop damage while conserving beneficial arthropods. The incorporation of resistance genes into elite cultivars also contributes significantly to reducing insecticide dependence and delaying the evolution of insecticide-resistant pest populations. Therefore, identification of resistant germplasm has become a major objective of sorghum breeding programmes worldwide.

Extensive germplasm screening conducted by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), USDA and several national breeding programmes has identified numerous sorghum accessions exhibiting resistance against shoot fly, stem borer, midge, aphids and other economically important insects. Resistant donor lines such as IS 18551, IS 2205, IS 2312, IS 5470, IS 5480 and several breeding derivatives have contributed substantially to the development of insect-resistant breeding populations. Nevertheless, the genetic and biochemical mechanisms responsible for durable resistance remain incompletely understood because insect resistance is a quantitative trait governed by multiple genes interacting with environmental factors. Therefore, detailed characterization of resistance-associated biochemical constituents is essential for accelerating marker-assisted selection and genomic breeding.

Plant resistance to herbivorous insects is mediated through three classical mechanisms proposed by Painter: antixenosis (non-preference), antibiosis and tolerance. Antixenosis reduces insect attraction, feeding and oviposition through morphological or chemical cues; antibiosis adversely affects insect survival, growth, fecundity and longevity after feeding; whereas tolerance enables plants to withstand insect injury without significant yield reduction. These resistance mechanisms often operate simultaneously and are regulated through complex interactions among structural, physiological, biochemical and molecular traits. Modern

molecular studies further indicate that resistance involves coordinated activation of phytohormonal signalling pathways, transcription factors, defence enzymes and secondary metabolite biosynthesis following insect attack.

Among various resistance mechanisms, biochemical defence constitutes one of the most important determinants of insect resistance in sorghum. Secondary metabolites synthesized through the phenylpropanoid and flavonoid pathways play critical roles in plant defence by reducing host suitability, impairing insect digestion and activating induced resistance responses. Phenolic compounds, condensed tannins, flavonoids, lignin precursors and related phytochemicals function as natural anti-feedants, feeding deterrents and toxic metabolites that interfere with insect growth and development. These compounds also contribute to oxidative defence by scavenging reactive oxygen species generated during herbivore attack and reinforcing structural barriers through lignification and cross-linking of cell wall polymers. Recent molecular investigations have shown that insect feeding activates WRKY transcription factors, MYB regulators, lipoxygenases, calcium-dependent protein kinases and jasmonic acid-mediated signalling pathways leading to enhanced biosynthesis of phenolics and flavonoids that contribute to resistance.

Phenolic compounds have consistently been identified as reliable biochemical markers associated with insect resistance in sorghum. Elevated phenolic concentrations reduce palatability, inhibit digestive enzymes, impair nutrient assimilation and suppress larval growth. Similarly, condensed tannins form stable complexes with proteins and digestive enzymes, thereby decreasing protein digestibility and reducing insect fitness. Numerous investigations have reported significant negative correlations between total phenol and tannin concentrations with shoot fly dead hearts, stem borer infestation, aphid colonization and grain-feeding insect damage. In contrast, soluble sugars generally improve host nutritional quality, enhance feeding preference and facilitate rapid multiplication of phytophagous insects. Consequently, susceptible genotypes frequently possess higher soluble sugar concentrations, whereas resistant germplasm is characterized by elevated phenolic and tannin contents coupled with comparatively lower soluble sugar levels. Recent experimental studies have further confirmed that stem borer-resistant sorghum genotypes contain lower moisture, nitrogen, protein and soluble sugar contents but higher phenolic concentrations than susceptible cultivars, highlighting the importance of biochemical defence mechanisms in host plant resistance.

Recent advances in metabolomics, transcriptomics and genomics have substantially improved understanding of the molecular architecture underlying insect resistance in sorghum. High-throughput sequencing and genome-wide association studies have identified quantitative trait loci and candidate genes controlling flavonoid biosynthesis, secondary metabolite accumulation and defence signalling pathways. Particular emphasis has been placed on MYB-regulated flavonoid biosynthesis, which governs the production of 3-deoxyanthocyanidins and related phenolic compounds associated with aphid resistance. Integration of biochemical phenotyping with genomic prediction, marker-assisted selection and artificial intelligence-assisted phenomics provides unprecedented opportunities for

accelerating the development of insect-resistant cultivars possessing durable and broad-spectrum resistance. Although substantial progress has been achieved in identifying resistant sorghum accessions, comprehensive studies integrating field resistance against multiple insect pests with biochemical characterization of defence metabolites under Indian agro-climatic conditions remain limited. Simultaneous evaluation of sorghum germplasm against shoot fly, stem borer and fall armyworm, together with quantification of total phenols, condensed tannins and soluble sugars, provides a robust framework for identifying stable sources of resistance and elucidating the biochemical mechanisms governing host plant defence. Such information is indispensable for resistance breeding programmes aimed at developing climate-resilient cultivars requiring minimal insecticide inputs.

Therefore, the present investigation was undertaken to evaluate diverse sorghum germplasm for resistance against major insect pests under field conditions and to elucidate the biochemical basis of resistance through quantitative estimation of total phenols, condensed tannins and total soluble sugars. The study further aimed to establish relationships between biochemical constituents and insect infestation, thereby identifying reliable biochemical markers that can facilitate resistance breeding and strengthen integrated pest management strategies in sorghum.

Materials and Methods

Experimental site

The field experiment was conducted during the kharif 2024 season at the Regional Agricultural Research Station (RARS), Nandyal, Andhra Pradesh, India (15°29' N, 78°29' E; 211 m above mean sea level). The experimental site is located in the scarce rainfall zone of Southern India and is characterized by a semi-arid tropical climate. The soil of the experimental field was medium black clay with good drainage. Standard agronomic practices recommended for sorghum cultivation were followed throughout the crop growth period.

Plant materials and experimental design

Twenty-five advanced sorghum (*Sorghum bicolor* (L.) Moench) germplasm lines were evaluated for resistance against major insect pests under natural field conditions. Four well-characterized genotypes, namely Swarna, DJ-6514, IS-2205, and IS-18551, were included as standard checks for comparison.

The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications. Each experimental plot consisted of a single row of 3 m length with a spacing of 45 cm × 15 cm (row × plant). Seeds were sown during the second fortnight of June 2024. All recommended agronomic practices were adopted uniformly across treatments except that no insecticidal or other plant protection measures were applied throughout the cropping season to permit natural infestation by insect pests.

Assessment of insect pest incidence

The germplasm was screened against three economically important insect pests, namely shoot fly (*Atherigona soccata* Rondani), stem borer (*Chilo partellus* Swinhoe), and fall armyworm (*Spodoptera frugiperda* J.E. Smith).

Shoot fly infestation was assessed by recording the percentage of dead hearts at 20 days after sowing (DAS). Stem borer incidence was recorded at 25 DAS based on the percentage of plants exhibiting characteristic dead-heart symptoms. Fall armyworm infestation was recorded at 25 and 45 DAS by estimating the percentage of plants showing typical feeding injury on the whorl and leaves.

The percentage infestation for each pest was calculated using the following equation:

$$[\text{Infestation (\%)}] = \frac{\text{Number of affected plants}}{\text{Total number of plants observed}} \times 100$$

Biochemical analysis

Based on field performance, germplasm exhibiting relatively high levels of resistance to the major insect pests was selected for biochemical analysis. Fresh leaf samples were collected during the vegetative stage from representative plants of each selected genotype and immediately transported to the laboratory for biochemical estimation.

Determination of total phenolic content

Total phenolic content was estimated using the Folin–Ciocalteu colorimetric method following the procedure described by Malick and Singh (1980) with modifications proposed by Singleton *et al.* (1999). Fresh leaf tissue (0.5 g) was homogenized in 80% ethanol, and the extract was centrifuged at 10,000 × g for 20 min. The supernatant was reacted with Folin–Ciocalteu reagent followed by sodium carbonate solution. After incubation, absorbance was measured using a UV–Visible spectrophotometer, and phenolic content was quantified using a catechol (or gallic acid) standard curve. The results were expressed as mg g⁻¹ fresh weight (FW).

Determination of tannin content

Tannin content was estimated using the Folin–Denis method as described by Schanderl (1970) and Price and Butler (1977). Fresh leaf samples were extracted with aqueous methanol, and the extract was reacted with Folin–Denis reagent in the presence of sodium carbonate. Following colour development, absorbance was recorded at 700 nm. Tannin concentration was determined from a tannic acid standard curve and expressed as mg tannic acid equivalents (TAE) g⁻¹ fresh weight.

Determination of reducing sugars

Reducing sugar content was estimated by the Nelson–Somogyi method (Nelson, 1944; Somogyi, 1952). Fresh leaf tissue was extracted using hot 80% ethanol. The extract was reacted with alkaline copper reagent followed by arsenomolybdate reagent, and the absorbance was measured at 620 nm using a UV–Visible spectrophotometer. Reducing sugar concentration was calculated using glucose as the standard and expressed as mg glucose g⁻¹ fresh weight.

Statistical analysis

Data on shoot fly dead hearts, stem borer infestation, and fall armyworm incidence were subjected to arcsine square-root transformation prior to statistical analysis to normalize percentage data. Analysis of variance (ANOVA) appropriate for a randomized complete block design was performed to

determine treatment effects. Mean comparisons were carried out using the least significant difference (LSD) test at the 5% significance level ($P \leq 0.05$).

Relationships between insect pest incidence and biochemical constituents were examined using Pearson's correlation analysis. To identify the major biochemical

variables associated with resistance and to classify the germplasm based on their biochemical profiles, principal component analysis (PCA) and hierarchical cluster analysis (HCA) were performed. All statistical analyses were conducted using appropriate statistical software.

Table 1: Details of sorghum genotypes

S.NO	Genotype
1	NJ-2704
2	NJ-2706
3	NJ-2707
4	AKR-150
5	NJ-2685
6	PVK-801
7	NJ-2686
8	NJ-2403
9	NJ-2668
10	NJ-2667
11	NJ-2669
12	NTJ-5
13	NJ-2672
14	NJ-2680
15	NJ-2679
16	NJ-2681
17	NJ-2682
18	NJ-2683
19	NJ-2652
20	NJ-2684
21	NJ-2662
22	NJ-2663
23	NJ-2664
24	NTJ-2
25	CSV-22-R
26	M-35-1
27	swarna
28	IS-18551
29	DJ-6514
30	IS-2205

Table 2: Damage scales & resistance reaction for shoot fly and stem borer in jowar

S. No	Damage % (Dead hearts)	Scale	Reaction
1	0-10	1	Resistant
2	10-20	2	Resistant
3	20-30	3	Resistant
4	30-40	4	Moderately resistant
5	40-50	5	Moderately resistant
6	50-60	6	Susceptible
7	60-70	7	Susceptible
8	70-80	8	Susceptible
9	80-90	9	Susceptible

Table 3: Screening of germplasm and advance cultures of sorghum against major insect pest (Per cent deadhearts by shootfly, stemborer and % plant infestation by fall armyworm on Sorghum)

S. No.	Entry	Shoot fly % dead hearts at 20 DAS	Reaction	Stem borer infestation @ 45 DAS (%)	Reaction	Fall armyworm plant infestation (%) 25 DAS	Fall armyworm plant infestation (%) 45 DAS	Yield (kg/ha)
1	NJ-2704	27.33(31.32)**	R	27.25(31.27)**	R	27.23(31.36)**	32.00(34.15)**	1743
2	NJ-2706	28.73(32.42)	R	25.73(30.43)	R	25.73(30.35)	37.83(37.87)	1773
3	NJ-2707	28.86(32.28)	R	18.13(24.72)	R	37.87(37.97)	28.97(32.57)	1643
4	AKR-150	3.00(9.83)	R	10.56(18.32)	R	10.56(18.32)	10.57(17.66)	2600
5	NJ-2685	21.19(27.08)	R	33.30(35.19)	MR	36.35(37.02)	24.23(29.37)	1764
6	PVK-801	37.83(37.94)	MR	31.79(33.77)	MR	39.37(38.83)	44.20(41.69)	1745
7	NJ-2686	30.50(33.47)	MR	31.73(34.24)	MR	18.13(25.21)	16.63(23.73)	2110
8	NJ-2403	4.50(12.24)	R	9.03(17.12)	R	22.53(28.32)	7.50(15.73)	2133
9	NJ-2668	30.27(33.23)	MR	36.33(36.96)	MR	33.03(35.03)	28.77(32.40)	1845
10	NJ-2667	33.32(35.09)	MR	34.83(35.75)	MR	36.30(36.97)	28.77(32.49)	1876
11	NJ-2669	12.09(19.72)	R	36.33(37.07)	MR	48.43(44.05)	34.80(35.84)	2083
12	NTJ-5	34.80(35.84)	MR	24.23(29.35)	R	22.69(28.39)	31.87(34.35)	1966
13	NJ-2672	21.19(27.08)	R	13.59(21.46)	R	18.13(25.12)	25.70(30.36)	2001
14	NJ-2680	34.82(36.11)	MR	51.49(45.91)	MR	34.82(35.98)	37.83(37.67)	1860
15	NJ-2679	19.66(26.12)	R	27.26(31.17)	R	31.79(34.30)	54.50(47.64)	1797
16	NJ-2681	43.90(41.49)	MR	40.87(39.70)	MR	27.23(31.28)	34.82(36.11)	1806
17	NJ-2682	34.83(35.73)	MR	9.04(17.13)	R	36.63(37.08)	39.37(38.83)	1809
18	NJ-2683	33.39(35.27)	MR	34.83(36.00)	MR	28.73(32.42)	42.37(40.61)	1931
19	NJ-2652	27.23(31.44)	R	33.30(35.19)	MR	54.50(47.64)	28.77(32.40)	1756
20	NJ-2684	18.13(24.92)	R	34.83(36.00)	MR	40.89(37.87)	33.30(35.08)	1905
21	NJ-2662	34.92(36.04)	MR	35.00(36.22)	MR	37.83(36.81)	51.50(45.91)	1670
22	NJ-2663	24.23(29.35)	R	27.26(31.17)	R	24.23(35.73)	36.43(37.12)	1571
23	NJ-2664	33.30(35.19)	MR	39.37(38.78)	MR	40.89(35.39)	27.60(31.61)	1547
24	NTJ-2	46.90(43.22)	MR	22.70(28.15)	R	26.23(30.63)	31.79(36.98)	1907
25	CSV-22-R	24.20(29.15)	R	30.27(33.23)	MR	34.83(37.02)	37.83(37.35)	1767
26	M-35-1	33.30(35.10)	MR	51.50(45.91)	S	34.82(41.38)	41.27(45.69)	1673
27	Swarna	80.27(64.30)	S	89.37(71.09)	S	61.47(43.51)	78.77(55.31)	1063
28	IS-18551	6.00(13.99)	R	42.50(40.65)	R	22.70(40.26)	40.27(44.48)	2066
29	DJ-6514	77.23(61.61)	S	83.30(66.26)	S	80.29(64.32)	57.56(42.31)	955
30	IS-2205	21.17(27.19)	R	9.03(17.12)	R	33.30(35.19)	36.33(36.81)	2037
F-Test		S		S		S	S	S
SEm		3.31		3.25		3.77	3.69	126.24
CD (5%)		9.37		9.20		10.69	10.44	357.38
CV (%)		17.66		16.32		18.26	17.69	12.06

Resistant (1-3), moderately resistant (4-5), susceptible (6-9)**Table 4:** Bio chemical composition of sorghum genotypes at 28 days after emergence

S. No	Genotype	Tannin %	Total soluble sugars %	Phenols mg/g
1	AKR-150	0.187	3.326	7.232
2	NJ-2669	0.177	3.652	6.802
3	NJ-2403	0.199	3.512	6.708
4	SWARNA	0.116	5.017	5.612
5	IS-18551	0.211	3.139	7.006

The biochemical characterization of selected sorghum genotypes demonstrated that resistance to major insect pests is closely associated with the accumulation of defensive secondary metabolites. Resistant genotypes (AKR-150, NJ-2669, NJ-2403, and IS-18551) were characterized by significantly higher phenol (6.708–7.232 mg g⁻¹) and tannin (0.177–0.211%) contents, together with lower total soluble sugar concentrations (3.139–3.652%), whereas the susceptible genotype Swarna exhibited the lowest phenol (5.612 mg g⁻¹) and tannin (0.116%) contents but the highest

soluble sugar concentration (5.017%). These biochemical characteristics corroborate the strong negative correlations of phenols and tannins with insect incidence and the positive correlation of soluble sugars with susceptibility. Collectively, the results identify high phenol and tannin contents, coupled with low soluble sugar concentration, as reliable biochemical markers for screening insect-resistant sorghum germplasm and provide valuable selection criteria for resistance breeding aimed at developing high-yielding, broad-spectrum insect-resistant cultivars.

Table 5: Simple correlation analysis between shoot fly, stem borer and FAW incidence with biochemical traits of sorghum genotypes

	phenols	Tannin	Reducing sugar
Shoot fly incidence	-0.958	-0.951	0.976
Stem borer incidence	-0.852	-0.807	0.832
Fall armyworm infestation	-0.825	-0.787	0.807

R = 0.878 P = 0.05

Pearson's correlation analysis revealed significant associations between the biochemical constituents of sorghum and the incidence of major insect pests, indicating that constitutive biochemical defenses play a pivotal role in host plant resistance (Table X). Among the biochemical parameters evaluated, total phenolic content exhibited the strongest negative association with insect infestation, followed by tannin content, whereas reducing sugars were positively correlated with pest incidence.

Total phenol content showed an extremely strong negative correlation with shoot fly incidence ($r = -0.958$), indicating that genotypes possessing elevated phenolic concentrations experienced substantially lower infestation. Similarly, phenols were negatively correlated with stem borer incidence ($r = -0.852$) and fall armyworm infestation ($r = -0.825$), demonstrating that phenolic compounds confer broad-spectrum resistance against multiple insect pests. The magnitude of these correlations suggests that phenols constitute one of the principal biochemical determinants governing host resistance in sorghum.

Tannin content also exhibited highly significant negative correlations with shoot fly ($r = -0.951$), stem borer ($r = -0.807$), and fall armyworm infestation ($r = -0.787$). These findings indicate that tannins substantially suppress insect colonization and feeding through their well-documented anti-nutritional properties, including protein precipitation, inhibition of digestive enzymes, and reduced nutrient assimilation. Although the correlations were slightly weaker than those observed for phenols, tannins consistently contributed to enhanced resistance across all three insect species.

In contrast, reducing sugar content displayed a strong positive association with insect infestation, exhibiting correlation coefficients of 0.976 with shoot fly incidence,

0.832 with stem borer incidence, and 0.807 with fall armyworm infestation. The exceptionally high positive correlation with shoot fly indicates that soluble carbohydrates markedly enhance host suitability by increasing tissue palatability and nutritional quality, thereby promoting oviposition preference, larval establishment, and subsequent feeding activity. Similar positive relationships with stem borer and fall armyworm further suggest that elevated reducing sugar concentrations predispose sorghum genotypes to increased herbivore attack.

Collectively, these results demonstrate a clear biochemical basis of insect resistance in sorghum, whereby increased accumulation of defensive secondary metabolites, particularly phenols and tannins, suppresses insect infestation, whereas elevated concentrations of primary metabolites such as reducing sugars enhance susceptibility. The stronger correlation coefficients observed for phenols relative to tannins suggest that phenolic compounds contribute more substantially to constitutive resistance mechanisms operating against shoot fly, stem borer, and fall armyworm.

Among the biochemical traits evaluated, phenolic content emerged as the most reliable biochemical indicator of insect resistance, followed by tannin content, whereas reducing sugar concentration served as a robust biochemical marker of susceptibility. These relationships explain the superior field performance of resistant genotypes such as AKR-150, NJ-2403, and NJ-2669, which combine elevated phenol and tannin levels with comparatively lower reducing sugar content, thereby minimizing infestation by shoot fly, stem borer, and fall armyworm.

Sorghum genotypes heat map

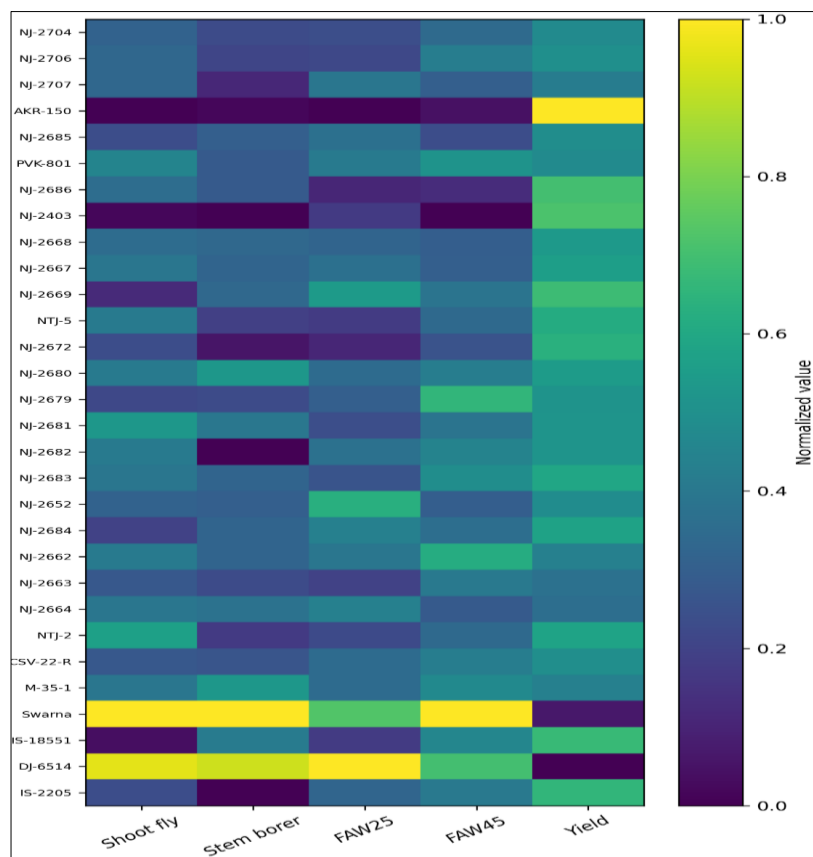


Fig 1: Heatmap illustrating the normalized variation in shoot fly, stem borer, FAW damage (25 and 45 DAS), and yield among different rice genotypes

The hierarchical colour distribution clearly distinguished resistant, moderately resistant, and susceptible genotypes

- **Highly resistant/tolerant genotypes:** akr-150, nj-2403, nj-2669 and nj-2686 exhibited consistently low normalized values for shoot fly, stem borer and FAW while maintaining high grain yield, indicating effective broad-spectrum resistance.
- **Moderately resistant genotypes:** NJ-2704, NJ-2706, NJ-2707, NJ-2668, NJ-2667, NTJ-5, NJ-2672, NJ-2680, NJ-2679, NJ-2681, NJ-2682, NJ-2683, NJ-2652, NJ-2684, NJ-2662, NJ-2663, NJ-2664, NTJ-2, CSV-22-R, M-35-1 and IS-2205 exhibited intermediate pest incidence and moderate yield.
- **Highly susceptible genotypes:** Swarna and DJ-6514 showed the highest normalized insect infestation coupled with poor yield, indicating extreme susceptibility.

The normalized heat map revealed substantial variability among sorghum genotypes for resistance to shoot fly, stem borer and fall armyworm (25 and 45 DAS), together with grain yield. AKR-150 consistently exhibited the lowest normalized values for all insect infestation parameters while recording the highest grain yield, demonstrating superior broad-spectrum resistance. NJ-2403, NJ-2669 and NJ-2686 also clustered among the resistant genotypes, characterized by reduced pest incidence and enhanced productivity. Most experimental entries displayed intermediate normalized values, reflecting moderate resistance. In contrast, Swarna and DJ-6514 exhibited the highest normalized insect infestation accompanied by markedly reduced grain yield, confirming their susceptibility. The inverse association between pest infestation and grain yield observed in the heat map suggests that resistance contributed significantly to yield stability under natural pest pressure. This visualization effectively discriminated resistant and susceptible germplasm and supports the identification of promising donor lines for sorghum resistance breeding.

Discussions

The present investigation demonstrated considerable genetic variability among the evaluated sorghum genotypes for resistance to shoot fly (*Atherigona soccata* Rondani), stem borer (*Chilo partellus* Swinhoe), and fall armyworm (*Spodoptera frugiperda* J.E. Smith) under natural field conditions. The differential response of the genotypes indicates the existence of constitutive resistance mechanisms that can be effectively exploited in sorghum improvement programmes. Among the twenty-six genotypes screened, AKR-150, NJ-2669, and NJ-2403 consistently exhibited lower infestation by all three insect pests while maintaining superior grain yield, suggesting that these genotypes possess broad-spectrum resistance. In contrast, the susceptible check Swarna recorded higher infestation levels and lower grain yield, confirming its susceptibility under field conditions.

The superior performance of resistant genotypes may be attributed to the cumulative effects of morphological and biochemical defense mechanisms that reduce insect colonization, feeding, and larval establishment. Host plant resistance has been recognized as one of the most sustainable components of integrated pest management because it minimizes dependence on chemical insecticides

while providing durable protection against multiple insect pests. Similar observations have been reported by Sharma *et al.* (2003) ^[5], who demonstrated that sorghum genotypes possessing inherent resistance mechanisms significantly reduced shoot fly infestation under field conditions. Likewise, Sharma (1993) ^[4] emphasized that host plant resistance remains one of the most economical and environmentally sustainable strategies for managing sorghum insect pests.

The biochemical analysis further demonstrated that resistant genotypes accumulated significantly higher concentrations of phenols and tannins, whereas the susceptible genotype accumulated greater amounts of total soluble sugars. Among the resistant entries, AKR-150 exhibited the highest phenolic content (7.232 mg g⁻¹), whereas IS-18551 accumulated the highest tannin concentration (0.211%). Conversely, Swarna recorded the highest soluble sugar concentration (5.017%) together with the lowest phenol and tannin contents. These results suggest that resistance is mediated through enhanced accumulation of defensive secondary metabolites coupled with reduced availability of nutritive primary metabolites.

Phenolic compounds are widely recognized as key biochemical determinants of host plant resistance owing to their multiple defensive functions. They reduce insect feeding, inhibit digestive enzymes, interfere with nutrient assimilation, induce oxidative stress in the insect midgut, and impair larval development. The strong accumulation of phenols in resistant genotypes observed in the present study therefore provides a plausible explanation for their reduced insect infestation. Similar findings were reported by War *et al.* (2012) ^[6], who concluded that increased phenolic content enhances plant resistance by reducing herbivore performance and survival. In sorghum, Sharma *et al.* (2003) ^[5] also reported that resistant genotypes contained significantly higher phenolic concentrations than susceptible cultivars.

Tannins exhibited a comparable pattern, with resistant genotypes consistently accumulating greater concentrations than susceptible entries. Tannins function primarily as anti-nutritional compounds that bind dietary proteins and digestive enzymes, thereby reducing protein digestibility and limiting insect growth and development. The higher tannin concentrations observed in AKR-150, NJ-2403, NJ-2669, and IS-18551 therefore likely contributed to reduced feeding efficiency and lower insect establishment. Similar observations have been reported by Bernays and Chapman (1994) ^[1], who demonstrated that condensed tannins reduce insect growth by interfering with digestion, and by War *et al.* (2018) ^[7], who highlighted tannins as important biochemical defenses involved in constitutive insect resistance.

In contrast, total soluble sugars were markedly higher in the susceptible genotype Swarna than in resistant genotypes. Soluble sugars represent readily available energy sources that improve host nutritional quality and increase tissue palatability, thereby enhancing insect feeding preference, oviposition, and larval survival. The inverse relationship observed between soluble sugars and resistance suggests that reduced carbohydrate availability may limit host suitability for phytophagous insects. Comparable findings have been reported by Painter (1951) ^[3] and War *et al.* (2012) ^[6], who proposed that increased concentrations of primary metabolites generally promote herbivore

development, whereas elevated secondary metabolites suppress insect performance.

Correlation analysis provided further evidence for the biochemical basis of resistance. Total phenolic content exhibited an exceptionally strong negative correlation with shoot fly ($r = -0.958$), stem borer ($r = -0.852$), and fall armyworm infestation ($r = -0.825$), indicating that increased phenolic accumulation substantially reduced pest incidence. Likewise, tannin content showed strong negative correlations with all three insect pests, whereas reducing sugars displayed strong positive correlations, particularly with shoot fly infestation ($r = 0.976$). These relationships clearly indicate that secondary metabolites contribute positively to resistance, whereas primary metabolites increase host susceptibility. Similar relationships between phenolic compounds, tannins, and insect resistance have been documented by War *et al.* (2012) [6], Mithöfer and Boland (2012) [2].

The heat map analysis effectively integrated insect incidence and grain yield, allowing simultaneous visualization of resistance patterns among genotypes. Resistant genotypes clustered together owing to their consistently low insect infestation and high productivity, whereas susceptible entries formed a distinct cluster characterized by greater pest incidence and lower grain yield. The clustering pattern demonstrates that insect resistance is closely associated with yield stability under natural infestation, emphasizing the value of multivariate visualization for identifying elite donor lines. Similar applications of hierarchical clustering and heat map analysis have been reported in crop resistance studies for efficient germplasm characterization and selection.

Collectively, the results demonstrate that broad-spectrum insect resistance in sorghum is associated with elevated concentrations of phenolic compounds and tannins together with reduced soluble sugar content. The complementary action of these biochemical constituents appears to constitute an effective constitutive defense mechanism against shoot fly, stem borer, and fall armyworm. Consequently, AKR-150, NJ-2669, and NJ-2403 represent promising sources of resistance that can be utilized in breeding programmes aimed at developing high-yielding insect-resistant sorghum cultivars suitable for sustainable crop production.

Conclusion

The multivariate heat map successfully integrated phenotypic responses to shoot fly, stem borer, fall armyworm, and grain yield, enabling comprehensive discrimination of sorghum genotypes based on their resistance profiles. The clustering pattern identified AKR-150, NJ-2403, NJ-2669, and NJ-2686 as elite genotypes possessing broad-spectrum resistance with minimal pest infestation and superior yield under natural field conditions. Conversely, Swarna and DJ-6514 were consistently associated with high pest pressure and reduced productivity, indicating pronounced susceptibility. The observed negative association between insect infestation and grain yield underscores the importance of host plant resistance in maintaining productivity under biotic stress. These findings demonstrate that heat map-based multivariate analysis is an effective tool for identifying stable resistant germplasm and provides a strong foundation for the selection of parental

lines in resistance breeding and for the development of climate-resilient, insect-resistant sorghum cultivars.

Author's contribution

Author Mr.M. Sivarama Krishna, working as a scientist in AICRP-Cotton entomology had conducted this trial for one season of 2024 at RARS, Nandyal and generated field data for further analysis. Second author helped in statistical analysis of data, third author helped in draft verification fourth author helped in collection of literature for the research article.

Declaration

I do hereby declare that above research article is based of field trial conducted for two seasons at RARS-Nandyala.

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