International Journal of Tropical Insect Science
https://doi.org/10.1007/s42690-024-01383-6

ORIGINAL RESEARCH ARTICLE

Phytochemical profiling of metabolites in commercial sugarcane (*Saccharum officinarum* L.) varieties that confer resistance to feeding of yellow sugarcane aphid (YSA) (*Sipha flava*) by using gas chromatography Mass Spectrometry (GC-MS) metabolomics approach

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Received: 16 July 2024 / Accepted: 21 October 2024
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Abstract
Secondary metabolites serve a variety of ecological purposes, including defense against biotic and abiotic stressors. The aim of this study was to quantify the total phenol and flavonoid contents in sugarcane leaves that mediate resistance to yellow sugarcane aphids (YSA) (*Sipha flava*). A 7 × 2 factorial experiment was conducted in a complete randomized block design (CRBD). Seven sugarcane varieties namely 00-1165, 96-1107, ZN 8, ZN 9, ZN 10, ZN 3 L, and N14 under two aphid treatments [(uninfested (control) and infested] were used. 00-1165 showed medium resistance, as shown by its aphid quantity ratio (AQR), which fell between 0.30 and 0.60. Moreover, ZN 10 is regarded a high sensitive variety because its AQR was more than 1.50. Highly significant ($p < 0.001$) differences were recorded in both uninfested and infested treatments on total phenol and flavonoid content. In the YSA infested plots, 96-1107 recorded the highest phenol content of 50.31 µg/g, while ZN 3 L had the lowest (25.92 µg/g). Furthermore, N14 recorded the highest flavonoid content of 6.47 µg/g, whereas ZN 3 L produced the lowest (1.60 µg/g) in YSA infested plots. Notably, there was a significant positive correlation between the percentage change in phenol concentration and aphid number ($p = 0.002$, $r = 0.58$), and between the percentage change in flavonoid concentration and aphid number ($p < 0.001$, $r = 0.70$). Moreover, the regression results showed a significant positive correlation ($p < 0.001$, $r = 0.70$) between the percentage change in flavonoid concentration and the number of aphids. Feeding by YSA increased phenol and flavonoid induced resistance. There is a need to incorporate sugarcane varieties with high phenol and flavonoid content into the existing YSA management strategies.

Keywords Sugarcane · Resistance · Flavonoids · Phenols · *Sipha flava*

Introduction

Eight African sugarcane producers reported the presence of Yellow Sugarcane Aphids (YSA) (*Sipha flava*) in their localities. In November 2006, the pest was first reported in Morocco in North Africa (Adbelmajid 2008). The insect pest made its way to southern Africa, specifically South Africa in May 2013 (Conlong and Way 2014). It then proceeded to other sugarcane-producing regions, including Zimbabwe, Swaziland, Malawi, and Zambia (Way et al. 2015; Conlong and Way 2014). It was also reported in Kenya in 2016 (Mutonyi and Babikha 2019), and Tanzania in 2019 (January et al. 2020). Along with the well-known Black Maize Beetle and Sugarcane Stalk Borer (*Eldana saccharina*), YSA is currently regarded as a significant sugarcane pest (Zimbabwe Sugar Association Experiment Station (ZSAES), unpublished).

YSA has emerged as a polyphagous insect pest, and its host range includes plants in the genera *Digitaria*, *Panicum*, *Paspalum*, and *Pennisetum*; cultivated cereal crops such as rice (*Oryza sativa*), wheat (*Triticum aestivum*), and

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Published online: 20 November 2024

sorghum (*Sorghum bicolor*); and non-crop members of the same genus (Blackman and Eastop 1984; Way et al. 2015). The host range of Zimbabwe consists of guinea grass, maize, sorghum, and sugarcane (ZSAES). Further investigation is necessary, as there is a chance that the pest's host range will expand as it becomes more entrenched. Cultural, biological, and chemical techniques are available as control strategies for YSA. In Zimbabwe, synthetic chemicals are the primary control method. Currently, Alice (Acetamiprid) and Actara (Thiamethoxam) have been approved for use. It is essential to create an environmentally benign strategy, such as the adoption of host plant resistance (HPRs), given the economic significance of YSA and the environmental concerns associated with employing chemicals to manage aphids. The only management strategy that has been tried thus far to control the YSA has not worked independently. To control YSA, it is crucial to incorporate the development of sugarcane varieties with high levels of secondary metabolites (phytochemicals) as a sustainable option to curb the rapid increase in YSA populations in the sugar industry.

Phytochemicals are secondary metabolites found in plant species and are chemical constituents of plants (Mercy et al. 2017). Although these substances do not hinder plant growth, they make tissues less appetizing to herbivorous insects (Howe and Jander 2008; Ahman et al. 2019). According to Nalam et al. (2021), aphid behavior and performance are affected by primary nutrients, as well as secondary chemicals. The effects of allelochemicals on the function of other organisms can be either beneficial or detrimental (Pejman et al. 2011; Thi et al. 2015; Rawat et al. 2017; Scavo et al. 2019). These substances may be utilized instead of traditional insecticides if they are hazardous to insects (Akbar et al. 2009). Synthetic insecticides are used frequently, such that insects have become resistant to them (Mulungu et al. 2007; Khater et al. 2012; Parwada et al. 2018), hence there is a need for natural substances that have been found to be effective against insect pests (Farooq et al. 2011; Ajayi et al. 2018). According to many researchers, the concept of allelopathy by utilizing host plant resistance has been successfully used to manage insect pests (Hongo and Karel 1986; Saljoqi et al. 2006; Farooq et al. 2011; Zia et al. 2011; Ajayi et al. 2018).

Phenol is the most prevalent compound in the Poaceae family, and several herbivorous insects have been shown to be affected when exposed to it (Kessler and Baldwin 2002; Sharma et al. 2009; Usha Rani and Jyothsna 2010; War et al. 2011b; Ahman et al. 2019). Some studies by Leszczynski et al. (1995), and Kessler and Baldin (2002) have demonstrated that aphid life-table parameters are affected by plants with high phenol concentrations. Additionally, phenols have been shown to have anti-feedant qualities against the cereal aphid (Urbanska et al. 2002). Numerous secondary metabolites, such as phenols, sterols, terpenoids, lignins, and policosanols (Singh et al. 2015) are found in sugarcane, particularly in its juice (Feng et al. 2014; Ali et al. 2019). Godshall and Legendre (1988) documented that sugarcane and its by-products contain phenols. Several authors including Mollyneux et al. (2007); Tinky et al. (2020), and Kerdchan et al. (2020) postulated that secondary metabolites promote development and activation of defensive mechanisms to safeguard plants. Moreover, it has been observed that they also give color, taste, and smell that deter pests. In support of this, War et al. (2012) indicated that these metabolites are constitutively generated by plants or induced in response to an insect attack. This may result in antixenosis (non-preference behavior), which discourages insects from feeding, ovipositing, and hiding on plants (Kogan and Ortman 1978; Smith and Clement 2012; Padmaja 2016; Puri et al. 2023). Additionally, plants may exhibit antibiosis, which has a deleterious effect on the biology of the insect when it feeds on the plant (Painter 1951; Padmaja 2016; Puri et al. 2023). Singh et al. (2015) reported the presence of phenolic compounds such as flavonoids in sugarcane leaves. Furthermore, Colombo et al. (2006) suggested that sugar cane juice contain flavonoids and those produced via the phenylpropanoid pathway, are among the major secondary metabolites (Falcone Ferreyra et al. 2012; Mierziak et al. 2014; Singh et al. 2021) involved in sugarcane defense. A well-known flavonoid called pisatin found in pea (Morkunas et al. 2016) offers protection against the pea aphid (*Acyrthosiphon pisum*). Similarly, resistance against corn leaf aphid (*Rhopalosiphum maidis*) in sorghum was recently shown to be conferred by the flavonoid 3-deoxyanthocyanidin (Kariyat et al. 2019).

The idea that insect damage could alter the phenolic chemicals in sugarcane was first proposed by Akbar et al. (2009). The total phenol content significantly increased when the root-sucking froghopper attacked sugarcane leaves (Silva et al. 2005). Furthermore, feeding by the white pit was found to significantly alter the amount and type of phenols in 15 sugarcane clones. The phenol and flavonoid response of sugarcane varieties to YSA herbivory has not been studied to date. Understanding the secondary metabolite response to YSA feeding on sugarcane would provide basic insights into the defensive mechanisms and resistance responses. According to Paudyal (2019), host plant resistance can assist raise economic thresholds (ETs), thereby eliminating the need for insecticide use. This makes it an efficient and least disruptive form of integrated pest management (IPM). The scientific method of examination, investigation, extraction, and testing by identifying many classes of metabolites present is known as phytochemical profiling (Tinky et al. 2020). Flavonoids in sugarcane leaves, juice, and bagasse were characterized by using a variety of

chromatographic techniques (Colombo et al. 2006, 2008). Therefore, the purpose of this study is to characterize the secondary metabolites in sugarcane (*S. officinarum*) leaves in response to *S. flava* feeding through Gas Chromatography Mass Spectrometry (GC-MS) profiling.

Materials and methods

The study was conducted in 2023/24 season at the Zimbabwe Sugar Association Experiment Station (ZSAES), which is owned by Tongaat Hullets, in the southeast Lowveld of Masvingo Province, in Chiredzi district. The site is situated on a 99 km peg along the Ngundu-Tanganda road. It is found in agro-ecological region V of Zimbabwe, which is characterized by very low and erratic rainfall of less than 500 mm per annum. It is located 430 m above sea level at latitudes of 200 01' S and longitude 280 38' E.

Agronomic practices

Seven different sugarcane varieties (ZN 10, N14, ZN 3 L, ZN 8, 96-1107, ZN 9, and 00-1165) (Sakadzo et al. 2024) were chosen and planted in accordance with the Zimbabwe Sugar Production Manual (ZSPM) (Clowes and Breakwell 1998).

Preparation of plant extract

For this investigation, the total visible dew lap (TVD) leaves of seven sugarcane varieties namely ZN 10, N14, ZN 3 L, ZN 8, 96-1107, ZN 9, and 00-1165 were used. TVD is the most active photosynthetic leaf an indicator of sugarcane productivity. Three months of sugarcane cultivation, as recommended by Rao et al. (2021), was used following a natural aphid infestation. The leaves of YSA-infested and uninfested sugarcane were defoliated from the plant (Fig. 1). Yellowing and purpling of leaves indicates damage symptoms inflicted by YSA in sugarcane. Following the procedure of Sanarat et al. (2021) the leaves were cleaned under running tap water to remove dust, dried in an oven at 60 °C for 18 h, crushed into small pieces, and stored at room temperature in a sealed bag. The crude leaf extract was later extracted by using methanol.

Sample preparation

Following the protocol used by TINNAC laboratories a 1 g of sugarcane leaf sample was weighed into a clean 100 ml Low Actinic Volumetric flask. Fifty milliliters of gradient-grade methanol was added and extracted with ultrasonic maceration for 1 h. Top up to volume with methanol. The mixture was then centrifuged at 1000 rpm for 15 min. The supernatant was collected and the residue was discarded. Concentration portion of the sample was placed in speed vacuum. The sample was reconstituted with gradient-grade chloroform in a 5 ml low actinic volumetric flask. The sample was passed through a solid-phase extraction vacuum station and a reverse-phase octadecyl (C18, 6 mL, 500 mg) column connected to a 3 kDa Amicron filtering device.

Standard concentration

The standard concentration was prepared by combining standard stock (100 ng/ml) diluted in chloroform to 10, 20, 40 and 50 ng/ml.

Fig. 1 YSA uninfested and infested leaves as exhibited by damage symptoms on sugarcane crop. Source (This study)

Sample analysis using Gas Chromatography-Mass Spectrometry (GC-MS) Shimadzu Nexis GC2030, GCMS TQ8040NX triple quadrupole mass spectrometer, HS-20NX mode

Gas chromatography mass spectrometry with electron ionization (Shimadzu Nexis GC2030, GCMS TQ8040NX triple quadrupole mass spectrometer, HS-20NX (Fig. 2) mode was used to examine the phenolic and flavonoid composition of both infested and un-infested sugarcane leaves. Seven sugarcane varieties under two aphid infestations were subjected to analysis by using GC-MS (Shimadzu Nexis GC2030, GCMS TQ8040NX Triple Quadrupole Mass Spectrometer, HS-20NX mode) replicated four times resulting in 56 samples. Following the extraction and filtration process, a split-mode injection port received 1 µl of the sample injected at a ratio of 1:10. Helium Carrier Gas Control at 32.0 cm/sec FR; 1.0 ml/min was used.The column of 30 m long, 0.25 mm internal diameter, and 0.25 µm thick fused in silica capillary column of BR-5MS (5% Diphenyl/95% Dimethyl poly siloxane) was used. The temperature of the oven was raised from 40 °C for two minutes to 160 °C at a rate of 20 °C/min without holding, then increased once more to 280 °C at a rate of 5 °C/min without holding, and finally to 300 °C at a rate of 12 °C/min with an 8-minute hold (Rajendran et al. 2017).

A 275 ^{o}C injector temperature of 275 °C and a GC operating duration of 41 min were observed. The purpose of this final increase was to thoroughly elute the components of the sample from the column and to remove any remaining residue. With an ionization energy of 70 eV, the mass spectrometer was operated in positive electron ionization (EI) mode and a 0–3.0 min solvent delay was observed. Fragments from m/z 50 to 500 kDa were programmed with a scan interval of 0.5 s. The temperature of the filament source used was 250 °C, and the transfer line temperature was set to 280 °C. The individual peak areas were compared with the total peak ion areas to determine the proportion of each component. Analytical work done at TINNAC Scientific Laboratory is thoroughly documented, providing full details of all analyses, including acceptance criteria and actual results, analytical methods and run conditions, chromatograms, and spectral data (if applicable), and analytical method validation and verification data are also available upon request (**Certificate of Analysis (COAs) No: TSL8330ZW**). Customer COAs and reports can be produced according to customer specifications for custom projects. Figure 3 shows a summary of the sample analysis flowchart.

Fig. 2 Shimadzu Nexis GC2030, GCMS TQ8040NX Triple Quadrupole Mass Spectrometer, HS-20NX mode. Source (This study)

Summaried analysis flow chart of secondary metabolites profiling

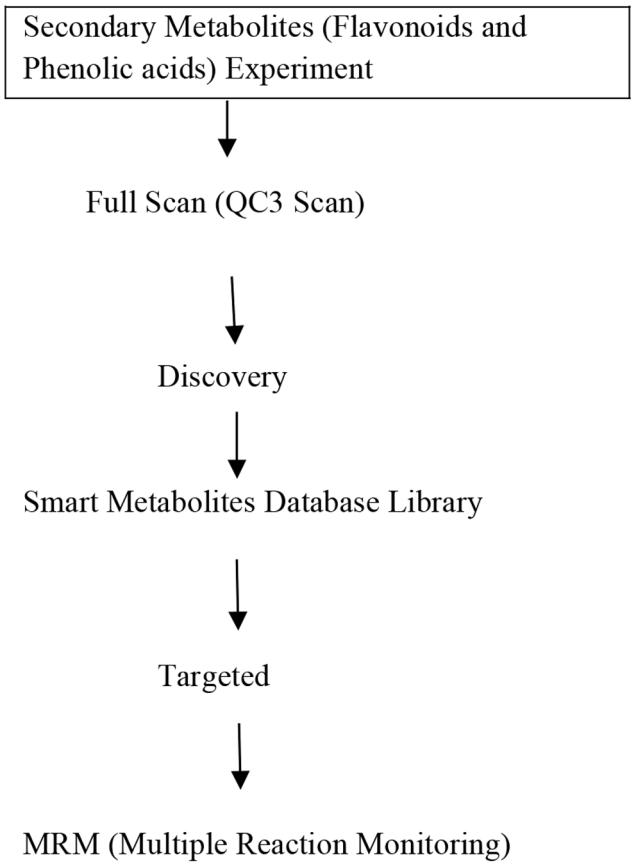

Fig. 3 Analysis flow chart of phenols and flavonoids

Phytochemical screening

The phytochemicals screened in this study included flavonoids, tannins, phenols, terpenoids, saponins, coumarins, and anthraquinones.

Test for flavonoids

The sodium hydroxide (NaOH) test was used to test for flavonoids. In this test, 1 ml of the stock solution was placed in a test tube, and a few drops of 1 M NaOH solution were added. The presence of flavonoids was indicated by the formation of an intense yellow color that disappeared after adding a few drops of 1 M hydrochloric acid (Hossain et al. 2013).

Testing for tannins

A ferric chloride test was performed to test for tannins. A few drops of 5% ferric chloride were added to a test tube containing 1 ml of the stock solution. Wait et al. (2011) inferred the presence of tannins when a greenish, blue-black, or blue-green color is obtained.

Testing for saponins

This was performed by adding 2 ml of distilled water to 2 ml of the extract solution in a test tube. The mixture was shaken in a test tube for 10s. Foam development indicates the presence of saponins (Kumar Bargah 2015).

Testing for coumarins

One milliliter of 10% NaOH (sodium hydroxide) was added to 1 ml of the extract in a test tube. Yellow color indicates the presence of coumarins (Vimalkumar et al. 2014).

Testing for terpenoids

This was performed by mixing 2 ml of chloroform and a few drops of concentrated sulfuric acid, which was later added to 0.5 ml of the extract in a tests tube. Raphael (2012) emphasized that a red-brown color indicates the presence of terpenoids.

Fig. 4 Aphid counting. Source (This study)

Testing for phenols

A combination of 2 ml distilled water and a few drops of 10% ferric chloride were added to 1 ml of the extract. The presence of blue or green indicates the presence of phenols (Gowri and Vasantha 2010).

Testing for anthraquinones

A few drops of 10% ammonia solution were added to 1 ml of the plant extract. Formation of a pink color indicated the presence of anthraquinones (Geetha and Geetha 2014).

Data collection

Aphid number

Aphids were physically counted on all leaves (Fig. 4) of five marked tillers to correlate aphid numbers with phenol and flavonoid contents.

Aphid quantity ratio (AQR)

The Chinese Agricultural Standard was used to determine the AQR (Chen et al. 2007; Xu et al. 2021). At the sampling stages, the number of aphids on the five plants in each treatment was counted. The following formula was used to determine AQR:

$$AQR = \frac{Average\ number\ of\ aphids\ on\ a\ variety}{Average\ number\ of\ aphids\ on\ all\ varieties}$$

Based on AQR, the following scale was used to evaluate aphid resistance (Xu et al. 2021) (Table 1).

Qualitative data on secondary metabolites

Qualitative data were obtained for phenols (P), flavonoids (F), terpenoids (T), saponins (S), coumarins (C), tannins (T), and anthraquinones (A).

Quantitative data on phenols and flavonoids

Total phenolic content (TPC) and total flavonoid content (TFC) were determined from 56 samples by GC-MS by adding the quantified phenols and flavonoids (Table 2). Furthermore, the relationship between aphid number and

Table 1 Aphid resistance scale based on AQR

Level	Description	AQR
1	High resistance	$0.01 < AQR \leq 0.30$
2	Medium resistance	$0.30 < AQR \leq 0.60$
3	Low resistance	$0.60 < AQR \leq 0.90$
4	Low sensitivity	$0.90 < AQR \leq 1.20$
5	Medium sensitivity	$1.20 < AQR \leq 1.50$
6	High sensitivity	$AQR > 1.50$

accumulation of total phenol content and flavonoid content in the seven sugarcane varieties was also determined.

Data analysis

Genstat version 18th edition was used to analyze data on aphid number, total phenol content (TPC), and total flavonoid content (TFC). Two way and one way analysis of variance was used to test the effects of variety and aphid treatment and their interaction (variety × aphid treatment). Separation of means was done by using Fishers Protected Least Significance Difference (LSD) at 5% Significance Level were significant differences ($p < 0.05$) were noted. Regression analysis was used to determine the correlation between the percentage change in total phenol and flavonoid contents and aphid number.

Results

The profiling of secondary metabolites revealed that the leaves of both infested and uninfested sugarcane leaves contained the following: phenols (P), flavonoids (F), terpenoids (T), saponins (S), coumarins (C), tannins (T), and anthraquinones (A) (Table 3). The identified and quantified phenols and flavonoids are listed in Table 2.

Yellow sugarcane aphid number

There was a highly significant interaction ($p < 0.001$) between sugarcane variety and aphid treatment (infested and uninfested) on aphid number. Aphid number showed highly significant differences ($p < 0.001$) among the sugarcane varieties in both the YSA uninfested (control) and infested treatments. The ZN 10 sugarcane variety recorded the highest YSA number (220) while the 00-1165 recorded the lowest (75). In aphid infested plots, ZN 10 recorded the highest YSA number (888) while 00-1165 recorded the lowest (309) (Fig. 5).

Evaluation of yellow sugarcane aphid resistance

There was a significant interaction ($p < 0.001$) between variety and aphid infestation on AQR. The AQR of variety 00-1165 was in the range of 0.30–0.60 indicating that it was a medium resistant variety to YSA stress, while ZN 8 and ZN 9 had low resistance. Furthermore, ZN 3 L and 96-1107 varieties showed low sensitivity. Moreover, N14 was moderately sensitive. Lastly, ZN 10 represents a highly sensitive variety because its AQR was more than 1.50 (Table 4).

Effects of sugarcane variety on total phenol content (TPC)

A highly significant interaction ($p < 0.001$) between sugarcane variety and aphid treatment on total phenol content was recorded. Highly significant differences ($p < 0.001$) in TPC among the sugarcane varieties were observed. The results showed that N14 had the highest phenol content of 35.79 µg/g while ZN 8 had the lowest phenol content of 20.71 µg/g in the YSA uninfested (control) treatment. In the YSA infested plots, 96-1107 sugarcane variety had the highest phenol content of 50.31 µg/g, while ZN 3 L had the lowest phenol content of 25.92 µg/g (Fig. 6).

Effects of sugarcane variety on total flavonoid content (TFC)

There was highly significant interaction ($p < 0.001$) between sugarcane variety and aphid treatment on TFC. Furthermore, highly significant ($p < 0.001$) differences in total flavonoid content were recorded among the sugarcane varieties in both uninfested and infested. 00-1165 sugarcane variety recorded the highest flavonoid content of 2.99 µg/g whereas ZN 9 had the lowest flavonoid content of 1.5 µg/g in the YSA uninfested (control) treatment. In the YSA infested plots, the N14 sugarcane variety had the highest total flavonoid content of 6.47 µg/g, whereas ZN 3 L had the lowest flavonoid content of 1.60 µg/g (Fig. 7).

Relationship between percentage change in phenol content and yellow sugarcane aphid number

The regression analysis showed a highly significant ($p = 0.002$) positive correlation between percent change in phenol content and YSA number (Fig. 8, $Y = 0.15X - 23.6$, $p = 0.002$, $r = 0.58$). An increase in YSA number or feeding stimulates the plant to produce more phenols as a defense strategy thereby causing a positive correlation between the two variables.

Table 2 Identified common phenols and flavonoids in sugarcane leaves amongst the seven sugarcane varieties in YSA uninfested and infested plots

Identified Compound Name	Match Score	Name
(1 S,3R,4R,5R)-3-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy-1,4,5-trihydroxycyclohexane-1-carboxylic acid	99.1	Chlorogenic Acid
(2E)-3-(3,4-dihydroxyphenyl)prop-2-enoic acid	99.36	Caffeic Acid
(2R,3 S,4 S,5 S)-2-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy-3,4,5-trihydroxyhexanedioic acid	99.22	2-O-caffeoylglucarate
1-(4-Hydroxy-3,5-dimethoxyphenyl)ethanone	99.8	Acetosyringone
Feruloyl quinic acid: 1,3,5-trihydroxy-4-[(E)-3-(4-hydroxy-3-	99.4	Feruloylquinic acid isomer 1
methoxyphenyl)prop-2-enoyl]oxycyclohexane-1-carboxylic acid	99.18	Feruloylquinic acid isomer 2
Unknown	98.27	Feruloylquinic acid isomer 3
Unknown	98.24	Feruloylquinic acid isomer 4
3,5-Dimethoxy-4-[(2 S,3R,4 S,5 S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxybenzoic acid	99.24	Glucosyringic acid
2-(4-Hydroxy-3-methoxyphenyl)acetic acid	98.21	Homovanillic acid
(1R,3 S,4 S,5 S)-1,3,4-trihydroxy-5-[(E)-3-(4-	98.36	1 p-Coumaroylquinic acid:
2 hydroxyphenyl)prop-2-enoyl]oxycyclohexane-1-carboxylic acid	99.15	p-Coumaroylquinic acid
3,4-Dihydroxybenzoic acid	97.92	Protocatechuic acid
4-Hydroxy-3-methoxybenzoic acid	98.96	Vanillic acid
4-Hydroxy-3,5-dimethoxybenzoic acid	99.54	Syringic acid
(E)-3-(4-hydroxy-3,5-dimethoxyphenyl)prop-2-enal	99.84	Sinapaldehyde
(2E)-3-(2-hydroxyphenyl)prop-2-enoic acid	97.77	o-Coumaric acid
(2E)-3-(3-hydroxyphenyl)prop-2-enoic acid	99.14	m-Coumaric acid
(2R,3 S)-2-(3,4-Dihydroxyphenyl)-3,4-dihydro-2 H-chromene-3,5,7-triol	98.84	Catechin
5,7-Dihydroxy-2-(3-hydroxy-4-methoxyphenyl)chromen-4-one	99.3	Diosmetin
5,7-Dihydroxy-2-(4-hydroxyphenyl)chromen-4-one	99.09	Apigenin
3-[[6-[2-(3,4-Dihydroxyphenyl)-5,7-dihydroxychromenylium-3-yl]oxy-3,4,5-trihydroxyoxan-2-	98.02	Cyanidin-3-malonyl-glucoside
2-(3,4-Dihydroxyphenyl)-5,7-dihydroxychromen-4-one	98.54	Luteolin
5,7-Dihydroxy-3-(4-hydroxyphenyl)chromen-4-one	99.63	Genistein
[(2R,3 S,4 S,5R,6 S)-6-[2-(3-Ethenyl-5-methoxy-4-methylphenyl)-5,7-dihydroxychromenylium-3-yl]oxy-3,4,5-trihydroxyoxan	99.25	Malvidin-3-caffeoyl-glucoside
5,7-Dihydroxy-2-(4-hydroxyphenyl)-6-[(2 S,3R,4R,5 S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]chromen-4-one	99.74	Isovitexin (apigenin-6-C-glucoside)
2-(3,4-Dihydroxyphenyl)-5-hydroxy-7-[(2 S,3R,4 S,5 S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxychromen-4-one	99.28	Luteolin-7-O-glucoside
3-[[6-[2-(3,4-Dihydroxyphenyl)-5,7-dihydroxychromenylium-3-yl]oxy-3,4,5-trihydroxyoxan-2-yl]methoxy]-3-oxopropanoic acid	99.08	Cyanidin-3-malonyl-glucoside
5-Hydroxy-2-(3-hydroxy-4-methoxyphenyl)-7-[(2 S,3R,4 S,5 S,6R)-3,4,5-trihydroxy-6-	99.73	Diosmin (diosmetin-7-Or-hamonglucoside)
[[(2R,3R,4R,5R,6 S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxymethyl]oxan-2-yl]oxychromen-4-one		
[(2R,3 S,4 S,5R,6 S)-6-[2-(3-Ethenyl-5-methoxy-4-methylphenyl)-5,7-dihydroxychromenylium-3-yl]oxy-	98.48	Malvidin-3-caffeoyl-glucoside
3,4,5-trihydroxyoxan-2-yl]methyl (E)-3-(3,4-dihydroxyphenyl)prop-2-enoate		
2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-6-[4-hydroxy-6-methyl-5-oxo-3-(3,4,5-trihydroxy-6-	99.03	Maysin (luteolin-6-C-diglycoside)
methyloxan-2-yl)oxyoxan-2-yl]chromen-4-one		
(2 S,4 S,5 S)-2-[7-Hydroxy-2-(4-hydroxy-3-methoxyphenyl)-3-[(2 S,5 S)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxychromenylium-5-yl]oxy-6-(hydroxymethyl)oxane-3,4,5-triol	99.5	Peonidin-3,5-diglucoside
6-[(2 S,3R,4 S,5 S,6R)-4,5-Dihydroxy-6-(hydroxymethyl)-3-[(3R,4R,5R,6 S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxyoxan-2-yl]-2-(3,4-dihydroxyphenyl)-5,7-dihydroxychromen-4-one	96.15	Isoorientin 2″-C-rhamnoside
5-Hydroxy-2-(4-hydroxy-3,5-dimethoxyphenyl)-7-[(3R,4 S,5 S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxychromen-4-one	95.11	Tricin-7-O-glucoside
3-O-(6''-succinyl)-rhamnoside	97.54	Petunidin
Vitexin (apigenin-8-C-glucoside) 5,7-Dihydroxy-2-(4-hydroxyphenyl)-8-[(2 S,3R,4R,5 S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]chromen-4-one	99.95	Vitexin (apigenin-8-C-glucoside)

Source This study

Table 3 Secondary metabolites screening of seven sugarcane varieties

Phytochemical screening							
Sugarcane variety	Phenols	Flavonoids	Terpenoids	Saponins	Coumarins	Tannins	Antraquinones
ZN 10	+	+	+	+	+	+	+
ZN 9	+	+	+	+	+	+	+
ZN 8	+	+	+	+	+	+	+
ZN 3 L	+	+	+	+	+	+	+
00-1165	+	+	+	+	+	+	+
96-1107	+	+	+	+	+	+	+
N14	+	+	+	+	+	+	+

+ indicates the presence of the tested: Phenols (P), Flavonoids (F), Terpenoids (T), Saponins (S), Coumarins (C), Tannins (T), Anthraquinones (A)

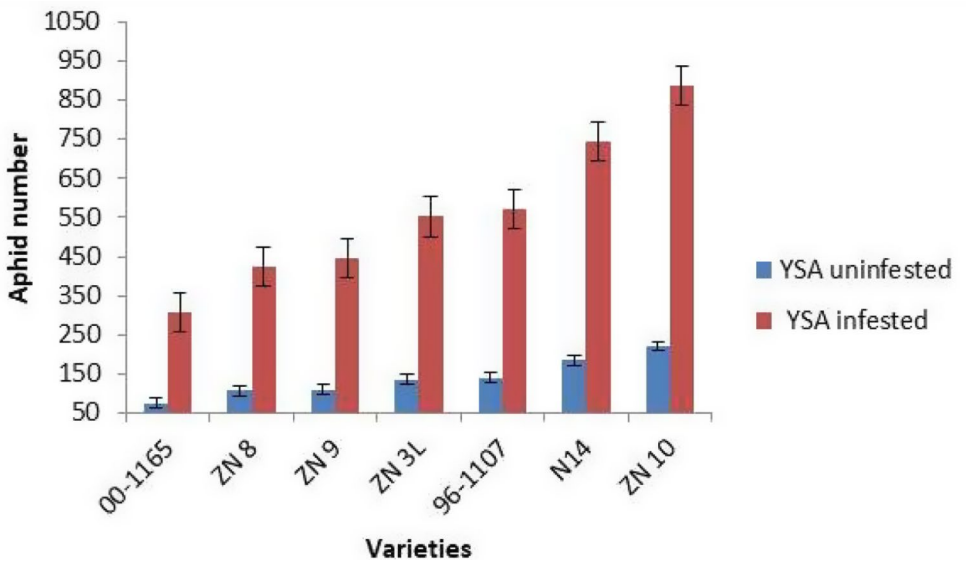

Fig. 5 Yellow sugarcane aphid number in uninfested (control) and infested plots of different sugarcane varieties

Table 4 The AQR and yellow sugarcane aphid resistance levels of different sugarcane varieties

Varieties	AQR	Aphid resistance
00-1165	0.55	Medium resistance
ZN 8	0.76	Low resistance
ZN 9	0.79	Low resistance
ZN 3 L	0.98	Low sensitivity
96-1107	1.02	Low sensitivity
N14	1.32	Medium sensitivity
ZN 10	1.58	High sensitivity

Relationship between percent change in flavonoid content and aphid number

Regression analysis showed a highly significant ($p < 0.001$) positive correlation between percent change in flavonoid content and YSA aphid number (Fig. 9, $Y = 1.17X - 50.2$, $p < 0.001$, $r = 0.70$). An increase in the number resulted in an increase in flavonoid content and served as a defense mechanism against YSA aphid infestation leading to a positive correlation between these two variables.

Discussion

The YSA number of the sugarcane varieties was influenced by secondary metabolites which in turn affected the AQR resistant evaluation. According to the AQR resistance evaluations, 00-1165 sugarcane variety is a medium resistant cultivar to YSA stress, while ZN 8 and ZN 9 are low resistant. Furthermore, ZN 3 L and 96-1107 showed low sensitivity. In addition, N14 is moderately sensitive, while ZN 10 variety is regarded as highly sensitive. This trend of aphid number and AQR might have been influenced by the differences in genetic map in relation to induced defense mediated by YSA. However, similar trend of results were reported by Xu et al. (2021) in wheat under cereal aphid stress on AQR resistant evaluations. Profiling of secondary metabolites in sugarcane showed that sugarcane contains a myriad of secondary metabolites (Tables 3 and 2) which act as anti-feedents and anti-repellents. Presence of secondary metabolites in sugarcane have been reported by a number of authors (Colombo et al. 2005, 2006, 2008; Duarte-Almeida et al. 2007; Feng et al. 2014; Singh et al. 2014; Pinheiro et al. 2017; Rajendran et al. 2017; Koch et al. 2018; Ali et al.

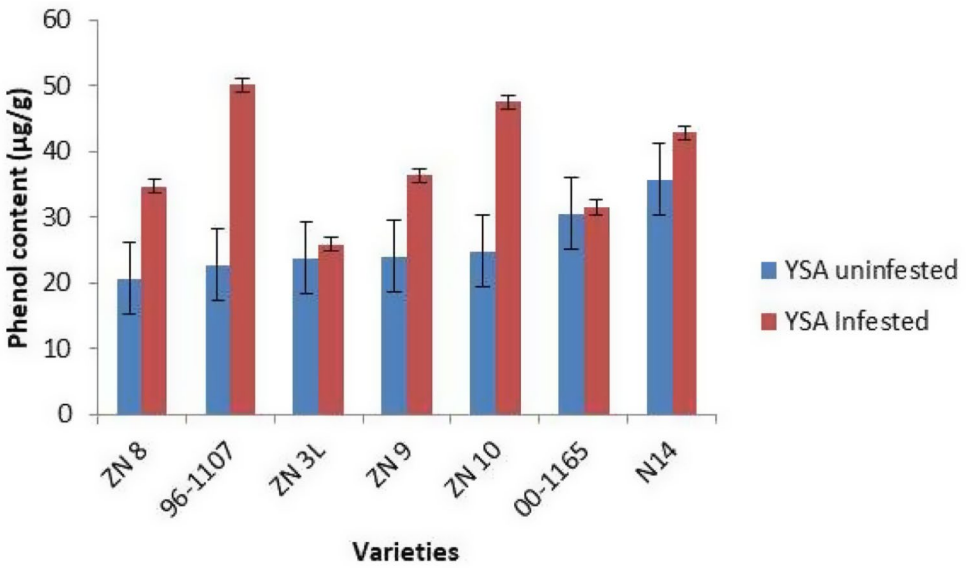

Fig. 6 Phenol content of different sugarcane varieties in yellow sugarcane aphid uninfested and infested plots

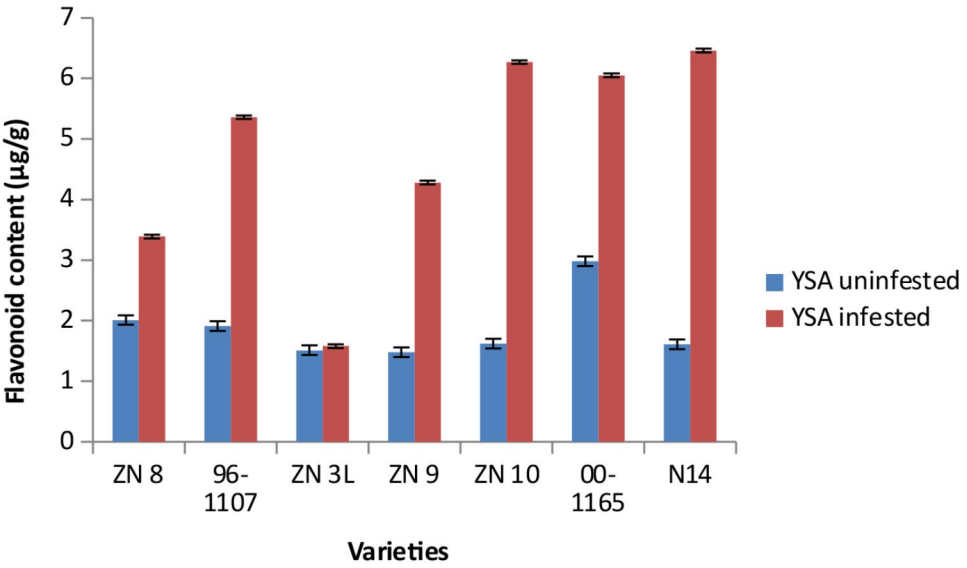

Fig. 7 Flavonoid content of different sugarcane varieties in yellow sugarcane uninfested and infested plots

2019; Salgado 2020; Kerdchan et al. 2020; Ni et al. 2021; Srihanam et al. 2021; Rao et al. 2022; Shafiqa-atikah et al. 2022. The study was restricted to sugarcane crop of ≤3 months which might have influenced the secondary metabolites in sugarcane. This is confirmed by Feng et al. (2014); Kraphankhieo and Srihanam (2016); Naowaset and Srihanam (2017) who suggested that type and location of sugarcane planting affects the secondary metabolite content of the crop. Identification and quantification of flavonoids and phenols was done. It has been observed that accumulation of phenols and flavonoids is part of the defense response of sugarcane to YSA feeding, with the aim of repelling or toxifying aphids. From this study, YSA feeding might have induced the expression of phenol and flavonoid biosynthetic genes, leading to increased production, although the biosynthetic pathway was not explored. Susceptible sugarcane varieties, such as 96-1107, N14, and ZN 10 were able to increase total phenolics and flavonoids in YSA-infested plots when compared to their control plots. In another study, a decrease in the body size of aphids was reported in wheat cultivars with high hydroxamic acid content (Fuentes-Contreras and Niemeyer 1998). In addition, plants generate several secondary metabolites known as phytoanticipins and or phytoalexins (War et al. 2012). These secondary compounds hinder insects' ability to feed continuously on plants (Nalam et al. 2019). In support of this, Malekshah et al. (2022) documented the impact of the physicochemical characteristics of sugarcane varieties on population dynamics of stem borer (*Sesamia nonagrioides*).

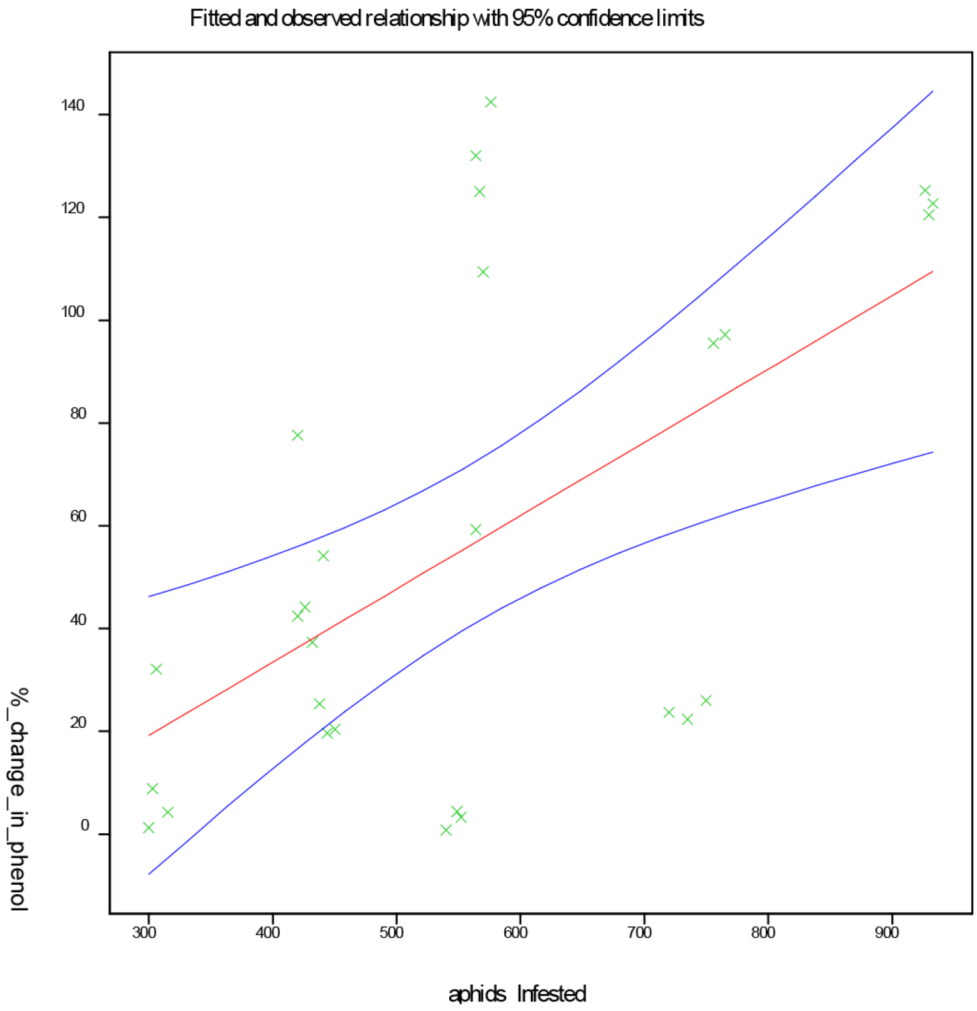

Fig. 8 Relationship between percentage change in phenol content and yellow sugarcane aphid number

In the present study, different sugarcane varieties exhibited varying levels of phenolic and flavonoid accumulation in response to YSA infestation. This significant increase between varieties is confirmed by Zhu et al. (2011). The N14 sugarcane variety seemed to be able to accumulate sufficient phenol and flavonoid content in response to YSA attack. Zhang et al. (2022) reported statistically strong associations between percent change in flavonoid content and aphid number, although a strong positive correlation were reported on both phenols and flavonoids. Moreover, Haviola et al. (2007) found positive correlations similar to this study. Additionally, Zhang et al. (2022) conjectured that correlations between total phenolic compounds and insect performance ranged from negative to zero. Similar results were reported by Green et al. (2003) on pigeonpea (*Cajanus cajan*) varieties' susceptibility to podworms (*Helicoverpa armigera*). Related results were reported by Chen et al. (2003), who indicated that wheat resistance to aphids improved when the total phenol content increased. The total phenol levels in the seven sugarcane cultivars were substantially higher than those in the control plants. Additionally, Chen et al. (2003), Sakihama and Yamasaki (2002), Ramos et al. (2017), and Xu et al. (2021) were in agreement with these findings.

From this study increased phenol is evidence that they are involved in host plant defense. Findings by Akbar (2009) reported increased phenolic content in response to aphid feeding in sugarcane. These results concur with the findings of Silva et al. (2005), who reported an increased phenol in response to root-sucking froghopper (*Mahanarva fimbriolata* Stal.). In support of this, insect herbivore have been reported to frequently cause changes in phenol and flavonoid levels in plants (Treutter 2007; Zhang et al. 2017; Wang et al. 2019). Higher phenol concentrations in plants have been shown to deter pests (Zhang et al. 2022). In addition, it was discovered that phenol content increased in 15 sugarcane clones that attacked by the white pit (*Antitrogus parvulus*) (Silva et al. 2005). Of the seven sugarcane accessions, 96-1107 and N14 displayed high total phenolic and flavonoid content, respectively. Furthermore, the susceptible varieties (96-1107, N14, and ZN 10) exhibited yellowish and purplish leaf color. These findings corroborate

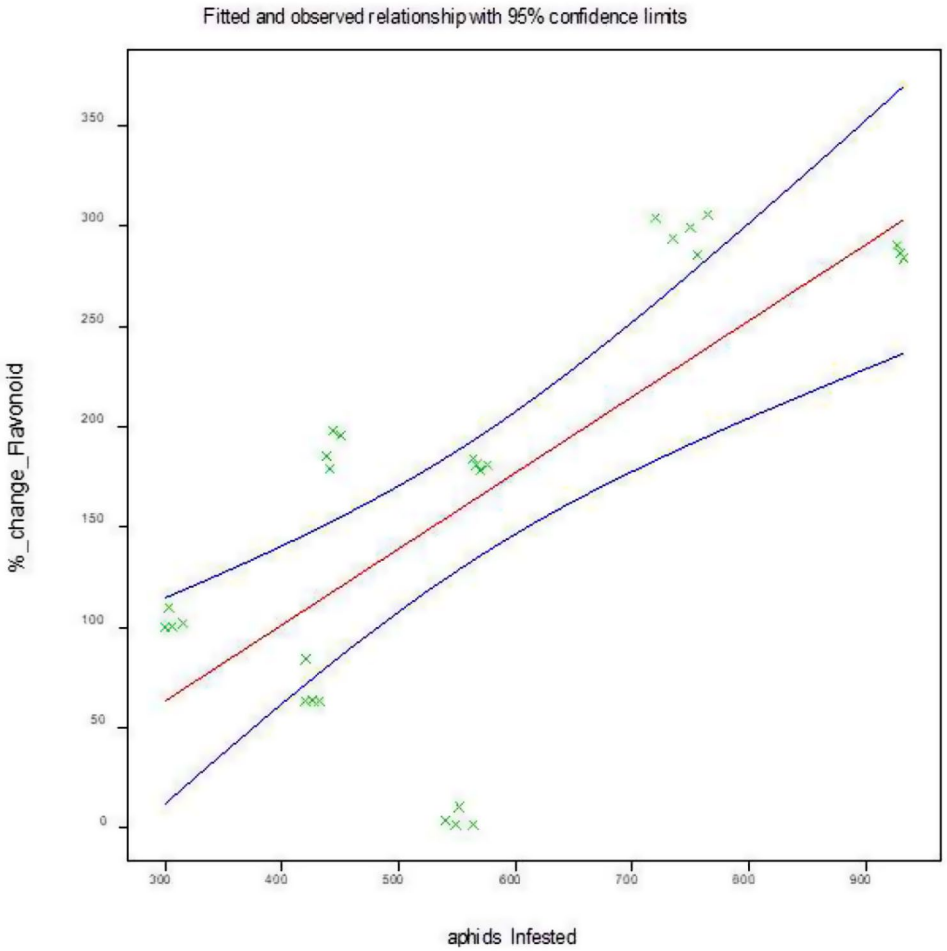

Fig. 9 Relationship between percent change in flavonoid content and yellow sugarcane aphid number

those of Haile et al. (1999) and Goławska et al. (2010), who observed that lower levels of chlorophyll in susceptible varieties may be linked to higher synthesis of defensive secondary metabolites. During feeding, aphids secrete phytotoxins that disrupt physiology and activate defense mechanisms (Botha et al. 2006; Smith et al. 2010). The leaf chloroplasts of aphid-infested plants can be broken down by enzymes in aphid saliva, resulting in longitudinal streaks that are white, yellow, purple, or reddish-purple on the leaves (Fouché et al. 1984; Pike and Allison 1991; Ma et al. 1998; Liu et al. 2020), although enzymes were not measured in this study.

The ability of sugarcane varieties to withstand the damaging effects of reactive oxygen species (ROS) caused by aphid infestation depends on their antioxidant capacity. Moreover, plants have evolved antioxidant systems to counteract the harmful effects of ROS. This mechanism eliminates excess ROS from the cell and shields the plant from oxidative damage (Shankar and Yinghua 2021). Smith and clement (2012) discovered that shikimate kinase was elevated in wheat infested by Russian wheat aphid. Therefore, the study findings suggest that the YSA infestation in our study could have triggered the shikimate route, which could subsequently trigger the creation of secondary metabolites via the plant defense mechanism pathway, thereby increasing the resistance of infested sugarcane types to aphids. However, the ROS and the shikimate route were not investigated by this study.

The increased phenolic and flavonoid contents in sugarcane leaves may correlate with enhanced antioxidant activity, helping to counteract the oxidative stress caused by YSA infestation. Elevated accumulation of ROS can also result in a defense response in the host plant, mediated by SARS-induced systemic acquired immunity (Wu et al. 1997; Cao et al. 1998; Zhang et al. 1999; Asada et al. 2006). Plants that experience ROS suppression are more vulnerable to aphid attacks, whereas ROS accumulation may result in aphid resistance. This concurs with the findings by Shoala et al. (2018). Such trend of results might be a similar case happening in YSA-resistant (00-1165) and moderately resistant (ZN 8, ZN 9, and ZN 3 L) sugarcane varieties. According to Shankar and Yinghua (2021), saliva is known to contain a variety of toxic chemicals that cause plants to perceive invasion by aphids. This may worsen the build-up of ROS, a precursor to oxidative stress. This condition ultimately

results in cell death (Mittler 2002; Gechev 2006; Shankar and Yinghua 2021). The resistant sugarcane genotype (00-1165) may have evolved as an antioxidant mechanism to combat the negative effects of ROS and to protect the plant from oxidative damage by removing excess ROS from the cell, which triggers the production of secondary metabolites. Moreover, resistant sugarcane varieties might have a regulatory antioxidant gene that regulates the toxic effects of the absence of ROS from susceptible sugarcane varieties.

The action of ROS scavenging enzymes neutralizes excessively generated ROS molecules to prevent oxidative damage that otherwise could result in needless cell death and maintain ROS homeostasis in plants (Ye et al. 2021). The resistant variety (00-1165) in this study might have had a higher concentration of the aforementioned detoxifying enzymes that regulated the exhibition of aphid-infested symptoms compared to susceptible varieties. These enzymes are referred to as detoxifying enzymes (Das and Roychoudhury 2014; Puri 2023) although they were not measured in this study.

In addition to being potent antioxidants, flavonoids play a variety of functions in plants, including defense against pathogens, resistance against insect pests and squelching of free radicals (Solovchenko 2010; Pourcel et al. 2007). Studies on a wide range of plant species have found that plants exhibit high levels of flavonoid content (Pieta 2000; Rao et al. 2019, 2020). A high concentration of antioxidant and flavonoids is generally linked with tolerance to biotic and abiotic stresses (Rao et al. 2020, 2021). Therefore, herbivores are less drawn to plant species with greater secondary metabolite levels (Rao et al. 2018, 2021).

In a nutshell, the study's findings showed that sugarcane leaves contain a wide variety of secondary metabolites, including anthraquinones, phenols, flavonoids, terpenoids, saponins, coumarins, and tannins (Singh et al. 2015; Sanarat et al. 2021). This is confirmed by Puri et al. (2023), who postulated that many secondary metabolites are produced for defense against invaders. War et al. (2012) stressed that these metabolites are constitutively or induced by pest attack. Phenols, flavonoids, and tannins exhibit antixenosis (Kogan and Ortman 1978; Smith and Clement 2012; War et al. 2012; Padmaja 2016; Puri et al. 2023) and antibiosis properties (Painter 1951; Padmaja 2016). This study highlights the importance of phenols and flavonoids in sugarcane defense against YSA, and suggests potential targets for breeding resistant varieties.

Conclusion

Secondary metabolites in sugarcane mediate resistance to YSA feeding. AQR evaluate level of resistance of sugarcane varieties in response to YSA incursion. The tested sugarcane varieties showed increased phenolic and flavonoid content in response to YSA infestation.

Acknowledgements We are indebted to Zimbabwe Sugar Association Experiment Station and sugar industry for providing resources during the experiment.

Author contributions Sakadzo, Nyasha: conceptualization; experimental design; data collection, analysis and interpretation; writing – original draft; writing and editing. Dr Mubvuma, Michael: supervision – review of content and editing. Ms Mukanga, Concilia: supervision; funding acquisition; writing - review and editing: Dr Mabveni, Audrey. R.S: supervision; funding acquisition. Prof Musundire, Robert: supervision - review and editing; revising critically for intellectual content: final approval of the version to be published.

Funding This research received no external funding.

Data availability The data that support the results of this study are in the manuscript and available on request from the corresponding author.

Declarations

Conflict of interest Authors declare no potential conflict of interest.

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