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Research article

Jasmonic acid and salicylic acid induced defensive response in wine grapes against *Drosophila suzukii* (Diptera: Drosophilidae)

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ABSTRACT

To better understand the elicitor induced defense in wine grapes against *Drosophila suzukii* (Matsumura), two varieties, Pinot Noir and Chardonnay, were sprayed with jasmonic acid (JA) and salicylic acid (SA). Total phenols, total flavonoids, total tannins and total soluble sugars were quantified. Oviposition preference by *D. suzukii* in response to JA and SA applications were also studied. Behavioral response of *D. suzukii* to various sugars was recorded. The effect of flavonoids (gallic acid, catechin, quercetin at 100 and 500 ppm) on mortality of *D. suzukii* was also studied in CAFE assay. Our results showed that JA and SA application significantly affected phenol, flavonoid and tannin contents of grapes. Reduced injury was observed in treated plants; this reduction was more pronounced in Chardonnay than Pinot Noir. The number of eggs laid by *D. suzukii* females was lower in JA and SA-treated plants; this reduction was more pronounced under no-choice conditions than under choice conditions. In prandial behavior, sucrose solution (5%), glucose solution (5%), fructose solution (5%), sucrose (5%) + yeast (5%) solutions and yeast solutions (5%) showed higher attraction of *D. suzukii* females compared to control treatments. Among flavonoids, catechin (100 ppm) showed higher mortality of *D. suzukii* than rest of the treatments. The results of this study can be used for developing management strategies of *D. suzukii* in wine grapes and related crops.

1. Introduction

Pomace flies are important pests of soft fruits and vegetables globally [1,2]. Strict quarantine regulations avoid the transfer of these pests geographically through export, however, their cryptic life cycle and their evolutionary tendency to start their progeny inside the fruits enables these insects to escape quarantine treatments/regulations [3–5]. The trade between countries and regions leads to their introduction to new geographic areas [6,7]. Drosophilid fruit flies are considered an important model organism to identify cures to many biological problems [8]. *D. suzukii* is commonly known as spotted-wing drosophila (SWD) due to the presence of a black spot on the anterior margin of wings in males. *D. suzukii* is a pest endemic to South Asia [9,10], in Europe and the United States [11]. The *D. suzukii* females pierce thin-skinned and overripe fruits with serrated ovipositor [9,12,13].

Drosophila suzukii has emerged as a major pest of wine grapes [14–16]. It causes a loss of over 314.3 million dollars is soft fruits in

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USA [1,17]. In addition to the physical damage, the larva remains inside the grapes, introducing microorganisms that cause sour rot in grapes, thereby, impacting the quality of the juice extracted from the grapes. Sorting fruits incurs additional handling costs. To our knowledge, the effective and sustainable pest management strategies are either not available for *D. suzukii* or are still in the developmental stage, and therefore, the management of this pest still relies heavily on chemical control. In the vineyard ecosystem, insecticides recommended for *D. suzukii* can be disruptive to the populations of beneficial arthropods [1,18,19].

The discovery of innovative bioinsecticides, which include microorganisms, animals, or plants that are naturally occurring, represents a promising new field in the organic chemical treatment of SWD pests. As an alternative to traditional chemical pesticides, bioinsecticides may pose less of a threat to human and environmental health, have a higher host specificity, and even be biodegradable [20]. Some of the natural-product chemistry, including both microbial-derived and plant-derived sources have been found effective against SWD [21,22]. For example, peppermint oils have been used as a repellent against *D. suzukii* under laboratory conditions [23]. Additionally, Actinidia extracts have shown oviposition deterrence towards SWD under laboratory conditions [20]. Jasmonic acid (JA) and salicylic acid (SA) are two potential molecules for induced resistance in plants against various insects [24,25]. Synthesis of JA is induced upon wounding and regulates many physiological and biochemical processes involved in direct and indirect defenses [26–28]. SA signals the oxidative stress response in plants against insects, which produces various antioxidative enzymes and other molecules that alter insect physiology [25,28], and this phenolic compound is an important secondary metabolite in grape berries promoting berry quality under stress conditions [29]. Pre- and post-harvest application of SA has been reported to increase the physical quality of grapes [30,31]. To control *D. suzukii*, there is a need for developing environmentally friendly strategies such as plant-based insecticides that are easily degradable and do not leave residues on the crop. Plants produce several secondary metabolites, which are important for defense against various stresses including insect herbivory [23,32].

D. suzukii is a major pest of grape vines in Virginia. No effective and sustainable pest management strategies are available for *D. suzukii* and the management of this pest relies heavily on chemical control. Thus, it is important to develop an efficient, eco-friendly pest management strategy against *D. suzukii*. Host plant resistance is considered an environmentally friendly and sustainable pest management strategy against insect pests. Host plant resistance can be constitutive (present naturally in the plants) or induced (by external agents such as herbivory or by the application of elicitors). Inducing resistance to insect pests is an environmentally selective plant defense strategy that allows them to either avoid and/or withstand insect damage by increasing toxic secondary chemicals [24, 28,33]. JA and SA are plant signaling molecules, which play an important role in plant defense against insect pests and pathogens. The plant signaling pathways mediated by JA and SA include octadecanoid and phenylpropanoid pathways, respectively [26,33]. *D. suzukii* feeds on fresh fruits and has emerged as a potential threat to grapes [14,16].

Our hypothesis is that the exogenous application of JA and SA induces plant metabolites in wine grapes that provide resistance against *D. suzukii*. Subsequently, the study reveals the defensive response of grapevines to two signaling molecules and infestation by *D. suzukii*. The study further focused on the quantification of the major defensive compounds (total phenol, total tannin and total flavonoid) contents in grape varieties in response to applied JA and SA, and the effect of these two compounds on oviposition and feeding by *D. suzukii*.

2. Materials and methods

2.1. Chemicals

Salicylic acid, jasmonic acid, gallic acid, quercetin, tannic acid, hydrogen peroxide, catechol, disodium hydrogen phosphate, and sodium dihydrogen phosphate were purchased from Sigma Aldrich, USA. Analytical grade chemicals were used in this study.

2.2. Insect rearing

The adults of *D. suzukii* were collected from grape wine fields of Virginia and reared on an artificial diet as per Dalton et al. [34], in the Department of Entomology, Virginia Tech, Blacksburg VA, USA. The diet consisted of agar (45 g), cornmeal (125 g), sugar (200 g) and nutritional yeast (70 g) mixed in 1 L of dH2O at room temperature. 2.8 L of boiling dH2O and the slurry were combined, and the resulting liquid was simmered at a gentle boil for 15 min while being constantly stirred. Propionic acid (17.7 ml at 1 M concentration) and ethanol (33.3 ml at 95% purity) were added when the mixture had cooled to 63 °C. Once sterile petri dishes and vials had been filled, the diet mixture was sealed with Parafilm (Pechiney Plastic Packaging, Inc., Chicago, IL), and stored at 4 °C. When the diet in the treatment cups began to dry out or degrade due to microbes, it was replaced. Cotton swabs were rehydrated with dH2O as required to keep the cups moist enough to avoid desiccation. The laboratory colony of *D. suzukii* was maintained in BOD (27 ± 1 °C, 75 ± 2% RH and 14 L: 10D h photoperiod). Newly mated females were used for all behavioral assays.

2.3. Field experimentation at vineyard

2.3.1. Study sites

The experiment was conducted at Ankida Ridge Vineyard, located in Bedford County, Virginia (37°42′ N x 79°10′W). The planting areas of the vineyards used for studies were Pinot Noir (red 0.56 ha) and Chardonnay (white- 0.7 ha). The plants were 7 years old, and the spacing between vines was 6–10 feet. Standard vineyard management and plant protection strategies were followed.

2.3.2. Treatments and sample collection

Three treatments for each variety with three replications per treatment were maintained in a randomly complete block design. To prepare 0.1 mM jasmonic acid (JA), the required quantity was dissolved in methanol (95%) and then diluted in water to prepare the desired concentration. Salicylic acid (SA) 1 mM concentration was prepared by dissolving SA in ethanol (95%) and diluting in water to a final concentration of 1 mM with water. The Pinot Noir vines were sprayed on August 4, 2015 and Chardonnay vines on August 11, 2015, using a CO_2-powered backpack sprayer equipped with an 8008 stainless steel spray tip at 40 psi. The plants were sprayed until run off. Untreated plants were maintained as a control. The plants were maintained at the spacing between vines and rows of 0.9×1.8 m. Both the varieties were harvested 21 days after spraying with JA and SA. The Pinot Noir variety was harvested on August 25, 2015 and Chardonnay variety was harvested on September 1, 2015. From each replicate/plant, two-three berries were harvested randomly from each of six clusters, placed together in 50 ml centrifuge tubes and immediately transferred to a to dry ice. The samples were then brought to the laboratory and stored at -80 °C. The samples were dried in a freeze dryer. The freeze-dried berries (500 mg) were subsequently ground in a pre-chilled mortar and pestle in 10 ml of ice-cold ethanol (80%). The extracts were transferred to a 15 ml tube. After centrifugation at $10{,}000 \times g$ for 10 min, the supernatant was collected in a 15 ml tube and the pellet was dissolved in 5 ml of ethanol (80%). The sample was kept for 24 h in a dark cold room. The sample was again centrifuged. The supernatant was taken in a 50 ml tube and diluted in 20 ml of ethanol and used to estimate the total phenol, total tannin and total flavonoid contents using a double beam spectrophotometer (ThermoFisher Biomate 35).

2.4. Estimation of phenols, flavonoids, tannin and sugar content

2.4.1. Total phenol content

Folin-Ciocalteu reagent (FCR) procedure was followed to determine phenol content. A 0.5 ml of FCR reagent was added to berry extract (0.5 ml). After incubation for 5 min, sodium carbonate (12 ml, 7.5 w/v) was added to the reaction mixture. The solution was vortexed and allowed to stand for 30 min. The absorbance was read at 760 nm. Gallic acid in methanol (80%) was used for calibrating the standard curve and the results were expressed as μg gallic acid equivalents g^{-1} DW (μg GAE g^{-1} DW).

2.4.2. Total flavonoid content

An aluminum chloride assay was followed to determine the total flavonoid content. To 0.5 ml of blueberry extract, 0.3 ml of $NaNO_2$ (5% w/v) was added and allowed to stand for 6 min. To this solution, 0.3 ml $AlCl_3$ (10%) was added. The solution was incubated for 5 min and 2 ml 1 M NaOH was added to it. The volume of the reaction mixture was made up to 10 ml with distilled water. The solution was vortexed, and the absorbance was read at 510 nm. Rutin was used as a standard for the total flavonoid content in wine grapes and the total flavonoid content was expressed as μg rutin equivalents g^{-1} DW (μg RE g^{-1} DW) on a dry weight basis.

2.4.3. Total tannin content

The vanillin-HCl method as described by Burns [35], with slight modifications was used to determine the tannin content from berry extract. Extracts (19 ml) were added to freshly prepared vanillin-HCl reagent (2.5 ml), which was prepared from 8% HCl in methanol and 4% vanillin in methanol. Both the components were of equal volumes and the solutions were mixed immediately before use. After incubation at room temperature for 20 min, the absorbance was read at 500 nm. The reaction mixture containing the reagent alone was used as a blank. Catechin diluted in methanol (80%) was used as the standard. The condensed tannin content was expressed as μg tannic acid equivalents g^{-1} DW (μg CE g^{-1} DW) on a dry weight basis.

2.4.4. Total soluble sugars

Immediately after harvesting to avoid transpiration loss, total soluble sugars (°Brix) were measured by a digital temperature-compensated refractometer (Atago, Japan) of treated (SA and JA sprayed) and untreated berries of both varieties. The treated plants of both these varieties were sprayed with jasmonic and salicylic acid at 1 mM concentration.

2.5. Fruit damage

At the time of harvesting, three grape clusters/replicate from each treatment from both varieties were randomly collected in field in disposable plastic containers and brought to the laboratory. After careful examination of berry clusters under a dissecting microscope (Wild M5), infested berries were excised from the rachis, counted and weighed on the scale to determine fruit injury from each treatment in both the varieties. The fruit damage was observed on numerical basis and on weight basis from these grape clusters.

2.6. Laboratory experiments

2.6.1. Oviposition preference assay

Three concentrations of salicylic acid (1.0, 2.0, 5.0 mM) were uniformly applied on a fly diet by using a handheld glass atomizer. The fly diet of 3 cm thickness was uniformly spread on the glass slides. The untreated fly diet was sprayed with distilled water was used as a control. Three replications were maintained for each treatment. Salicylic acid-treated glass slides, containing fly diet and a control were placed into these oviposition cups and five newly mated females of *D. suzukii* were released into each cup for 48 h for oviposition under choice and no choice conditions. The number of eggs laid on the fly diet on glued glass slides were counted under the stereomicroscope.

2.6.2. Olfactometer bioassays with analytical carbohydrates

The behavioral response of *D. suzukii* adults toward a sucrose solution (5% wt/vol) was studied using Y- tube olfactometer according to Damodaram et al. [36]. The treatments used were (i) sucrose solution 5% (wt/vol), (ii) glucose solution 5% (wt/vol), (iii) fructose solution 5% (wt/vol), (iv) sucrose 5% (wt/vol) + yeast 5% (wt/vol) solution and (v) yeast solution 5% (wt/vol). The known concentration of each test sample was made by dissolving in distilled water. The filter paper strips sprayed with the tested solutions were placed in treated side arm toward the end in Y-olfactometer. Filter paper strips with distilled water (30 μl) served as a control. Five adult females of *D. suzukii,* starved for 10–12 h, were released in the base arm of the olfactometer. The experiment was replicated 20 times. The observation was taken until the adults made a decision/no decision into arms of the respective treated samples in 10 min.

2.6.3. Effect of flavonoids on adult D. suzukii

The mortality of *D. suzukii* by exposure to flavonoids was determined using CAFE assay according to William et al. [37] with slight modifications. Adults of *D. suzukii* were fed with quercetin, gallic acid and catechin-incorporated diet. These flavonoids were weighed and mixed with the sucrose solution 5% (wt/vol) just after its preparation. The sucrose solution % (wt/vol) mixed with correct dosages of flavonoids in each treatment was loaded in the capillaries with a micropipette and monitored daily to avoid air bubbles in the capillaries and the top end of the capillaries were closed with three layers of parafilm. Five adults of *D. suzukii* were introduced in each replicate/treatment with 100 and 500 ppm of each flavonoid. Each treatment was represented in three replicates. The control consisted of adults fed on sucrose solution (5%) alone. After 5 days after treatment (DAT), adult mortality was recorded.

3. Statistical analysis

The experimental design followed was randomized complete block design (RCBD). The data were analyzed by a two-way analysis of variance (ANOVA). The varieties, treatments, and their interaction as fixed effects and the replication was taken as a random effect in SPSS v15.1 (SPSS, Inc., Chicago, IL, USA). The means were separated by a Tukey's test in statistically significant treatments. Percentage data of damage, mortality and response were subjected to normalization by arcsine square-root transformation. The A Chi-Square was used to analyze the olfactory attraction of *D. suzukii* in Capillary Feeder (CAFE) in response to various solutions. An alpha value of 0.05 was used to determine significance.

4. Results

4.1. Total phenol content

JA and SA-treated plants exhibited greater levels of phenol (186.16 and 185.36 μg/g DW), and control plants showed 184.33 μg/g DW of total phenol content for Pinot Noir variety ($F_{(2,14)} = 281.72$, $P \leq 0.0001$) (Fig. 1). Among both wine grape varieties, untreated control plants exhibited the lowest phenol content (184.33 and 180.56 μg/g for Pinot Noir and Chardonnay, respectively). The treated and untreated control vines exhibited significant differences in total phenol content ($F_{(1,8)} = 1246.15$, $P \leq 0.0001$) in both wine grape varieties. The interaction effects between variety x treatment (VxT) were also significantly different ($F_{(4,17)} = 138.47$, $P \leq 0.0001$).

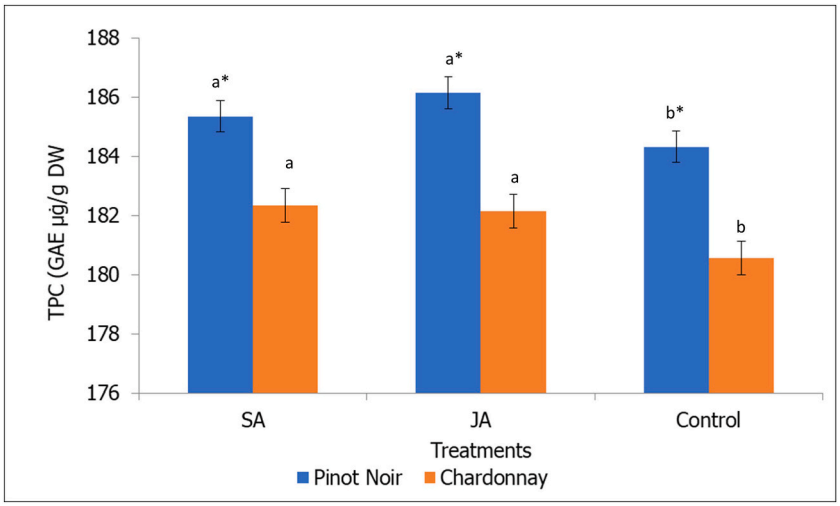

Fig. 1. Total phenol content (GAE μg/g DW) of grape berries of Pinot Noir and Chardonnay varieties treated with jasmonic acid (JA) and salicylic acid (SA). All values are given as Mean ± SE. Bars with asterisk* within a treatment show that the two varieties differ significantly. Bars with similar letters of the same variety do not differ significantly at P ≤ 0.05 (Tukey's HSD test).

4.2. Total flavonoid content

Both Pinot Noir and Chardonnay wine grape cultivars responded to applied JA and SA with changes in their total flavonoid content. The SA-treated Pinot Noir vines showed significantly higher flavonoid content (217.66 μg/g DW) followed by vines treated with JA (192.66 μg/g DW) ($F_{(1,8)} = 33.49$, $P \leq 0.05$) (Fig. 2). Chardonnay vines exhibited the highest flavonoid content (104.33 μg/g DW) when treated with JA followed by SA (97.66 μg/g DW), respectively ($F_{(1,8)} = 59.27$, $P \leq 0.05$). In both Chardonnay and Pinot Noir, unsprayed control vines contained the lowest flavonoid content (111.0 and 82.86 μg/g DW in Pinot Noir and Chardonnay, respectively. While comparing the flavonoid content among the treatments between the two wine grape varieties, Pinot Noir contained significantly higher levels of flavonoid than Chardonnay in all treatments ($F_{(2,14)} = 33.49$, $P \leq 0.0001$). The interaction effects between variety x treatment (VxT) were significantly different ($F_{(4,17)} = 18.80$, $P \leq 0.0001$).

4.3. Total tannin content

Both Chardonnay and Pinot Noir contained significantly higher levels of condensed tannins in treated plants compared to control plants ($F_{(1,8)} = 477.16$, $P \leq 0.05$) (Fig. 3). Untreated control vines in both cultivars contained the lowest levels of tannins (15.6 and 9.36 μg CE g^{-1} DW for Pinot Noir and Chardonnay, respectively). The condensed tannin content of SA-treated vines showed a significant difference in both cultivars ($F_{(2,14)} = 42.67$, $P \leq 0.05$). In JA-treated vines, condensed tannin content did not differ significantly between Chardonnay and Pinot Noir ($P > 0.05$). The interaction effects between variety x treatment (VxT) were not significantly different ($F_{(4,17)} = 2.34$, $P = 0.138$).

4.4. Effect of jasmonic acid and salicylic acid on total soluble sugars (°Brix)

Significantly higher total soluble sugars (°Brix) content was detected in SA-treated berries in both cultivars (22.92 and 22.72 °Brix) for Pinot Noir and Chardonnay, respectively (Fig. 4). Minimal levels of total soluble sugars were detected in untreated plants in both Chardonnay and Pinot Noir. No significant differences were recorded in total soluble sugars between the two cultivars in any treatments ($F_{(2,14)} = 35.8$,$P \leq 0.05$).

4.5. Effect of jasmonic acid and salicylic acid on berry damage

The treatments showed a significant effect on fruit injury compared to the control ($F_{(2,14)} = 2.504$, $P \geq 0.05$). For Pinot Noir grapes, fruit injury on weight/number basis was highest in unsprayed plants compared to treated plants, spraying with SA and JA ($F_{(1,8)} = 16.34$, $P \leq 0.05$) (Fig. 5). The same trend was observed on number/weight basis for the fruits injured by *D. suzukii* in Chardonnay grapes. The interaction effects were not significantly different.

Effect of different concentrations of salicylic acid on fly diet for oviposition preference *by D. suzukii* in choice and no-choice tests.

In choice tests, the number of eggs laid on the fly diet sprayed with 1, 2 and 5-mM concentrations of SA was significant in different treatments compared to the control ($\chi^2 = 30.88$, df = 3, $P < 0.001$). The lowest number of eggs was observed in 5 mM SA treatment followed by 2 mM and 1 mM SA treatments, respectively. The control treatment recorded the highest number of eggs laid by adult females of *D. suzukii* compared to SA-treated fly diet (Fig. 6). In no-choice tests, the number of eggs laid on the fly diet treated with different concentrations was statistically significant compared to the control treatment ($\chi^2 = 15.80$, df = 3, $P < 0.001$) (Fig. 6).

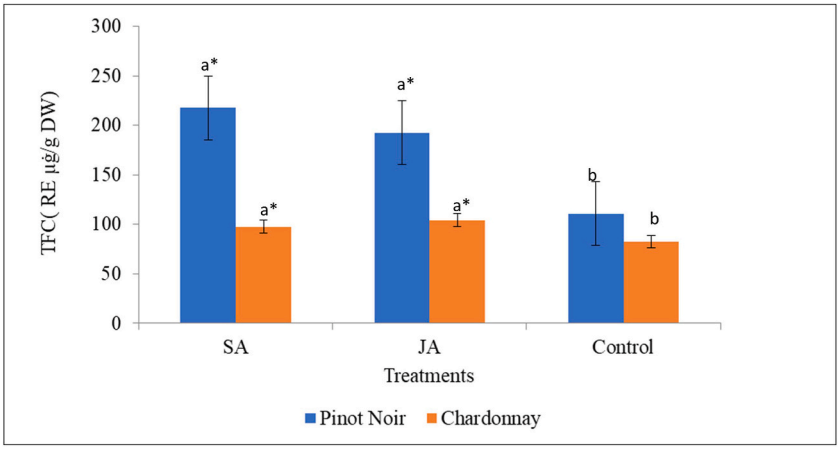

Fig. 2. Total flavonoid content (RE μg/g DW) of grape berries of Pinot Noir and Chardonnay varieties treated with jasmonic acid (JA) and salicylic acid (SA). All values are given as Mean ± SE. Bars with asterisk* within a treatment show that the two varieties differ significantly. Bars with similar letters of the same variety do not differ significantly at $P \leq 0.05$ (Tukey's HSD test).

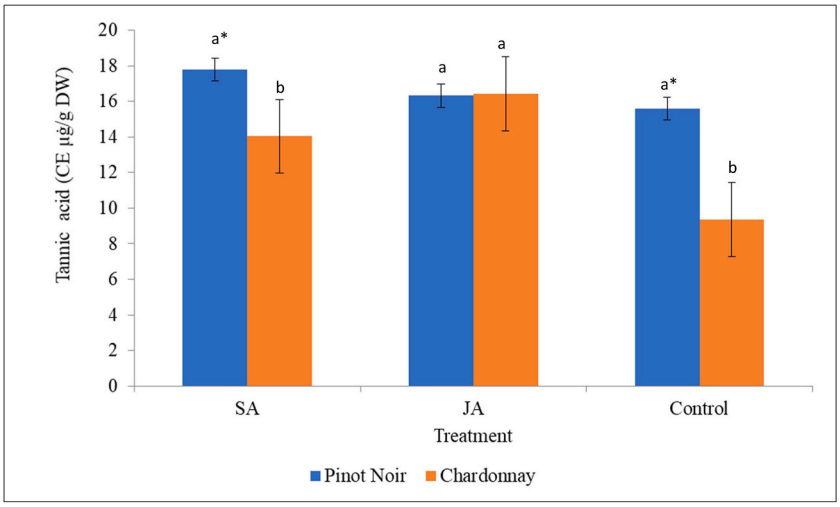

Fig. 3. Total tannin content (CE µg/g DW) of grapevine berries of Pinot Noir and Chardonnay varieties treated with Jasmonic acid (JA) and Salicylic acid (SA). All values are given as averages Mean ± SE. Bars with asterisk* within a treatment show that the two varieties differ significantly. Bars with similar letters of the same variety do not differ significantly at $P \leq 0.05$ (Tukey's HSD test).

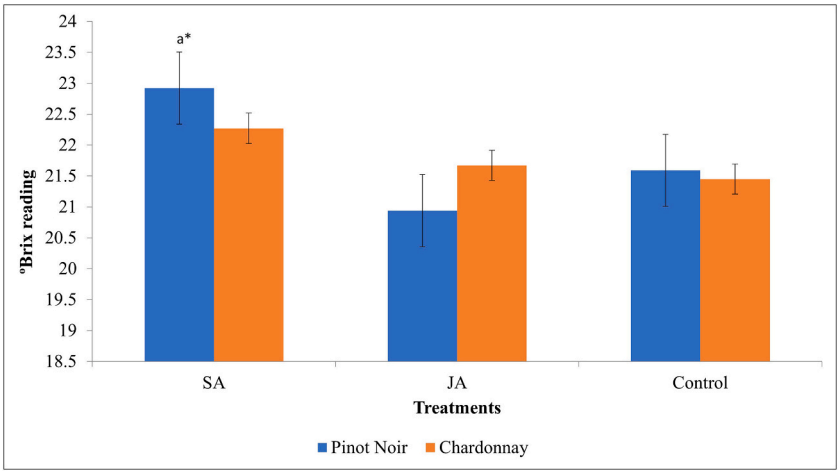

Fig. 4. Total soluble sugars (Brix^0) of grapevine berries of Pinot Noir and Chardonnay varieties treated with Jasmonic acid (JA) and Salicylic acid (SA). All values are given as Mean ± SE. Bars with asterisk* within a treatment show that the two varieties differ significantly. Bars with similar letters of the same variety do not differ significantly at $P \leq 0.05$ (Tukey's HSD test).

4.6. Olfactometer bioassay for the attraction of D. suzukii to different analytical carbohydrates

The preference of *D. suzukii* females in olfactometer to different types of carbohydrates compared to control treatments was significantly different ($\chi^2 = 24.76$, df = 7, $P < 0.001$) (Fig. 7). Among the treatments, sucrose, glucose, fructose, sucrose + yeast and yeast solution (each 5% wt/vol) were more attractive to *D. suzukii* females compared to control ($P = 0.05$). The sucrose solution 5% (wt/vol) followed by glucose solution 5% (wt/vol) showed greater attraction in females of *D. suzukii* when tested individually. However, the combination of sucrose 5% + yeast 5% (wt/vol) showed greater attraction than individual sucrose 5% (wt/vol) and yeast 5% (wt/vol).

4.7. Effect of tested flavonoids on the mortality of D. suzukii by CAFE assay technique

Among the tested flavonoids (gallic acid, quercetin and catechin), higher mortality of *D. suzukii* at 5 DAT was observed using CAFE assay in the adult flies fed on sucrose 5% (wt/vol) incorporated with 100 ppm of quercetin (57.77%) followed by gallic acid (46.66%) and catechin (30.00%) ($\chi^2 = 10.41$, df = 5, $P < 0.05$) (Fig. 8). At 500 ppm, significantly higher mortality occurred in adult *D. suzukii* fed on sucrose 5% (wt/vol) solution incorporated with catechin (93.33.0%) and quercetin (88.88%) relative to the other treatments.

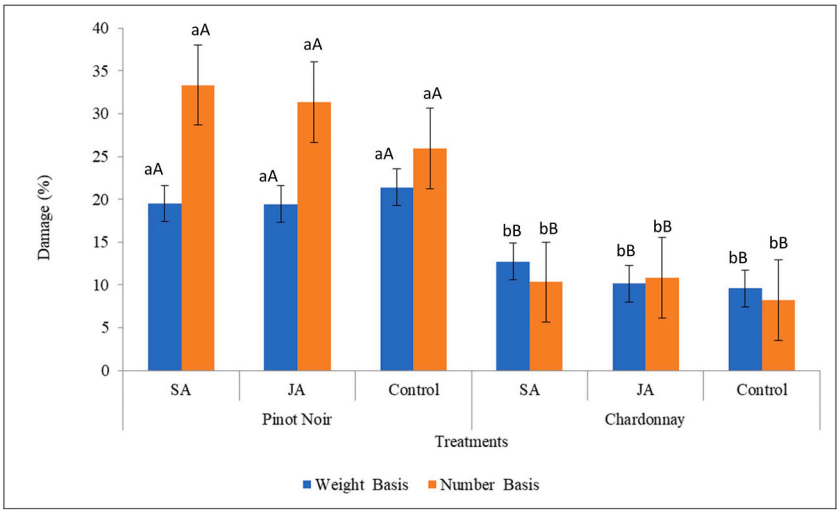

Fig. 5. Damage (%) on weight and number basis of grape berries of Pinot Noir and Chardonnay varieties by *Drosophila suzukii* treated with jasmonic acid (JA) and salicylic acid (SA). All values are given as Mean ± SE. Bars with similar small letters are not significantly different in terms of damage on weight and number basis within a treatment in a variety. Bars of same color with similar capital letters are not significantly different within a treatment between the varieties ($P \leq 0.05$, Tukey's HSD test). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

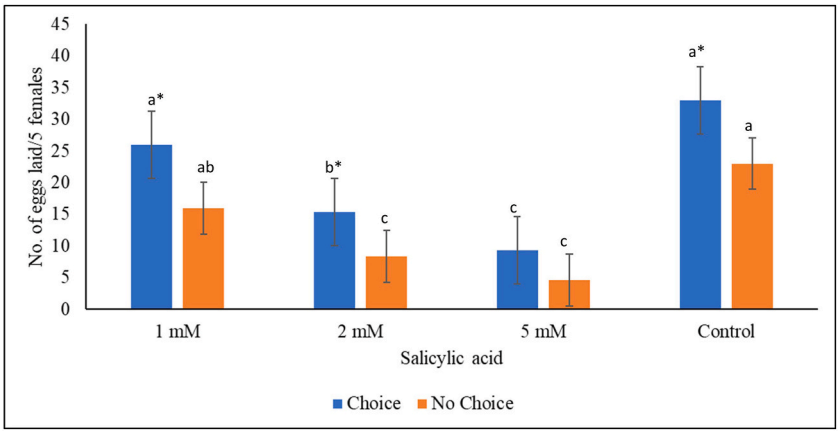

Fig. 6. Eggs laid by five female *Drosophila suzukii* on artificial diet treated with salicylic acid after 48 h under choice and no choice test. (SA = Salicylic acid). All values are given as Mean ± SE. Bars with asterisk* within a treatment show significant different between choice and no-choice method. Bars within a method with similar letters of different treatments do not differ significantly at $P \leq 0.05$ (Tukey's HSD test).

Significant differences in fly mortality were observed at 100 and 500 ppm in different treatments; control treatment recorded 0.02% mortality.

5. Discussion

Our results showed that application of JA and SA increased the levels of phenols, tannin and flavonoid content in two wine grape varieties compared to untreated plants. Pinot Noir berries showed greater levels of phenols, tannin and flavonoid content than the Chardonnay berries, both in JA and SA-treated plants compared to untreated control plants. Pinot Noir had greater total flavonoid content in SA-treated plants compared to the JA-treated plants. The toxicity of flavonoids against insect pests makes them an important component of plant defense [23,26,33]. The higher levels of flavonoids in treated plants can be attributed to the strong induction of the plant signaling pathways by JA and SA [23]. The phenols induced in plants in response to insect attack are important plant defensive traits [23,28]. Phenols commonly accumulate in plants when attacked by herbivores, such as insect pests and by some abiotic factors, thus enabling plants to withstand herbivory [23].

The pomace fly damage on number and weight basis was significantly lower in treated than the untreated berries, but no significant difference was observed between JA and SA treated vines in both Pinot Noir and Chardonnay. Among the two tested wine grape

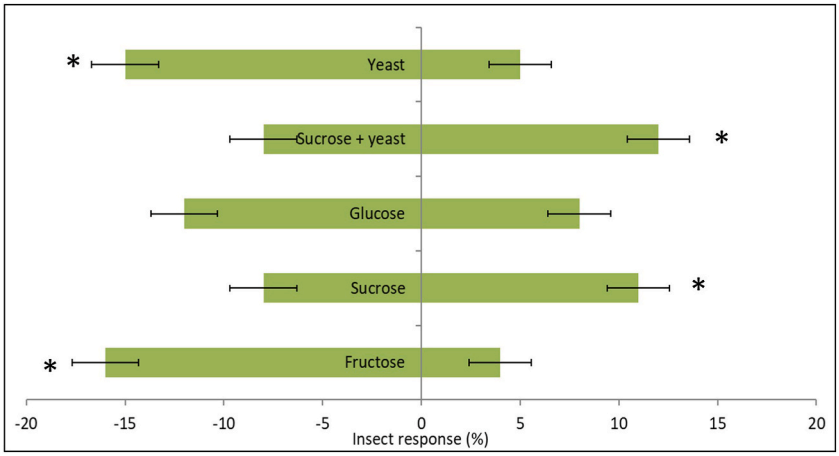

Fig. 7. Behavioral response of *Drosophila suzukii* usiny Y-tube olfactometer in response to (i) sucrose solution 5% (wt/vol), (ii) glucose solution 5% (wt/vol), (iii) fructose solution 5% (wt/vol), (iv) sucrose 5% (wt/vol), + yeast 5% (wt/vol) solution and (v) yeast solution 5% (wt/vol). n = 10 flies in each treatment, 20 replications. All values are given as averages Mean ± SE. * Denotes significant differences between the treatments at $P < 0.05$, ns = non-significant (Chi- Square test).

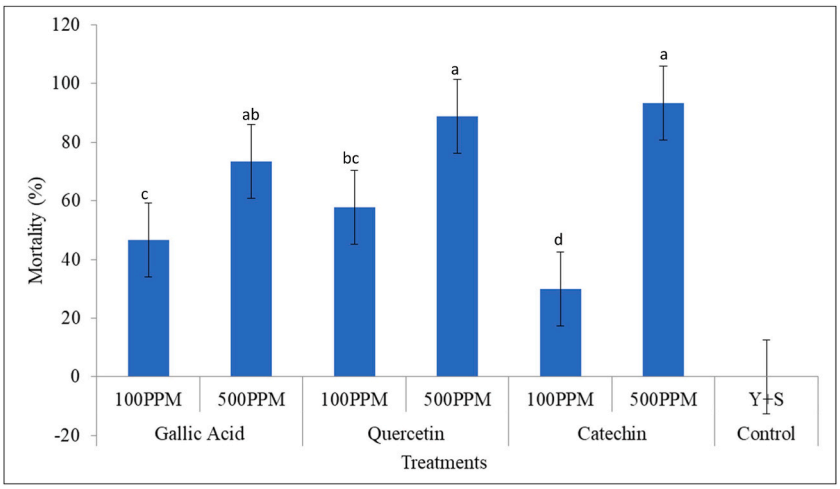

Fig. 8. Mortality (%) of *Drosophila suzukii* adults on diet incorporated with flavonoids at 05 days after treatment. n = 5, replications = 3, Values (mean ± SE), Y + S = yeast + sucrose. Bars with similar letters of different treatments do not differ significantly at $P \leq 0.05$ (Tukey's HSD test).

varieties, Pinot Noir showed higher insect injury compared to Chardonnay. The SA and JA-sprayed plants received less insect injury in both the tested wine grape varieties under field conditions, signifying that SA and JA treated fruits were less suitable to *D. suzukii*. Exogenous spraying of SA and JA induced plant defensive traits in tested wine grape berries; these included increased flavonoid, phenolic and tannin contents, which may be responsible for host protection from *D. suzukii*. SA and JA potentially generate a broad array of metabolic responses in plant parts including fruits at low concentrations and these complex responses can make the berries unsuitable for *D. suzukii* by amending chemical content to avoid fruit fly host finding and selection [36]. Further, the exogenous application of SA and JA on two wine grape varieties reduced fruit injury compared to the control and can be attributed to the accumulation of defensive compounds, which enhance berry tolerance to adults of *D. suzukii.*

5.1. Oviposition preference by D. suzukii in choice and no-choice tests by applying SA on fly diet

A large, serrated ovipositor is present on abdominal tip of *D. suzukii* females, which is pierced into the soft ripening fruit. The eggs are laid inside the fruit and the developing maggots consume the fruits and make them vulnerable to microbial infection [1,38,39]. In choice and no choice assays, our results showed that SA application on a fly diet resulted in reduced oviposition by *D. suzukii* in a dose-dependent manner. Our results agree with earlier reports where *Helicoverpa armigera* Hübner oviposition was reduced in groundnut cultivars after application of JA and SA [24] and a separate study resulted in reasonable mite control in *Phaseolus vulgaris* with improved yields [40]. It has been reported that the application of phytohormones on fruits altered the oviposition behavior in

Ceratitis capitata (Wiedemann) and *Anastrepha suspensa* (Loew) [41,42]. The host plant is recognized by insect olfaction (Pickett et al., 1988). SA inhibits the host attraction of female oriental fruit fly, *Bactrocera dorsalis* Hendel and larval development and adult emergence are drastically reduced [36]. Verghese et al. [43] reported that phenolics act as chemical barriers for oriental fruit flies. The application of SA and induction of resistance to biotic stresses have been well studied in vegetables and other crops but limited work has been done so far on fruit crops.

5.2. Olfactometer bioassay for the attraction of D. suzukii to different analytical carbohydrates

The preference of *D. suzukii* females in olfactometer studies to different types of carbohydrates as compared to control treatments recorded a significant difference ($P \leq 0.05$). Among the five treatments, sucrose (5% wt/vol) attracted more females of *D. suzukii* when tested individually. Also, the combination of sucrose (5% wt/vol), + yeast (5% wt/vol) attracted more females of *D. suzukii* than individually. It was observed that *D. suzukii* infestation/attraction is linked to sequestration of sugars in soft-skinned wine grapes and towards yeasts. The SWD adults are attracted to ripe and overripe fruits, when sugar content is high. Though, SWD females are least attracted to unripe fruits, they possess a nasty ovipositor to lacerate the immature or unripe fruits. Further, it has been reported that life history traits, fecundity, insecticide tolerance, etc., depend on sucrose concentrations in fruits [39]. The total soluble sugars influence the oviposition behavior of SWD. More eggs are laid on fruits with higher sugar content and the larval growth is faster on the fruits with high sugar levels [18,39,44]. Further research to quantify the total sugars from different wine grape varieties is needed to understand the effects of °Brix and penetration pressure [16]. Furthermore, sugars are added to volatiles to enhance the trapping of SWD as an effective management strategy for this pest.

5.3. CAFE assay technique to study the mortality of D. suzukii to different flavonoids

Plants produce several kinds of secondary metabolites, which are involved in plant protection against pathogens and herbivores [23,24,45]. They are found in active form in all parts of the plant, including leaves, unripe fruits and flowers. This study revealed that the three metabolites, gallic acid, quercetin and catechin are more toxic to adult *D. suzukii* at both the tested concentrations compared to a control. It has been reported that in *Eriosoma lanigerum* (Hausmann), mortality caused by quercetin dehydrate, rutin hydrate and naringine are 85, 93 and 86%, respectively [46]. Midgut toxicity in insects has been reported due to caffeic and chlorogenic acids and has been attributed to protein oxidation and free ion release [47]. War et al. [23] reported that chlorogenic acid, gentisic acid, caffeic acid, catechin, trihydroxyflavone, and protocatechuic acid, and lectins are toxic to *H. armigera*. Various studies reported the effect of naturally occurring compounds on insect pests, but few studies have been conducted for the genotoxic effect of gallic acid [48].

Environmentally selective strategies including plant-based insecticides and other control methods are required for effective management of this pest. Among them, plant secondary metabolites are promising alternatives. Metabolic pathways in plants produce several secondary byproducts with insecticidal effects and can contribute to the development of crop protection strategies [23,24]. However, few data are available on the use of plant metabolites from wine grapes against *D. suzukii* [49]. In *B. dorsalis*, high total phenol and flavonoid contents resulted in reduced larval growth and development, and adult emergence [36]. Our study demonstrates the occurrence of metabolic changes specific from veraison through berry maturation stages that lead to the attraction of *D. suzukii* and piercing of wine grapes at the later stage of berry development. The results of this study will form important biochemical, physiological and behavioral indicators for managing spotted-winged drosophila in wine grapes and related crops. However, further in-depth studies on the role of plant secondary metabolites in wine grapes in protection against *D. suzukii* is needed for strategic management of this pest.

Author contribution statement

Barkat Hussain: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper. Douglas G Pfeiffer: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data. A R War: Designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Data availability statement

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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