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Posted Date: 31 May 2024

doi: 10.20944/preprints202405.2121.v1

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Effects of Conventional and Organic Fertilization on the Chemical Profile of *Sorghum bicolor* and the Olfactory Preference of Sugarcane Aphid (*Melanaphis sacchari*)

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Abstract: The type of fertilization used influences plant compounds and pest infestation. We measured, through chemical stimuli, the preference of *Melanaphis sacchari* for sorghum plants fertilized by means of conventional fertilization (CF) or organic fertilization (OF). Leaves were collected from sorghum plants fertilized with 200 kg N ha^{-1} using ammonium sulfate and poultry manure. Extracts were obtained using Soxhlet extraction, and the compounds were identified using a gas chromatograph coupled with mass spectrometry (GC/MS). Sorghum extracts were individually tested through bioassays to determine *M. sacchari* preference. The abundance and number of compounds in sorghum differed depending on the type of fertilization used. *M. sacchari* showed a preference for the extract from CF sorghum plants (76.66%) over the extract from OF plants (23.34%). Therefore, the type of fertilization can be used as a tactic to prevent higher infestations of *M. sacchari*. The biological activity of the compounds identified here with *M. sacchari* should be determined for future pest management strategies using allelochemicals, given that the sugarcane aphid uses chemical signals to locate its host plant.

Keywords: alellochemicals; bioassays; chemical stimuli; fertilization; plant extracts**;** sorghum; yellow aphid

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1. Introduction

Sorghum bicolor (L.) Moench, commonly called sorghum, belongs to the family Poaceae. Mexico contributes 10.6% of the world's production and imports 5.01 million tons of this grain utilized mainly as feed for cattle, pigs, and poultry [1]. The sugarcane aphid (*Melanaphis sacchari*) poses a persistent challenge in sorghum cultivation; it is distributed globally and, specifically in Mexico, is found in 27 states [2]. This insect negatively affects sorghum and other important crops. Sugarcane aphids are economically important; they feed on plant sap, leading to reduced yields and, in some cases, total sorghum losses [2,3]. Pest management strategies proposed for *M. sacchari* include biological control, determination of optimal planting dates, elimination of alternative hosts, and the use of pesticides, with the latter being the primary control measure for this insect [4]. Pesticides can potentially enter the environment, contaminating both the environment and food, thereby affecting biodiversity and human health [5]. Therefore, it is crucial to implement new strategies, such as utilizing insect behavior to compounds emitted by plants or also called allelochemicals. [6]. These compounds serve as chemical signals for insects to identify their host plants, yet their potential

 doi:10.20944/preprints202405.2121.v1

benefits in *M. sacchari* management remain poorly explored [7]. Nitrogen (N) fertilization, mainly synthetic or conventional N, is essential for obtaining high crop yields. However, this practice causes contamination, similar to the use of pesticides, and has been associated with a higher incidence of pest and aphid species, such as *Brachycaudus cardui*, which has shown increased attraction to fertilized host plants [8,9]. In addition, there is evidence that insects have a lower preference for organically fertilized (OF) plants. Organic fertilization has recently gained importance because it shows less nutrient loss and is more environmentally friendly than conventional fertilization (CF) [10]. In line with the above, it has been reported that the type of fertilization used affects plant chemical compounds [11,12].

The aim of the present study was to determine whether the type of fertilization affects the compounds found in sorghum plants and the preference of the sugarcane aphid. To do this, compounds from S. bicolor were identified and bioassays were conducted to assess the behavior of *M. sacchari* in response to chemical stimuli, which could serve as another important factor in the management of this pest.

2. Materials and Methods

2.1. Plant Material

S. bicolor seeds were sown in 4 L polyethylene bags in a greenhouse of the Colegio de Postgraduados (COLPOS), Campus Puebla (19°04′26.5′′N; 98°15′41.3′′W). Plants were grown for 60 days at 20-25 °C and 60 ± 10% humidity, and watered every 3 days. The experiment followed a completely randomized design consisting of 3 treatments, with 3 replicates (Table 1).

Table 1. Fertilization treatments in *S. bicolor*.

Treatments	Description
F0	Soil (4.4% organic material; nitrogen 100 ppm; phosphorus 0.80 ppm; potassium 5.50 ppm and pH 7.4)
CF	200 kg ha^{-1} N (ammonium sulfate) + soil
OF	200 kg ha^{-1} N (poultry manure) + soil

F0: Zero fertilization; CF: Conventional fertilization; OF: Organic fertilization.

2.2. Insect Breeding

Sugarcane aphid nymphs were obtained from sorghum plots in the municipality of Izúcar de Matamoros, Puebla (18°36′10′′N; 98°27′5′′W). The nymphs were transported to the greenhouse and maintained on healthy sorghum plants (under the above-mentioned conditions) for reproduction until their use in bioassays using extracts.

2.3. Extraction and Identification of Compounds

The third and fourth alternate leaves of sorghum plants (300 g) were ground using a mill, wrapped in filter paper, and then placed in a Soxhlet extractor mounted atop a distillation flask containing 150 mL of 90% ethanol (Sigma Aldrich). The extraction process was carried out for 3 h. Then, 1 µL of the extract was injected into an Agilent Technologies 7890A gas chromatograph coupled with an Agilent Technologies 5975C mass spectrometer (Santa Clara, CA, USA). The system was equipped with a 30 m x 0.25 mm HP-5MS column with a film thickness of 0.50 µm (Agilent J&W, Santa Clara, CA, USA). The GC-MS parameters were as follows: helium as the carrier gas, injector temperature of 250 °C in splitless mode, initial oven temperature of 36 °C for 1 min and then increased by 10 °C per min until reaching 250 °C, which was maintained for 3 min. Compounds were identified through comparison with mass spectra from the National Institute of Standards and Technology library (NIST 8 and NIST 11).

2.4. Bioassays Using Extracts from *S. bicolor*

Healthy-looking 1.2 mm-long, 13-day-old females of *M. sacchari* were fasted for 1 h prior to individual bioassays. A glass Petri dish (10 cm in diameter) was used as the behavioral arena (Figure 1). Bioassays were carried out under laboratory conditions (22 ± 3 °C and 60 ± 10% RH). Briefly, 10 μL of extract were placed on 1 cm x 1 cm filter paper pieces (Whatman No. 1) and allowed to evaporate for 30 s. For the control (C), an equivalent volume of solvent (ethanol) was used, following the same procedure. Each piece of filter paper was placed end to end of the Petri dish randomly in each bioassay. A 5 min response time was given, or until it moved towards the extract (past the response line). A total of 30 individual bioassays were conducted. After 3 bioassays, the Petri dish used was replaced by a clean one. The extracts tested were C, F0 (unfertilized plant), CF, and OF. Six combinations of extracts were made, as described in Table 2.

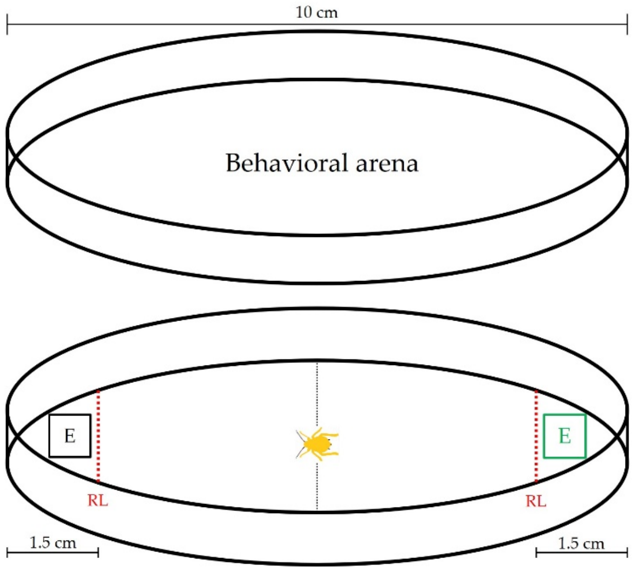

Figure 1. Behavioral arena for bioassays with sorghum extracts. RL: Response line; E: Extract.

Table 2. Combinations for behavioral bioassays.

Bioassay	Combinations extracts
1	CF vs. OF
2	F0 vs. OF
3	F0 vs. CF
4	C vs. OF
5	C vs. CF
6	C vs. F0

C: Control (etanol); F0: Zero fertilization; CF: Conventional fertilization; OF: Organic fertilization.

2.5. Statistical Analysis

The frequencies of behavioral responses were analyzed through an exact binomial test using the R Studio software.

3. Results

3.1. Extraction and Identification of Compounds

The number of compounds identified in sorghum leaf extracts were 12 in F0, 34 in CF, and 16 in OF. Butanedioic acid, 1-tetradecene, (Z)-7-hexadecene, and phenol were found in leaves from all three treatments. Butanedioic acid and 1-tetradecene were most abundant in F0, followed by CF and

OF. In contrast, phenol was most abundant in CF (23.35%), followed by F0 (22.64%) and OF (14.01%). The abundances of (E)-5-octadecene and benzophenone were higher in CF than in OF. On the other hand, 1,2-benzenedicarboxylic acid was more abundant in OF (1.62%) than in CF (0.77%). The most abundant compound in F0 and OF was (4-methoxy-phenyl)-(5-p-tolyl-furan-2-ylmethylene)-amine (46.89% and 68.02%, respectively). In CF, 5-[[[3,4,5-trimethoxyphenyl]imino]methyl]-2,4-pyrimidinediamine was the most abundant compound (37.73%). The least abundant compounds were (2,2-dichlorocyclopropyl) methanol in F0 (0.06%), 5,6-dihydro-2-(4-nitrophenyl)-4H-1,3-oxazin-5-one in CF (0.11%), and 3-methyl-1-(4-toluidino)pyrido [1,2 -a]benzimidazole-4-carbonitrile in OF (0.2%). A total of 28 compounds were found exclusively in CF sorghum plants.

Table 3. Area of compounds detected in *S. bicolor* leaves.

Number	Compound	Area F0 (%)	Area CF (%)	Area OF (%)
1	Butyric acid	ND	0.33	ND
2	Tridecyl trifluoroacetate	ND	0.15	ND
3	(R)-2-octanol	ND	0.21	ND
4	Propanoic acid	ND	0.36	ND
5	1-Methyldecylamine	1.43	ND	ND
6	Ethylamine	ND	0.28	ND
7	4-methyl-2-Pentanamine	ND	0.33	ND
8	4-fluorohistamine	ND	0.61	ND
9	1-undecanol	7	ND	2.69
10	2H-pyran-2-one	ND	5.48	ND
11	2-heptanol	ND	0.35	ND
12	Acetic acid	ND	0.63	ND
13	(2,3-dimethyloxiranyl) methanol	ND	0.39	ND
14	2,3-diethoxy-propionic acid, ethyl ester	ND	0.42	ND
15	2-nonanol	ND	0.24	ND
16	Butanedioic acid	2.24	1.83	1.09
17	1-tetradecene	6.36	5.47	2.5
18	1-(1-propynyl)-cyclohexene	ND	0.42	ND
19	(Z)-7-hexadecene	3.4	2.85	1.46
20	Phenol	22.64	23.35	14.01
21	4-(2-methylamino)ethyl)pyridine	ND	0.04	ND
22	2-fluoro-2′,4,5-trihydroxy-N-methyl-benzenethanamine	ND	0.89	ND
23	(E)-5-octadecene	ND	0.93	0.55
24	N,N′-dimethyl-2-butene-1,4-diamine	0.68	ND	ND
25	1-dodecanamine	ND	1.53	ND
26	4-hydroxy-benzeneacetonitrile	ND	5.29	ND
27	N,N-dimethyl-dimethylphosphoric amide	ND	0.39	ND
28	2-Octyl benzoate	ND	0.72	ND
29	Benzophenone	ND	1.06	0.69
30	N-(3-pyridinylmethylene)benzenamine	1.09	ND	ND
31	4-amino-2-oxy-furazan-3-carboxylic acid	ND	0.20	ND
32	Piperidin-4-ol, 1,3,3-trimethyl-4phenyl	ND	0.37	ND
33	Benzene, 4-bromo-1,3-dimethoxy-6-(4-acetylphenyliminomethyl)	ND	ND	0.93
34	Phthalic acid, isobutyl 4-isopropylphenyl ester	ND	0.24	ND
35	9-hydroxy-3,4-dihydro-2H-1,4-ethanoquinoline-9-carboxylic acid	ND	ND	1.14
36	5,6-dihydro-2-(4-nitrophenyl)-4H-1,3-oxazin-5-one	ND	0.11	ND
37	1H-pyrrolo [1,2-a]benzimidazolium,2,3-dihydro-4-(1,2,3,4-tetrahydro-6-hydroxy-1,3-dimethyl-2,4-dioxo-5-pyrimidinyl)-, hydroxide	ND	ND	1.07
38	5-[[[3,4,5-trimethoxyphenyl]imino]methyl]-2,4-pyrimidinediamine	ND	37.73	ND
39	(4-methoxy-phenyl)-(5-p-tolyl-furan-2-ylmethylene)-amine	46.89	ND	68.02

40	1,2-benzenedicarboxylic acid	ND	0.77	1.62
41	2-methyl-benzothiazole	0.55	ND	ND
42	2-bromo-N-methyl-2-propen-1-amine	ND	0.35	ND
43	4-methyl-2-pentanamine	0.83	ND	ND
44	(2,2-dichlorocyclopropyl)methanol	0.06	ND	ND
45	1,3-benzenedicarboxylic acid	ND	0.34	ND
46	4-phenoxy-2-phenyl-1-naphthalenol	ND	ND	1.06
47	3-methyl-1-(4-toluidino)pyrido [1,2-a]benzimidazole-4-carbonitrile	ND	ND	0.2
48	5-(p-aminophenyl)-4-(p-nitrophenyl)-2-thiazolamin	ND	ND	0.3
49	2-oxo-1,4,5-triphenyl-4-imidazolin	ND	ND	0.68
	Number of compounds detected per treatment	**12**	**34**	**16**

ND: Not detected.

3.2. Bioassays Using Extracts from S. bicolor

M. sacchari females showed a preference for *S. bicolor* extracts compared to the control (C), with higher response in F0 (93.34%), CF (86.66%), and OF (76.66%). However, bioassays comparing F0 (20%) with CF (80%) and F0 (26.67%) with OF (73.33%), exhibited preference for extracts from fertilized *S. bicolor* (CF and OF). Finally, when comparing CF (76.66%) with OF (23.34%) extracts, CF was preferred (Figure 2).

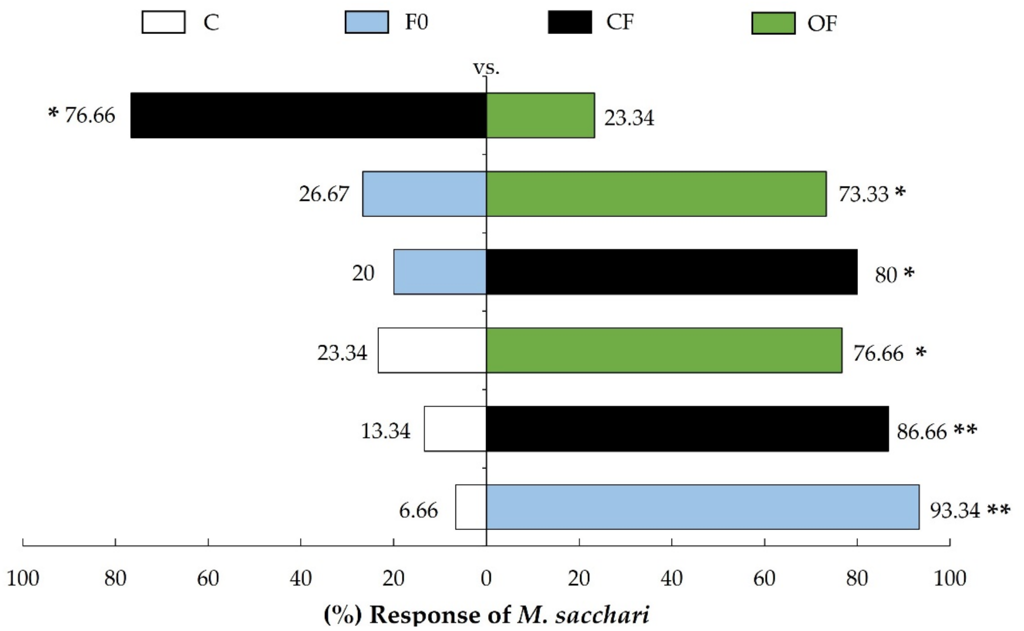

Figure 2. Behavioral bioassays of *M. sacchari* in response to *S. bicolor* extracts. C (control), F0 (unfertilized plant), CF (plant fertilized with ammonium sulfate), and OF (plant fertilized with poultry manure). * denotes significant differences (Binomial test: $p < 0.05$); ** denotes significant differences (Binomial test: $p < 0.0001$).

4. Discussion

The type of fertilization affected the abundance and number of compounds found in sorghum plants. This is in agreement with observations in *Rubus idaeus* plants, where the type of fertilization had both qualitative and quantitative effects on the compounds emitted [13]. In other plants such as willow, strawberry, and tomato, the compounds were affected by CF with N [12–14]. In addition, OF has been shown to modify the abundance of compounds compared to unfertilized tomato plants [14]. In line with previous reports for other species, we found that the number of compounds in *S. bicolor* was higher in CF, followed by OF and F0. These results are attributed to the availability of N, which

 doi:10.20944/preprints202405.2121.v1

leads to increased protein synthesis and, therefore, increased synthesis of secondary metabolites that affect plant defense [15]. CF has been associated with a higher abundance of compounds [12,16], as evinced by the results here obtained for (E)-5-octadecene, benzophenone, and phenol. However, said trend was not observed when comparing F0 with CF. Butanedioic acid, 1-tetradecene, and (Z)-7-hexadecene were more abundant in F0. In this regard, it has been reported that compounds exhibit individual responses to fertilization, which could be attributed to different biosynthetic pathways and environmental factors [12]. Eleven of the compounds exclusively identified in CF were mostly aromatic compounds, which act as chemical signals involved in the attraction of insects, such as *Drosophila melanogaster*, *Eupeodes corolla*, and *Sitona humeralis* [17–19]. These compounds, which were not common to other treatments, could be a key factor in the attraction of *M. sacchari* to extracts from CF sorghum plants.

Some of the compounds identified in this study have been reported to have biological activity in certain insects, including some found exclusively in CF plants, such as acetic acid. Acetic acid has been related to high-dose N fertilization in *Brassica napus* plants and serves as a potential attractant of *Meligethes aeneus* and *D. melanogaster* [11,17]. Butyric acid is a key attractant of pests such as *Holotrichia paralela* and *Bubas bison* [20,21]. A compound induced by herbivory, 2-octanol, has been reported as an attractant of *Spilosoma obliqua* [22]. In *Camellia sinensis* plants, 1,3-benzenedicarboxylic acid has been related to N fertilization doses and infestation of the aphid *Toxoptera aurantii* [23]. The most abundant compound in CF was phenol, which acts as an attractant of beetles of the species *B. bison* [21].

In OF, we found butanedioic acid, a component of the silkworm cocoon, which may be involved in its protection [24]. Likewise, higher levels of butanedioic acid were found in chickpea plants and were linked to increased resistance to the leaf miner *Liriomyza cicerina* [25]. Another compound that could have repellent activity against *M. sacchari* is 1,2-benzenedicarboxylic acid, which has potential as a natural insecticide [26]. In *Capsicum* spp. plants infested with *Aphis gossypii* aphids, 1-undecanol has been identified as a compound involved in plant's defense [27]. In the present study, 1-undecanol was exclusively found in F0 and OF sorghum plants, suggesting its potential role as a repellent against *M. sacchari*. Finally, 1-tetradecene exhibits repellent activity against the aphids *Acyrthosiphon pisum* and *Myzus persicae* [28], while showing attractant activity towards *Apolygus lucorum*, *Adelphocoris suturalis*, and *Megalurothrips sjostedti* [29,30]. Although previous reports on the compounds identified in this study may provide an indication of their activity against or towards *M. sacchari*, further research is needed to confirm their biological activity.

Based on our results, *M. sacchari* has a preference for CF and OF treatments. In this regard, it has already been reported that there is a preferential relationship of *M. sacchari* for plants fertilized with higher levels of N [4]. Here, we compared the preference for CF or OF plants. The release of nutrients in organic fertilization is slower compared to chemical fertilizers. This differential rate impacts sap-sucking insects, as leaf sap composition is affected by fertilizer sources and dosages [31,32]. To date, only *M. persicae* has been tested using chemical stimuli. Olfactometer tests revealed that this species was more attracted to volatile compounds from cabbage plants with higher N doses [33]. A higher incidence and preference for fertilized plants has also been observed in other aphids, such as *M. persicae*, which preferred capsicum plants with higher N doses [34]. Similarly, the aphids *T. aurantii*, *Lipaphis erysimi*, *Bemisia tabaci*, *Rhopalosiphum padi*, and *Sitobion avenae* were more attracted to host plants fertilized with high N doses compared to unfertilized plants. This preference affected their fecundity and longevity [23,35–37].

Insect preference for fertilized plants is attributed to physiological changes in the plants, which cause changes in their metabolites and, consequently, in the chemical signals they emit [37]. In addition, *M. persicae* showed a preference for white clover CF plants over plants fertilized with poultry manure [38]. It has also been reported that maize plants fertilized with synthetic fertilizers had a higher percentage of infestation by *R. maidis* aphid than plants fertilized with animal manure [39]. In this regard, chemical nitrogen fertilizers enhance the vigorous qualities of plants, making them attractive to insects [40,41]. On the other hand, organic fertilization, characterized by the slow release of nutrients and consequently a lower N dose, may affect the production of toxic compounds, helping to maintain pest populations at low levels and to enhance plant resistance [42–44].

There are no reports indicating the existence of a chemical signal involved in the interaction between *M. sacchari* and *S. bicolor*. Moreover, it has been claimed that this insect relies primarily on visual signals. However, aphids have large antennae, suggesting that olfactory signals must be important factors in locating host plants [45,46]. Our findings confirm the above, as chemical signals were used to evaluate the preference of *M. sacchari* for *S. bicolor* extracts.

5. Conclusions

The type of fertilization used affected the compounds extracted from *S. bicolor* plants, which is related to the attractant activity shown by the extracts from fertilized sorghum plants. The sugarcane aphid showed a preference for extracts from CF plants, confirming that *M. sacchari* uses chemical stimuli to locate its host plant, and that CF makes sorghum plants susceptible to attack by this aphid. Therefore, OF represents a key method for pest prevention.

The type of fertilization and its relationship with the chemical compounds of sorghum plants should be taken into account when devising management strategies for *M. sacchari*. It is evident that sugarcane aphids rely on allelochemicals to locate the sorghum plant. We recommend further studies to determine the biological activity of the compounds identified here against *M. sacchari*.

Author Contributions: Conceptualization, E.N.S. and E.S.C.; methodology, E.N.S. and M.D.C.A.; software, E.N.S.; validation, E.S.C., M.D.C.A., A.D.A., A.H.d.l.P. and I.O.F.; formal analysis, E.N.S.; investigation, E.N.S.; resources, E.N.S. and M.D.C.A; data curation, E.N.S.; writing—original draft preparation, E.N.S.; writing—review and editing E.N.S., M.D.C.A., A.D.A., A.H.d.l.P. and I.O.F.; visualization, E.N.S. and E.S.C.; supervision, E.N.S. and M.D.C.A.; project administration, E.N.S., E.S.C. and M.D.C.A.; funding acquisition, E.N.S., M.D.C.A. and E.S.C. All authors have read and agreed to the published version of the manuscript.

Funding: This study is a product of the doctoral dissertation of the first author, who is grateful to the Consejo Nacional de Humanidades, Ciencias y Tecnologías (CONAHCyT) for grant number 814396 in support of her doctoral studies.

Data Availability Statement: Data are contained within the article.

Conflicts of Interest: The authors declare no conflicts of interest.

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