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Communication

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

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Abstract: *Melanaphis sacchari* is a cosmopolitan pest that causes losses in sorghum crops, so new management methods are needed. In addition, the type of fertilization used influences plant compositions and pest infestation, and allelochemicals are a promising method for the possible management of *M. sacchari*. In this work, we measured the preference of *M. sacchari* through chemical stimuli towards sorghum plants grown under greenhouse conditions without fertilization (F0), conventional fertilization (CF), and 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: allelochemicals; bioassays; chemical stimuli; fertilization; plant extracts; sorghum; sugarcane aphid

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1. Introduction

Sorghum bicolor (L.) Moench, commonly called sorghum, originated in Africa approximately 8000 years ago, later distributed to countries in the Middle East and Asia, and finally in the 19th Century to the American continent [1]. This crop is considered the fifth most important grain crop in the world [2]. Mexico contributes 10.6% of the world's production and imports 5.01 million tons of this grain and it is utilized mainly as feed for cattle, pigs, and poultry [3]. However, currently, this crop has taken relevance as a food source in African, Asian, and South American countries [4]. A recurrent problem in sorghum crops is the sugarcane aphid (*Melanaphis sacchari*), a pest that is distributed worldwide [5]. It was introduced to the American continent in 2013, mainly affecting sorghum crops in the United States and Mexico, being distributed in the latter in 27 states [5,6]. This insect negatively affects sorghum and other important crops. Sugarcane aphids are economically important due to the fact that they feed on plant sap, leading to reduced yields and, in some cases, total sorghum losses [5,7]. Pest management strategies proposed for *M. sacchari* include biological control, determination of optimal planting dates, elimination of alternative hosts, and use of pesticides, with the latter being the primary control measure for this insect [8]. Pesticides can potentially enter the environment, contaminating both the environment and food, thereby affecting biodiversity and human health [9]. Therefore, it is crucial to implement new strategies, such as utilizing insect behavior to compounds emitted by plants or also called allelochemicals [10]. These compounds serve as chemical signals for insects to identify their host plants and can be used for crop protection and productivity as part of pest management [11,12]. Still, their potential benefits in *M. sacchari* management remain poorly explored. On the other hand, nitrogen (N) fertilization practices, particularly of a synthetic or conventional origin, are essential for crop yields [13]. However, this practice causes contamination, similar to the use of pesticides; it generates several environmental problems such as greenhouse gas emissions, soil acidification and degradation, groundwater contamination, and loss of biological diversity, among others [14]. In addition, it is also a practice that is related to a higher incidence of pests and aphid species, such as *Brachycaudus cardui*, *Myzus persicae*, and *Toxoptera aurantii*, which showed increased attraction to fertilized host plants [15–18]. On the contrary, there is evidence that aphids such as *M. persicae* and *Rhopalosiphum maidis* have a lower preference for organically fertilized plants [19,20]. Organic fertilization (OF) has recently gained importance because it is considered an alternative fertilization that can help improve environmental sustainability, due to its advantages such as improving soil organic matter, soil structure, and moisture; improving soil microbial activity; and showing less nutrient loss than conventional fertilization (CF) [21,22]. In line with the above, it has been reported that the type of fertilization used affects plant chemical compounds [23,24].

Based on the above, it is hypothesized that the type of fertilization affects the compounds of sorghum plants and the preference of the aphid *M. sacchari*. The aim of the present study was to identify the compounds of *S. bicolor* and to perform bioassays to evaluate the behavior of *M. sacchari* to sorghum extracts as a chemical stimulus, which could serve as another important factor for the management of this pest.

2. Materials and Methods

2.1. Plant Material

In October 2023, *S. bicolor* seeds (Dekalb 45) 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 16:8 photoperiod 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 matter; nitrogen at 100 ppm; phosphorus at 0.80 ppm; potassium at 5.50 ppm; and pH 7.4)
CF	200 kg ha^{-1} N (ammonium sulfate) + soil
OF	200 kg ha^{-1} N (poultry manure: 57% organic matter; nitrogen at 400 ppm; phosphorus at 230 ppm; potassium at 200 ppm; and pH 7.8) + soil

F0: Plant without fertilization; CF: Conventional fertilization; OF: Organic fertilization.

2.2. Insect Breeding

In August 2023, 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 in cages made of wood and polyethylene mesh (50 cm × 50 cm × 80 cm) (under the above-mentioned conditions) for reproduction during 5 generations until their use in bioassays with the extracts [25].

2.3. Extraction and Identification of Compounds

The third and fourth alternate leaves of sorghum plants were rinsed with sterile distilled water and weighed (300 g). Then, they 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, Seelze, Germany). The extraction process was carried out for 3 h [26]. Then, 1 µL of the extract was injected into an Agilent Technologies (Santa Clara, CA, USA) 7890A gas chromatograph coupled with an Agilent Technologies 5975C mass spectrometer (Santa Clara, CA, USA). The system was equipped with a 30 m × 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 the splitless mode, and 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) [27].

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 performed under laboratory conditions (22 ± 3 °C and 60 ± 10% RH) between 10:00 a.m. and 1:00 p.m. Briefly, 10 µL of the extract was placed on 1 cm × 1 cm filter paper pieces (Whatman No. 1) and allowed to evaporate for 30 s [25]. For the control (C), an equivalent volume of solvent (ethanol) was used, following the same procedure [28]. Each piece of filter paper was placed end to end of the Petri dish randomly and the Petri dish was closed 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.

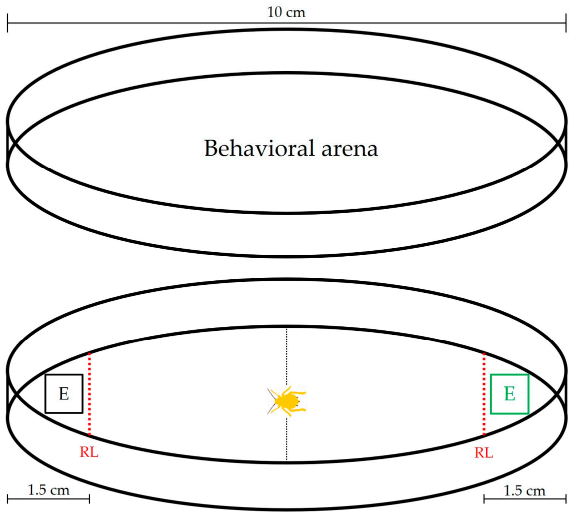

Figure 1. Behavioral arena for bioassays with sorghum extracts. RL: Response line; E: Extract.

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 (ethanol); F0: Plant without fertilization; CF: Conventional fertilization; OF: Organic fertilization.

2.5. Statistical Analysis

The frequencies of behavioral responses were analyzed through an exact binomial test using R Studio software (version 4.4.0).

3. Results

3.1. Extraction and Identification of Compounds

The numbers of compounds identified in sorghum leaf extracts were 12 in F0, 34 in CF, and 16 in OF (Table 3). 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. Compounds detected in *S. bicolor* leaves.

Number	Retention Time	Compound	Peak Area F0 (%)	Peak Area CF (%)	Peak Area OF (%)
1	3.95	butyric acid	ND	0.33	ND
2	3.96	tridecyl trifluoroacetate	ND	0.15	ND
3	3.98	(R)-2-octanol	ND	0.21	ND
4	4.00	propanoic acid	ND	0.36	ND
5	4.14	1-methyldecylamine	1.43	ND	ND
6	5.38	ethylamine	ND	0.28	ND
7	5.59	4-methyl-2-pentanamine	ND	0.33	ND
8	5.66	4-fluorohistamine	ND	0.61	ND
9	6.04	1-undecanol	7	ND	2.69
10	6.05	2H-pyran-2-one	ND	5.48	ND
11	6.40	2-heptanol	ND	0.35	ND
12	6.93	acetic acid	ND	0.63	ND
13	7.00	(2,3-dimethyloxiranyl) methanol	ND	0.39	ND
14	7.08	2,3-diethoxy-propionic acid, ethyl ester	ND	0.42	ND
15	7.55	2-nonanol	ND	0.24	ND
16	7.77	butanedioic acid	2.24	1.83	1.09
17	8.14	1-tetradecene	6.36	5.47	2.5
18	8.63	1-(1-propynyl)-cyclohexene	ND	0.42	ND
19	10.12	(Z)-7-hexadecene	3.4	2.85	1.46
20	10.88	phenol	22.64	23.35	14.01
21	11.21	4-(2-methylamino)ethyl)pyridine	ND	0.04	ND
22	11.41	2-fluoro-2′,4,5-trihydroxy-N-methyl-benzenethanamine	ND	0.89	ND
23	11.99	(E)-5-octadecene	ND	0.93	0.55
24	11.99	N,N′-dimethyl-2-butene-1,4-diamine	0.68	ND	ND
25	12.27	1-dodecanamine	ND	1.53	ND
26	12.73	4-hydroxy-benzeneacetonitrile	ND	5.29	ND
27	12.91	N,N-dimethyl-dimethylphosphoric amide	ND	0.39	ND
28	13.23	2-octyl benzoate	ND	0.72	ND
29	14.20	benzophenone	ND	1.06	0.69
30	14.21	N-(3-pyridinylmethylene)benzenamine	1.09	ND	ND
31	14.37	4-amino-2-oxy-furazan-3-carboxylic acid	ND	0.20	ND
32	14.85	piperidin-4-ol, 1,3,3-trimethyl-4phenyl	ND	0.37	ND
33	16.16	benzene, 4-bromo-1,3-dimethoxy-6-(4-acetylphenyliminomethyl)	ND	ND	0.93
34	16.17	phthalic acid, isobutyl 4-isopropylphenyl ester	ND	0.24	ND
35	16.58	9-hydroxy-3,4-dihydro-2H-1,4-ethanoquinoline-9-carboxylic acid	ND	ND	1.14

Table 3. *Cont.*

Number	Retention Time	Compound	Peak Area F0 (%)	Peak Area CF (%)	Peak Area OF (%)
36	16.84	5,6-dihydro-2-(4-nitrophenyl)-4H-1,3-oxazin-5-one	ND	0.11	ND
37	16.86	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	17.21	5-[[[3,4,5-trimethoxyphenyl]imino]methyl]-2,4-pyrimidinediamine	ND	37.73	ND
39	17.21	(4-methoxy-phenyl)-(5-p-tolyl-furan-2-ylmethylene)-amine	46.89	ND	68.02
40	17.42	1,2-benzenedicarboxylic acid	ND	0.77	1.62
41	17.42	2-methyl-benzothiazole	0.55	ND	ND
42	17.61	2-bromo-N-methyl-2-propen-1-amine	ND	0.35	ND
43	21.47	4-methyl-2-pentanamine	0.83	ND	ND
44	21.51	(2,2-dichlorocyclopropyl)methanol	0.06	ND	ND
45	22.04	1,3-benzenedicarboxylic acid	ND	0.34	ND
46	22.05	4-phenoxy-2-phenyl-1-naphthalenol	ND	ND	1.06
47	23.60	3-methyl-1-(4-toluidino)pyrido[1,2-a]benzimidazole-4-carbonitrile	ND	ND	0.2
48	26.96	5-(p-aminophenyl)-4-(p-nitrophenyl)-2-thiazolamin	ND	ND	0.3
49	27.02	2-oxo-1,4,5-triphenyl-4-imidazolin	ND	ND	0.68
		Number of compounds detected per treatment	12	34	16

ND: Not detected; F0: Plant without fertilization; CF: Conventional fertilization; OF: Organic fertilization.

3.2. Bioassays Using Extracts from *S. bicolor*

M. sacchari females showed a statistically significant preference ($p < 0.0001$) 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 a statistically significant ($p < 0.05$) preference for fertilized extracts of *S. bicolor* (CF and OF). Finally, when comparing CF (76.66%) with OF (23.34%) extracts, *M. sacchari* showed a statistically significant preference ($p < 0.05$) for the CF extract (Figure 2).

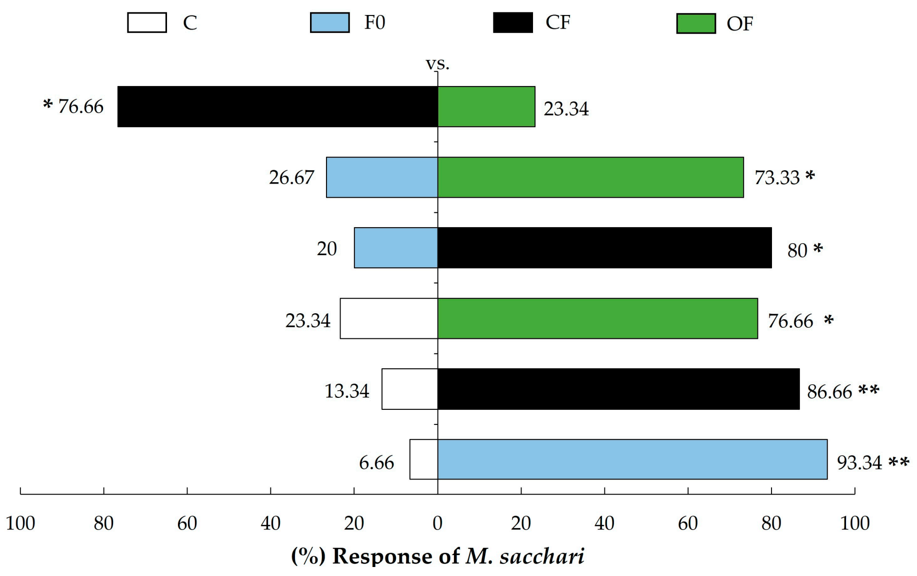

Figure 2. Behavioral bioassays of *M. sacchari* in response to *S. bicolor* extracts. C (control), F0 (plant without fertilization), CF (plant fertilized with ammonium sulfate), and OF (plant fertilized with poultry manure). * denotes significant differences (binomial test: $p < 0.05$); ** denotes significant 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 [24,29,30]. In addition, OF has been shown to modify the abundance of compounds compared to unfertilized tomato plants [30]. 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 leads to increased protein synthesis and, therefore, increased synthesis of secondary metabolites that affect plant defense [31]. CF has been associated with a higher abundance of compounds [24,32], as evinced by the results obtained here 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 [24]. Eleven of the compounds exclusively identified in CF were aromatic compounds, which act as chemical signals involved in the attraction of insects, such as *Drosophila melanogaster*, *Eupeodes corolla*, and *Sitona humeralis* [33–35]. Also, eight of the compounds only identified in the FO are alkaloids, which are associated with nutrient availability and also function as an indicator for host plant acceptance by insects such as *Manduca sexta* and *Formica polyctena* [36,37]. Likewise, these compounds are used by aphids such as *Macrosiphum euphorbiae*, *Macrosiphum albifrons*, *Aphis cytisorum*, and *Aphis jacobeae* to detect their food [38]. Another way that aphids use alkaloids is through feeding on plants with high levels of these compounds to accumulate them in their bodies (sequestration) and serve as a defense against predators [38]. 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* [23,33]. Butyric acid is a key attractant of pests such as *Holotrichia paralela* and *Bubas bison* [39,40]. A compound induced by herbivory, 2-octanol, has been reported as an attractant of *Spilosoma obliqua* [41]. In *Camellia sinensis* plants, 1,3-benzenedicarboxylic acid has been related to N fertilization doses and the infestation of the aphid *T. aurantii* [18]. The most abundant compound in CF was phenol, which acts as an attractant of beetles of the species *B. bison* [40].

In OF, we found butanedioic acid, a component of the silkworm cocoon, which may be involved in its protection [42]. Likewise, higher levels of butanedioic acid were found in chickpea plants and were linked to increased resistance to the leaf miner *Liriomyza cicerina* [43]. Another compound that could have repellent activity against *M. sacchari* is 1,2-benzenedicarboxylic acid, which has potential as a natural insecticide [44]. In *Capsicum* spp. plants infested with *Aphis gossypii* aphids, 1-undecanol has been identified as a compound involved in the plant's defense [45]. 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 *M. persicae* [46], while showing attractant activity towards *Apolygus lucorum*, *Adelphocoris suturalis*, and *Megalurothrips sjostedti* [47,48]. 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 [8]. 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 [49,50]. 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 [15]. 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 [16]. 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 and this preference affected their fecundity and longevity [17,18,51–53].

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 [53]. Likewise, the growth and reproduction of aphids is influenced by the quality of the sap obtained from the host plants, which translates into a higher concentration of amino acids and, as a consequence, aphids attack these plants to a greater extent [54]. In addition, *M. persicae* showed a preference for white clover CF plants over plants fertilized with poultry manure [19]. It has also been reported that maize plants fertilized with synthetic fertilizers had a higher percentage of infestation by a *R. maidis* aphid than plants fertilized with animal manure [20]. In this regard, chemical nitrogen fertilizers enhance the vigorous qualities of plants, making them attractive to insects [55,56]. 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 [57–59].

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 to orient themselves to their host plant, because plant color indicates the food value of the plant [60–62]. However, aphid antennae are as large as their bodies and are provided with sensilla that have olfactory neurons, suggesting that olfactory signals must be important factors in locating host plants [61,63]. 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 an attack by this aphid. Therefore, OF is a sustainable alternative that should be considered as part of a method for pest prevention or to alternatively start combining both types of fertilization.

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. However, having demonstrated the above, and in order to have a broader picture, we recommend further studies to determine the biological activity of the compounds identified here against *M. sacchari*. It is necessary to conduct tests at the physiological level with aphids of *M. sacchari* and also take into account other environmental factors that may influence the *M. sacchari*–*S. bicolor* interaction.

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