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Honey bees and honeydew honey production from oat aphid *Rhopalosiphum padi* on sorghum plants in Egypt

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Abstract

Climate changes may play an important role in the level of plant growth and the degree of their response and sensitivity to insect infections and may affect the distribution and spread of insects. The current study draws attention to the emergence of honeydew honey produced from sorghum plants, which has spread in some governorates of Egypt, including Fayoum, Assiut, and Sohag. Samples of aphids were collected from *Sorghum bicolor* (L.) plants in Fayoum Governorate during the infestation. Samples were also taken from the beehives after the honeydew honey harvest. The aphids were classified, and the honey samples obtained were analyzed for some physical and chemical characteristics. The results of the research showed that the *S. bicolor* were infected with oat-birdcherry aphid, *Rhopalosiphum padi* (L.) (*Hemiptera*: Aphididae) which caused the appearance of honeydew. The honeybee colonies collected them and produced honeydew honey. Most samples showed high electrical conductivity, and physical and chemical analyses were in accordance with Egyptian standard specifications, while acidity showed a noticeable increase, ranging from 36.0 meq. /kg to 66.5 meq. /kg with a range of 48.83 meq. /kg. Thus, some newly harvest honeydew honey samples exceeded the permissible limits of Egyptian standard specifications while molds and yeasts isolation, enumeration and identification from honeydew honey produced a unique marker from containing fungi *Penicillium apimei* that produces penicillin. This study may open further future studies on the possibility of the emergence of another type of honeydew honey in Egypt. It may also be good to track these types and set specifications in a way that their precision is consistent with their composition and production conditions without tampering with the quality of the product.

Introduction

The oat aphid, initially described by Linnaeus in 1758, was classified as *Aphis padi* at that time. Later taxonomic revisions led to its reclassification under the genus *Rhopalosiphum*, as *Rhopalosiphum padi*) (*Hemiptera*.: Aphididae) (Matsishina *et al*. 2021). Linnaeus's work laid the foundation for modern biological taxonomy, and his descriptions remain influential in the classification of organisms.

Aptera 1–2.4 mm, olive mottled with darker green (Schroder, 2014 and Kamal and Ali, 2022). Siphuncular area and nearly also tip of abdomen are rust-red. Juveniles paler, matt, with thin wax dusting. Siphunculus is almost straight, longer than cauda, with subapical contraction and apical flange. Dioecious, alternating between *Prunus* and graminoids (Poaceae, Cyperaceae, Juncaceae) (Singh *et al.,* 2020), rarely on other hosts, exceptionally on Equisetum. Recorded by E. sylvaticum. This species is known for its wide host plant range, with a preference for cereal crops such as oats, barley, wheat, and rye (Starks and Webster, 1985; Andrews and Sinha, 1991; Hockett, 2000; Saheed, 2007; TaheRi *et al.,* 2010; Gavloski and Meers, 2011 and Zafrullah Khan *et al.,* 2017). It also infests various grasses and some trees in the Rosaceae family, including bird cherry (*Prunus padus*) (Rogerson, 1947; Halarewicz and Gabryś, 2012; Nam and Hardie, 2012 and Nestby, 2020), which gives rise to its common name.

The oat-bird cherry aphid (*Rhopalosiphum padi*) is known to infest various cereal crops, including *Sorghum bicolor* (L.), commonly known as sorghum (Nae *et al.,* 2021). Sorghum is a widely cultivated cereal crop known for its resilience to drought and its versatility in uses ranging from human consumption of animal feed and biofuel production.

When sorghum is infested by oat-bird cherry aphids, the aphids feed on the plant's sap using their piercing-sucking mouthparts. This feeding can lead to various detrimental effects on the sorghum crop, including stunted growth, reduced vigor, and yield losses (Rochow and Eastop, 1966; Zapata *et al.,* 2016 and Nae *et al*., 2021). Additionally, aphid infestations can also make sorghum plants more susceptible to certain diseases.

Farmers often employ various management strategies to mitigate aphid infestations and minimize crop damage. These strategies may include the use of insecticides, biological control agents such as ladybugs or parasitic wasps, cultural practices like crop rotation, and the planting of aphid-resistant sorghum varieties.

Research into aphid resistance mechanisms in sorghum and the development of sustainable pest management strategies are ongoing efforts aimed at helping farmers combat aphid infestations effectively while minimizing environmental impact.

Honeydew honey can indeed be produced from sorghum plants. When certain sap-sucking insects, such as aphids (Heidari and Copland, 1993 and Leroy *et al.,* 2011), feed on the sap of sorghum plants, they excrete a sugary substance called honeydew. Bees may then collect this honeydew and convert it into honey (Harris-Shultz *et al.,* 2022).

Sorghum honeydew honey may not be as common or well-known as other varieties, but it is valued by some for its distinctive taste and potential health benefits. It's worth noting that honeydew honey, regardless of its source, is often prized for its higher mineral content and antioxidant (Tomczyk *et al.,* 2022) properties compared to floral honey. Honeydew

honey produced from sorghum honeydew has a unique flavor profile that can be influenced by the specific types of insects feeding on the sorghum, as well as the local flora and environmental conditions. It tends to have a darker color and richer taste compared to floral honey (Persano and Piro, 2004; Yurukova *et al.*, 2008; Primorac *et al.*, 2009; Purcărea *et al.*, 2014 and Seraglio *et al.*, 2019), higher fructose contents than glucose keep it from crystallization (Campos *et al.*, 2003; Bobis *et al.*, 2008; Kaškonienė *et al.*, 2010; Olga *et al.*, 2012 and Seraglio *et al.*, 2019). July 2020-2021 specific information about the first detection research for honeydew honey in Egypt isn't readily available. Research on honey types and production in Egypt tends to focus more on traditional floral varieties, particularly those derived from plants like clover, citrus, and cotton. However, honeydew honey may have been studied in the broader context of honey production and beekeeping practices.

Materials and methods

Samples were collected for the aphid stage from *S. bicolor* leaves surface and honeydew honey was harvested from beehive during season 2021-2022, Fayoum Governorate.

1. Aphid taxonomy:

All stages of aphid classification were carried out with the assistance of specialized scientists, Insect Identification Unit, Plant Protection Research Institute, Agricultural Research Center.

2. Honeydew honey analysis: A. O. A. C.

Five samples of honeydew honey were collected from Fayoum Governorate; Determination of the moisture content of honey was carried out by measuring its refractive index value (Abbe refractometer at 20 ºC) (A. O. A. C., 1995), The optical density of all the samples was determined and the color was measured by using the relation between optical density and USDA standards, as indicated by White (1978), electrical conductivity Based on the method of Vorwhol (1964), Determination of Total Soluble Solids (TSS) of honey (A. O. A. C., 1980), Determination of Hydroxymethylfurfural (HMF) was determined according to Winkler (1955), Determination of pH, free acids, lactone content and total acidity Based on the method of White *et al.*(1962). Sugars analysis using High-Performance Liquid Chromatography (HPLC) according to Ni *et al.* (2016), Determination of the amount of sediment in honey Based on the method of Louveaux *et al*. (1978).

3. Molds and yeast identification:

This work is carried out on Animal Health Research Institute, Mycology Department, and Agricultural Research Center (ARC): molds and yeasts isolation, enumeration and identification according to Baily and Scott (1998); ISO 6611 (2004) and Pitt and Hocking (2009), respectively.

Results and discussion

The taxonomy of aphids, like other organisms, is based on their hierarchical classification, which includes various levels such as kingdom, phylum, class, order, family, genus, and species. Aphids belong to the kingdom Animalia, phylum Arthropoda, class Insecta, order Hemiptera (True bugs), and family Aphididae. Within the family Aphididae, there are numerous genera and species. These are often distinguished based on characteristics such as body shape, coloration, antennae structure, host plant preferences, and reproductive strategies.

1. Aphid taxonomy:

The results of the classification of the aphids that were monitored and samples collected on sorghum in Fayoum Governorate resulted in one of the Governorates in which honeydew appeared (Figure 1). Carried out insect Identification Unit, Plant Protection Research Institute, Agricultural Research Center. Those samples for oat insect *R. padi.*

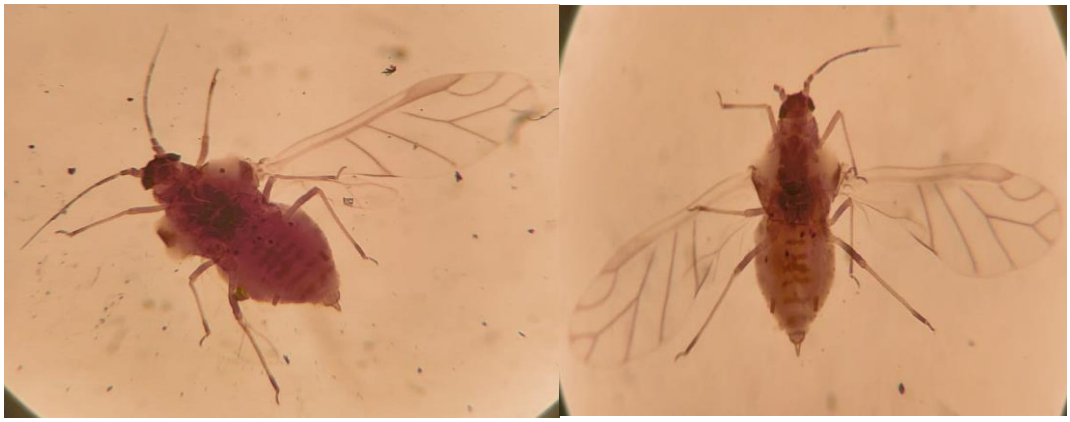

Figure (1): Oat-birdcherry aphid (*Rhopalosiphum padi)*.

According to the difference in the plant authentication of honey between nectar honey and honeydew honey, the international standard specifications set standard limits for this honey, each according to its production conditions.

2. Honeydew honey analysis:

Physical analysis from honeydew honey that produced an infestation of *S. bicolor* with oat-bird cherry aphid (*R. padi*) ranged from 15.8 to 19.0% of moisture percentage with a mean of 17.39%, Based on this, the percentage of total solids was recorded 84.2% to 81.0% as a range with a mean 82.61% and electrical conductivity noted a range from 0.023% to 0.08% with a mean 0.045%, and chemical analysis that included pH showed a range from 4.11 to 4.86 with a mean 4.52; while free acidity and lactone measured as a mean 38.67 meq./kg and 10.17 meq./kg with a range from 25.0 to 61.0 meq./kg, 5.0 to 13.5 meq./kg respectively, and total acidity ranged from 36.0 to 66.5 meq./kg with a mean of 48.83 meq./kg; sugar profiles as fructose, glucose, sucrose, and maltose showed a means 37.53%, 32.38%, 1.41% and 1.95%, respectively. And ranged from 32.7 to 41.3% for fructose, from 28.0 to 36.7% for glucose, <LOQ to 4.33% for glucose and from 1.41 to 3.05 % for maltose. Hydroxy methylfurfural as a sharp indicator for heating or storage of honey indicated that all samples ranged from 0.0 to 32.64mg/Kg with a mean of 8.32mg/Kg (Table 1).; honeydew honey showed absence for pollen grain while marked with some fungus and yeasts that can use a clear fingerprint for sorghum honeydew honey authenticity.

Table (1): Physical-Chemical analysis for honeydew honey from *Sorghum bicolor* yield in Egypt.

Parameters	Samples									Mean	Range
	1	2	3	4	5	6	7	8	9		
Moisture (%)	18.00	18.00	19.00	17.00	16.80	17.50	15.80	16.40	18.00	17.39	15.80: 19.00
Total soluble solid (%)	82.00	82.00	81.00	83.00	83.20	82.50	84.20	83.60	82.00	82.61	84.20: 81.00
Electrical conductivity (%)	0.045	0.049	0.063	0.023	0.047	0.024	0.028	0.080	0.045	0.045	0.023: 0.08
pH	4.52	4.86	4.84	4.69	4.11	4.33	4.30	4.83	4.17	4.52	4.11: 4.86
Free acidity	27.50	38.50	53.00	30.50	25.00	38.00	43.50	61.00	31.00	38.67	25.00: 61.00
Lactone	12.50	12.00	13.50	9.50	11.00	5.00	10.50	5.00	12.50	10.17	5.00: 13.50
Total acidity	40.00	50.50	66.50	40.00	36.00	43.00	54.00	66.00	43.50	48.83	36.00: 66.50
Fructose (%)	36.60	40.10	40.40	37.90	40.50	33.40	34.90	32.70	41.30	37.53	32.70: 41.30
Glucose (%)	31.30	35.40	36.70	31.00	31.90	32.00	30.70	28.00	34.40	32.38	28.00: 36.70
Sucrose (%)	1.55	0.60	0.50	1.07	1.40	1.29	<LOQ	4.33	0.52	1.41	<LOQ: 4.33
Maltose (%)	1.41	1.50	1.50	2.07	3.05	1.64	2.25	2.53	1.60	1.95	1.41: 3.05
HMF (mg/Kg)	1.92	5.78	11.52	0.00	0.00	5.76	32.64	7.68	9.60	8.32	0.00: 32.64

3. Molds and yeast identification:

The total count of molds was recorded as 650cfu/g and the total count of yeast were 650cfu/g, and identification of the molds and yeasts that was based on the morphology of the colony showed that honeydew honey contaminated with *Penicillum apimei, Aspergillus niger, Aspergillus flavus* fungus and *Candida parapsilosis* yeast (Figures 2, 3, 4, 5 and 6).

Figure (2): Honeydew produce from Oat-birdcherry aphid (*Rhopalosiphum padi* L.) from *Sorghum bicolor*.

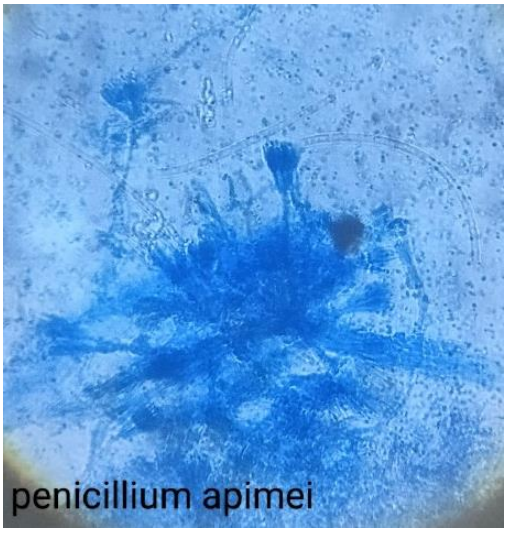

Figure (3): *Penicillium apimei* after Isolation and classification from sorghum honeydew honey.

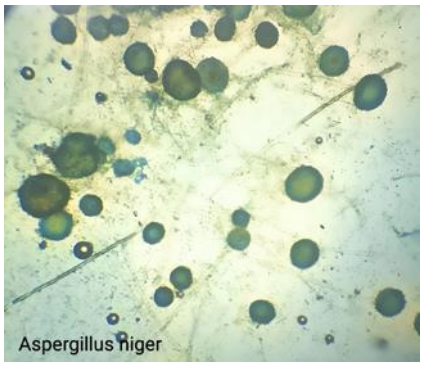

Figure (4): *Aspergillus niger* after Isolation and classification from sorghum honeydew honey.

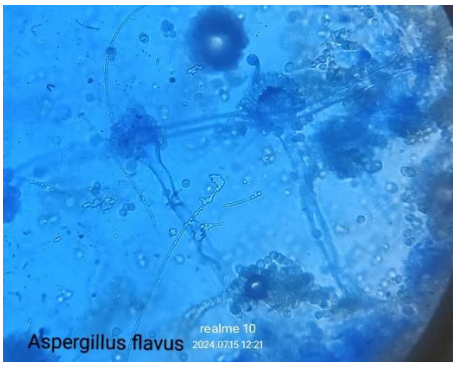

Figure (5): *Aspergillus flavus* after Isolation and classification from sorghum honeydew honey.

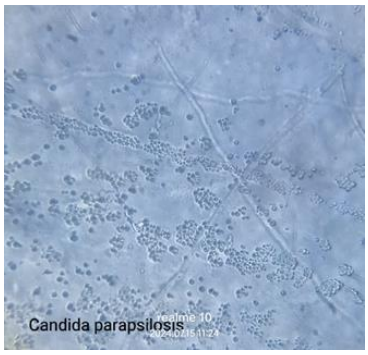

Figure (6): *Candida parasilosis* after Isolation and classification from sorghum honeydew honey.

Sorghum infestation with Oat-bird cherry aphid (*R. padi*) results for samples classified by insect Identification Unit, Plant Protection Research Institute, Agricultural Research Center., may cause aphids to respond to short (UV) and long (Green-yellow) wavelength stimuli during host-plant searching behavior. Although many aphids are attracted to yellow, the bird cherry-oat aphid, *R. padi*, is attracted to green (Schroder *et al.*, 2014), common *S. bicolor* may be susceptible (Starks and Mirkes, 1979) so aphid, sugarcane aphid (SCA; *Melanaphis sacchari*) grows faster on sorghum than a generalist aphid species (Puri *et al.*, 2023), changes in gene expression that correlated with the up-regulation of genes for two glutathione transferases, lactoyl glutathione lyase, peroxidase, catalase, glutathione and quinone oxidoreductase (Park *et al.*, 2006), and climate change may be an

important for bird cherry-oat aphid *R. padi*, attacking many host plants, which be recorded not only in most localities of Egypt but also along a year. Numbers of wintering and summery generations of *R. padi* ranged between 1–27 and 11-42 generations, respectively, under current conditions (Tabikha, 2016).

When aphids are fed sap that passes through the phloem, they absorb essential nutrients and excrete the sugar-rich sticky liquid known as honeydew. These exudates are collected by honeybees and turned into honey known as honeydew, fir or spruce honey. Therefore, the chemical structure of honeydew honey originates from phloem sap (Botanical origin), followed by the honeydew produced from aphid (Zoological origin), and the final product is collected by *Apis mellifera* workers as honeydew honey (Another zoological origin) (Shaaban *et al.,* 2021).

Chemical analysis of honeydew honey samples collected from Fayoum Governorate, which represented the average of moisture as one of the quality measures through which the degree of ripeness of honey within bee hives determined, as well as the extent of the honey's ferment eventuality grounded on the count of yeasts associated with the plant. honeydew honey is characterized by appear low water content, which was on average 17.39%, but did not exceed 19.0% and identically low percentage of water content in Polish honeydew honey was reported by Madejczyk and Barałkiewicz (2008); Rybak-Chmielewska *et al.* (2013); Jara-Palacios *et al., (*2019); Seraglio *et al. (*2019) and Tomczyk *et al. (*2022).

Electrical conductivity clearly indicates, that to classify honey as honeydew, its conductivity ranged (0.023%: 0.08%. with means of 0.045%, this parameter can be helpful in identifying honeydew honey as a result of the high mineral elements in it (Tomczyk *et al.*,2022). Similarly, it represents higher values of electrical conductivity, pH, darker color, pH, and acidity while high values of acidity indicate sugar fermentation with the formation of acetic acid by hydrolysis of alcohol (Geană *et al.,* 2020 and Seraglio *et al.*,2021) upon loading of yeasts (Mccleskey and Oertel, 1950; Belitz and Grosch, 1988 and Sanz *et al.*, 1995). The presence of *Penicillium apimei* fungi that produces penicillin fermentation fungus *Penicillium* (Barreiro *et al.*,2023), it is one of the oldest antibiotics for gram-positive bacteria with an outer cell wall containing a peptidoglycan layer and some negative bacteria that contain peptidoglycan between the membranes while human cells do not contain this component so it makes penicillin penetration easier (Yip and Gerriets, 2024), Which may increase the therapeutic and marketing value of honeydew produced from sorghum and undoubtedly may open the way for many future studies related to this matter.

According to the current Egyptian (Egyptian Standard, 2005) and codex (Codex Alimentarius Commission, 2001) standards, reducing sugars (Fructose and glucose) minimum value for honeydew honey, is 45gm/100gm with a sucrose not more than 10gm/100gm (Puścion-Jakubik *et al*., 2020 and Cavia *et al*., 2002). HMF levels did not exceed the permissible limits and an indicator of freshness, unheated honeydew honey (Farag, 2007, 2013; Karabournioti and Zervalaki, 2001 and Salome *et al*., 2022).

This is the first record of honeydew honey in Egypt, including a general view of its production conditions, chemical composition, and distinctive content of some important

fungal strains such as *Penicillium*, which allows the develop a new vision of its economic and therapeutic importance and employ that in the service of Egyptian standard specifications. It may also illuminate the way for one of the methods used in judging honey, which is the total fungal count, which may not be appropriate in these cases, so it is necessary to classify the fungi and shed light on the sp.

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