



## Original article

Effectiveness of naturally occurring *Aphis gossypii* on tomato plants as a bio-indicator for heavy metals in Riyadh and Hafar Al-Batin, Saudi ArabiaReem Alajmi<sup>a,\*</sup>, Mona Al-Shammari<sup>b</sup>, Rewaida Abdel-Gaber<sup>a,c</sup>, Dina Metwally<sup>a,d</sup>, Manal F. El-Khadragy<sup>e</sup>, Albandary Alrajeh<sup>a</sup><sup>a</sup> Zoology Department, College of Science, King Saud University, Riyadh, Saudi Arabia<sup>b</sup> Department of Biology, Faculty of Science, Hafar Al-Batin University, Hafar Al-Batin, Saudi Arabia<sup>c</sup> Zoology Department, Faculty of Science, Cairo University, Cairo, Egypt<sup>d</sup> Department of Parasitology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt<sup>e</sup> Department of Biology, Faculty of Science, Princess Nourah Bint Abdulrahman University, Riyadh, Saudi Arabia

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## ABSTRACT

Although certain pollutants can be biologically degraded by microorganisms, rendering their impact short-term, others can not be impaired, such that their effect persists. The present study evaluates the effectiveness of using a field-collected aphid, *Aphis gossypii*, as a bio-indicator for heavy metals in tomato farms in Riyadh and Hafar Al-Batin, Saudi Arabia. Heavy metals were selected (Cd, Cu, Zn, and Pb) and measured for comparative screening in field-collected plants, soil, and aphids using inductively coupled plasma-mass spectrometry (ICP-MS). Field-collected aphids from both studied regions were identified as *Aphis gossypii*. In Riyadh, there was no significant difference observed for Cd, Cu, and Zn for all experimental samples, while, Pb was showed differences among samples especially tomato leaves. None of the studied samples in Hafar Al-Batin were showed statistically significant differences in Cd, in reverse to significant differences in the other heavy metals. Comparing concentrations of selected heavy metals between the two studied regions was showed that neither region showed a significant difference in heavy metals except for Cu. This study demonstrates that tomato leaf samples showed the highest concentrations of most studied heavy metals, followed by soil, then aphids. Aphids were utilized as a bio-indicator of heavy metals in the studied regions.

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## 1. Introduction

Environmental pollution, one of the most important and widely distributed challenges worldwide, includes environmental changes with negative impacts on living organisms. It can persist for decades and, in some cases, cannot be mitigated (Ramamohana, 2017). Pollution by heavy metals has become a global concern since their environmental concentrations have increased during the last decades. Since they have accumulated in many environments—including water, soil, and air—resulting from anthro-

pogenic activities (Sanità di Toppi and Gabbrielli, 1999). Metals tend to accumulate in soils, sediments, and living tissues (Arsalanm and Mashhood, 2011). Their impact is widespread due to the ability to be transferred for long distances from their sources (Kehinde et al., 2017). Several studies have demonstrated the long-term, pervasive negative impact of heavy metals on public health, such as their effects on reproduction, the function of organs—including the liver, kidney, and lungs—and their impact on the nervous and digestive systems (Magajim, 2012). Chromium, Copper, Zinc, Nickel, Arsenic, Lead, Cadmium, and Mercury are the most common metal pollutants, of which the latter three metals pose the most significant cause for concern (Stankovic and Stankovic, 2013; Iqra et al., 2015).

Plants represent the most critical route for transferring heavy metals from soil to herbivores (Gazi, 2007; Durán-Lara et al., 2020; Magaji et al., 2020). Suppose herbivorous animals feed on plants contaminated with heavy metals. In that case, these metals tend to accumulate within animal bodies—including those of phytophagous insects—thereby heightening the potential for introduc-

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ing metals into the food chain and increasing their subsequent usage by consumers (Devkota and Schmidt, 2000). The development and application of plants and animals (birds, mollusks, and mammals) as bioindicators have occurred primarily since the 1960s (Holt and Miller, 2011). Several Insecta orders—including Lepidoptera (Devkota and Schmidt, 2000), Coleoptera (Green et al., 2003), Ephemeroptera, Megaloptera, and Trichoptera (Neumann et al., 2003), and Diptera (Jose et al., 2010)—have been used as heavy metal bioindicators. Research has focused on insects as pollution bioindicators for several reasons: they are responsible for many processes in the ecosystem; they are the most abundant animals in almost all ecosystems; and their diversity facilitates the assessment of differences between habitats on an acceptably refined scale (Jose et al., 2010). Many studies have also proved the negative impact of heavy metals on insects, including affecting their developmental period, weight, fecundity, mortality, and populations (Wu et al., 2003; Hayford and Ferrington, 2005).

Aphids (Hemiptera), which are identified as more than 4700 species, are globally considered pests (Remaudiere and Remaudiere, 1997; von Dohlen et al., 2006). *Aphis gossypii* (Glover) (Hemiptera: Aphididae) is a notorious pest distributed globally in an extensive range of host plants (Singh et al., 2014), causing severe damage in commercial fields and urban green landscapes. The honeydew excreted by aphids decreases plants' photosynthetic activity and contaminates fruits, severely reducing their quality (Emden et al., 2007). Moreover, *A. gossypii* is a carrier for many viral diseases that cause severe crop losses (Sileshi et al., 2008). Few studies have evaluated the uptake of heavy metals by aphids and their effect on aphid tissues and biology (Merrington et al., 1997; Green et al., 2003; Harvey et al., 2003). The sparsity of information on the use of aphids as heavy metal bioindicators motivated the present study, which aims to conduct comparative assessments of selected heavy metals (Cd, Pb, Zn, and Cu) in aphids, soils, and plant specimens collected from two geographically distinct localities, Riyadh and Hafar Al-Batin, Saudi Arabia, to investigate whether aphids could be utilized as heavy metal bio-indicators.

## 2. Materials and methods

### 2.1. Study area

A survey was carried out during November 2018 to study heavy metal contamination on two tomato farms located in Riyadh (24°46'27.3540"N, 46°44'18.9096" E) and Hafar Al-Batin (28°26'1.79"N 45°57'29.39"E), Saudi Arabia. Riyadh is located in the center of the Kingdom of Saudi Arabia at 600 m above sea level, featuring several valleys and dunes. Whereas, Hafar Al-Batin, in North-Eastern Saudi Arabia, is located 307.848 m above sea level and is relatively flat except on the slope of the Wadi Al-Batin area.

### 2.2. Sample collection

Samples of soil, tomato leaves, and aphids were collected from both farms with their owners' permission. At the time of collection in Riyadh, temperatures were 30 °C, and relative humidity was 55%; in Hafar Al-Batin, the temperature was 15 °C, and the relative humidity 65%. Soil samples were collected by removing a 15 × 15 cm portion of the soil to a depth of approximately 10 cm, which was then crushed with a wooden pestle and mortar for it to pass through a 2 mm pore sieve (Alajmi et al., 2019). Collected soil and tomato leaf samples were preserved in sterilized polythene bags (Iqra et al., 2015), while aphid insects were preserved in ethanol (Lars and Joachim, 2010). Collected specimens were transferred to the laboratory and stored at 4 °C until they were used for heavy metal investigations (Kehinde et al., 2017).

### 2.3. Morphological identification of collected aphids

Aphid insects were processed for morphological identification using taxonomic keys, according to Blackman (1987).

### 2.4. Evaluation of heavy metals

Samples of soil, tomato leaves, and aphids were oven-dried at 105 °C for 1 h, ignited at 550 °C for 6 h in a muffle furnace, digested with 3 ml concentrated HCl then diluted to the appropriate volumes with deionized H<sub>2</sub>O. Targeted heavy metals were measured using an inductively coupled plasma-mass spectrometer (ICP-MS, Agilent 710-ES Instrument Software, USA), and their dry weights were expressed in mg/g. Heavy metals were detected against wavelengths and normal limits of 214.439 nm and 0.04 ppm for Cd, 327.395 nm and 0.05 ppm for Cu, 220.353 nm and 0.13 ppm for Pb, and 213.857 nm and 0.04 ppm for Zn (Alajmi et al., 2019).

### 2.5. Statistical analysis

Statistical analyses were performed using SPSS software program (version 22.0, SPSS Inc., Chicago, IL, USA). Data were initially tested for normality using the Kolmogorov-Smirnov and Shapiro-Wilk tests. All data were analyzed as a completely randomized design using one-way Analysis of Variance (ANOVA) to study the effect of the experimental sample factor. Duncan's test (Duncan and Duncan, 1955) was used to compare the significance between treatment means at a significance level of  $p \leq 0.05$ , and results were presented as a mean ± standard error (SE).

## 3. Results

### 3.1. Aphid identification

The collected aphid samples were identified as *A. gossypii* according to their morphological characteristics. Two random sequences obtained from each region in the present study were deposited in GenBank under the accession numbers: Riyadh sequences (MN893395 and MN893400) and Hafar Al-Batin sequences (MN893389 and MN893394).

### 3.2. Effect of different experimental samples from Riyadh on metal concentrations

There was no significant differences between the means of experimental samples for Cd, Cu, and Zn (Table 1). In Pb's case, there was a substantial difference between all experimental samples showed that tomato leaves had higher significant means ( $2.45 \pm 0.45$  mg/g) than soil and aphid samples ( $1.09 \pm 0.00$  and  $0.08 \pm 0.01$  mg/g, respectively).

### 3.3. Effect of different experimental samples from Hafar Al-Batin on metal concentrations

The mean concentrations of heavy metals in the samples from Hafar Al-Batin (Table 2) showed no significant Cd differences. However, there were significant differences between the mean concentrations in aphid and others studied for Zn and Pb. On the contrary, Cu showed significant differences between the tomato leaves and other samples from soil and aphid. In general, all heavy metals were high in tomato leaves, except for Zn.

**Table 1**  
Mean values of heavy metal concentrations in different samples from Riyadh.

Experimental samples	Heavy metals concentrations (mg/g) *			
	Cd	Cu	Zn	Pb
Soil	0.05 ± 0.01 <sup>a</sup>	0.18 ± 0.03 <sup>a</sup>	1.41 ± 0.01 <sup>a</sup>	1.09 ± 0.00 <sup>b</sup>
Tomatoes leaves	0.12 ± 0.03 <sup>a</sup>	0.53 ± 0.39 <sup>a</sup>	0.80 ± 0.36 <sup>a</sup>	2.45 ± 0.45 <sup>a</sup>
Aphids	0.12 ± 0.01 <sup>a</sup>	0.45 ± 0.11 <sup>a</sup>	0.74 ± 0.02 <sup>a</sup>	0.08 ± 0.01 <sup>c</sup>

\* Mean values with superscripts (a, b, c) in the same column differ significantly at  $p \leq 0.05$ .

**Table 2**  
Mean values of heavy metal concentrations in different experimental samples from Hafar Al-Batin.

Experimental samples	Heavy metals concentrations (mg/g) *			
	Cd	Cu	Zn	Pb
Soil	0.04 ± 0.00 <sup>a</sup>	0.40 ± 0.05 <sup>b</sup>	1.69 ± 0.25 <sup>a</sup>	1.56 ± 0.40 <sup>a</sup>
Tomato Leaves	0.11 ± 0.04 <sup>a</sup>	4.18 ± 0.24 <sup>a</sup>	1.28 ± 0.38 <sup>a</sup>	1.82 ± 0.39 <sup>a</sup>
Aphids	0.09 ± 0.00 <sup>a</sup>	0.11 ± 0.04 <sup>b</sup>	0.11 ± 0.00 <sup>b</sup>	0.12 ± 0.02 <sup>b</sup>

\* Mean values with superscripts (a, b, c) in the same column differ significantly at  $p \leq 0.05$ .

3.4. Comparisons between Riyadh and Hafar Al-Batin

Statistical analysis of data from Riyadh and Hafar Al-Batin, focusing on heavy elements (Fig. 1) showed no significant differences ( $p \leq 0.05$ ) between the two cities regarding Cd, Zn, and Pb. The only significant difference in the Cu, where the average concentration in Hafar Al-Batin was  $1.56 \pm 0.66$  mg/g, and  $0.42 \pm 0.1$  mg/g in Riyadh.

3.5. Effect of different experimental samples on heavy metal concentrations

In general, heavy metal concentrations were higher in tomato leaves than in soil and aphid samples (Fig. 2). Since the results showed significant differences between the mean heavy metal

concentrations in these samples, the soil significantly different from tomato leaves for Cd and Cu only. In contrast, soil differed significantly from aphids in Cd and Zn only. Additionally, tomato leaves differed significantly from aphids in Cu and Pb only, as shown in Fig. 2.

4. Discussion

A bio-indicator was defined as a species or group of species that readily reflects the abiotic or biotic state of an environment or represents the impact of environmental change on a habitat, community, or ecosystem (Folgarait, 1998). Heavy metal pollution is an environmental risk that, due to natural phenomena or anthropogenic activity, is distributed worldwide (Nieboer and Yassi, 1998), with highly negative impacts on the environment and vari-

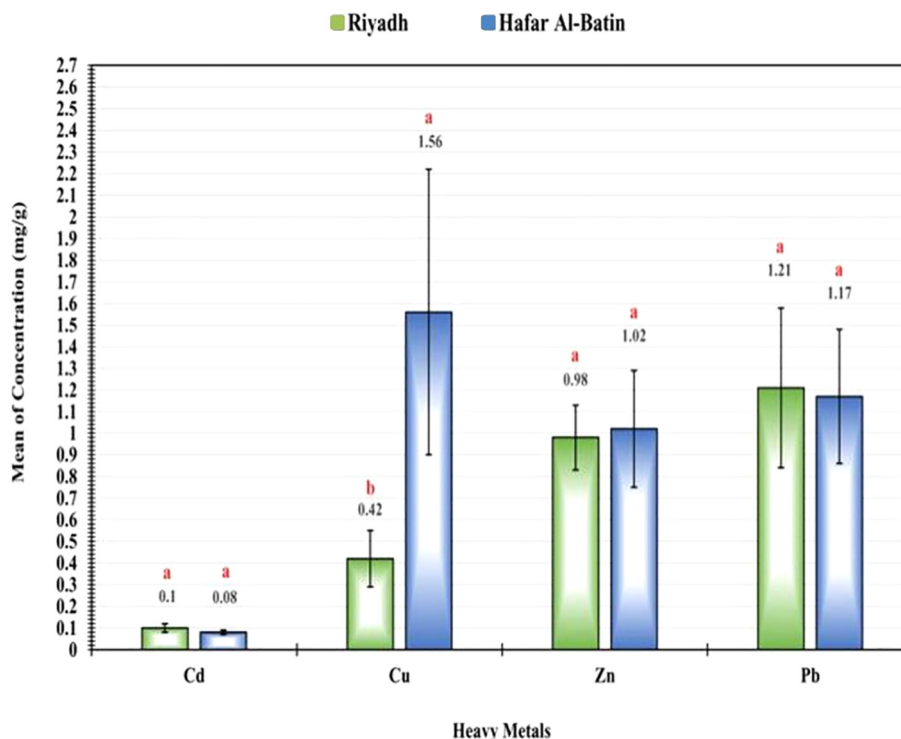


Fig. 1. Comparisons between the city-specific effects of Riyadh and Hafar Al-Batin on heavy metal concentrations at  $p \leq 0.05$ .

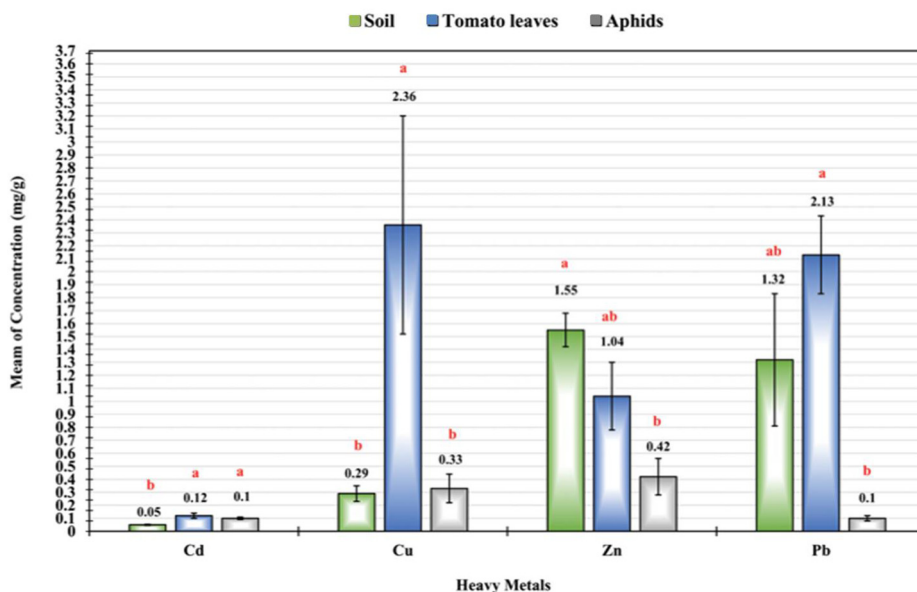


Fig. 2. Heavy metals concentrations in different experimental samples at  $p \leq 0.05$ .

ous organisms within, including humans. In the present study, different samples of soil, aphids, and tomato leaves from two farms were analyzed for the presence of selected heavy metals (Cd, Zn, Pb, and Cu). By studying each source separately, the concentration of Cd in soil was the lowest among the studied elements. In contrast, Cu and Zn were the highest in both regions. This following previous studies conducted in Saudi Arabia, which recorded Zn as the most concentrated and Cd was the least metal in soils from several areas, including Jeddah, Riyadh, Wadi Hanifa, and Al-Kharj (Mahmoud, 2011; Al-Hammad and Abd El-Salam, 2016; Balkhair and Ashraf, 2016; Al-Sihany et al., 2019). Additionally, studies conducted in China, Nigeria, and Korea have also recorded the same results (Yang et al., 2008; Hu et al., 2011; Qiao et al., 2011; Cai et al., 2012; Pingguo et al., 2014; Akande and Ajayi, 2017). However, other studies conducted in Saudi Arabia have recorded Cu as the lowest metal concentration in soil in Jeddah (Al-Mur et al., 2017). Both El-Nakhlawy et al. (2017) and Bai et al. (2018) recorded high Pb concentrations in the soil. In contrast, Cd was the least concentrated in their studies in Saudi Arabia and China, respectively.

In the present study, it was found that, in general, Hafar Al-Batin is more contaminated with Cu, possibly due to the uncontrolled use of pesticides and fertilizers by non-specialist farmers in the region. Global metal concentrations in soil vary due to different anthropogenic and geogenic factors (Butt et al., 2018). Heavy metal solubility, mobility, and availability in soils were controlled by reactions between metals and soil constituents, which depend upon levels of contamination, element origin, metal speciation, and physicochemical properties of the studied soils (Harter and Naidu, 1995).

In the present study, the lowest Cd concentrations were recorded in tomato leaves collected from Riyadh and Hafar Al-Batin. In contrast, Pb and Cu were reported at the highest concentrations in both locations. Our results agreed with other studies in Saudi Arabia (Al-Hammad and Abd El-Salam, 2016; Balkhair and Ashraf, 2016; El-Nakhlawy et al., 2017). Studies conducted in India, China, Brazil, and Egypt also recorded Cd as the least abundant heavy metal present in various plant species (Valitutto et al., 2007; Yang et al., 2008; Yadav and Chandra, 2011; Fawzy et al., 2012). Unfortunately, Cd was considered a highly toxic metal even when present in low concentrations (Emre et al., 2013). Al-Sihany et al. (2019) recorded high concentrations of Zn in several species

of plants (such as *Ochradenus baccatus*, *Rhazya stricta*, and *Ziziphus spina-christi*) in Wadi Hanifa. Studies in Mahad AD'dahab (Al-Farraj and Al-Wabel, 2007), Riyadh, Dammam, Jazan, Tabuk (Ali and Al-Qahtani, 2012), and Jeddah (Balkhair and Ashraf, 2016), showed that, when compared with concentrations of Cu, Cd, and Pb in plants, Zn showed the highest concentrations. In Poland, Markunas et al. (2018) recorded higher concentrations of Zn than Cu and Cd in *Typhaceae*. In China, Bai et al. (2018) recorded high concentrations of Zn compared to Cu and Pb in *Eichhornia crassipes*. The difference in heavy metal concentrations in these cited studies could be due to different plant species' tendencies to absorb different concentrations of heavy metals (Awoyemi et al., 1996). Plants with the capacity to accumulate too high levels of specific heavy metals show increased concentrations of these metals in leaf tissues (Stolpe et al., 2017).

The presence of heavy metals in tomato leaves reflects vegetable species' ability to absorb metals through their root system—particularly those essential for development and growth—from soil and water. Vegetables accumulate considerable quantities of heavy metals—especially Pb, Cr, Cu, and Zn—in their roots and leaves (Stankovic and Stankovic, 2011; Piscitelli et al., 2020). The variation in heavy metal concentrations in plants between our study and others may be ascribed to variable heavy metal concentrations in different localities. As we have demonstrated here, both soil and tomato leaves carry significant concentrations of most heavy metals. This could be due to the watering system, which may play a role in transporting heavy metals over long distances and using wastewater in irrigation for extended periods, which may lead to an accumulation of heavy metals in agricultural soils and plants (Al-Mur et al., 2017). Wastewater, considered a rich source of organic matter and other nutrients, leads to elevated concentrations of heavy metals, such as Fe, Mn, Cu, Zn, Pb, Cr, Ni, Cd, and Co in the receiving soil. The use of wastewater in irrigation therefore leads to the contamination of soil and plants in the food chain (Mchuweti et al., 2006). Irrigation with industrial waste could explain the high concentrations of Pb and Cd in soil and tomato leaves since industrial waste usually contains high amounts of such metals (Dikinya and Areola, 2010). Variation in the concentrations of measured heavy metals in plants is due to differences in the uptake of these toxic metals according to their synergistic or antagonistic effects, which depend on their concentration in soil (Alina and Pendias, 2001).

The role of insects in the trophic chain is often as food for other organisms and may therefore constitute an essential pathway for the bioaccumulation of heavy metals. Invertebrates, including insects, are good models for studying the toxicity of heavy metals and, as such, are essential bioindicators of environmental contamination (Nummelin et al., 2007). Accumulation of heavy metals in herbivores' bodies through feeding on heavy metal-contaminated plants heightens the potential for the introduction of metals into food chains and their subsequent usage by consumers (Smith, 1995). Herbivore insect–host plant relationships can be considered model systems for studying the potential of heavy metal effects at population and individual levels over short timescales. The importance of using phytophagous insects as bioindicators for heavy metal accumulation—especially phloem feeders such as aphids—has been shown in several laboratories, greenhouse, and field studies (Crawford et al., 1990). Our results showed that, in aphids collected from Riyadh, Pb was present at the lowest concentration of all studied heavy metals. In contrast, the difference between other metals was statistically non-significant. By comparison, in Hafer Al-Batin aphids, the heavy metal at the highest concentration was Pb. Surprisingly, our results showed that the examined aphids had lower concentrations of Zn and Pb compared to soil and plants. Butt et al. (2018) showed that aphids of the species *Sitobion avenae* were not a good Cd bioindicator. Naikoo et al. (2019) documented that an increased elimination of Pb via aphid excreta (honeydew) and pupal exuviae in a dose-dependent manner suggested that these were possible detoxification mechanisms operating at two different trophic levels enabling the control of Pb bioaccumulation along the food chain. The potential for the uptake of heavy metals by aphids and other herbivorous insects were demonstrated in several laboratory and field studies, showing that aphids accumulate heavy metals to a greater degree in their tissues than in the host plant body (Crawford et al., 1995; Merrington et al., 2001). However, insects differ in their ability to accumulate specific heavy metals, and such abilities may be heavy metal-specific (Gazi, 2016).

It could be concluded that the aphid species *A. gossypii* could be used as a bioindicator for the presence of certain heavy metals (Cd, Cu, Zn, and Pb). However, tomato leaves were better bioindicators for these heavy metals. Since different insect species tend to accumulate different heavy metals, further studies should evaluate the uptake of other heavy metals in *A. gossypii*. According to WHO (2004) standards, our results showed that all tested heavy metal concentrations were above limits considered acceptable for the environment. These results should motivate the government and related persons and bodies to put in place control and mitigation strategies to reduce the concentration of heavy metals in the environment and eliminate their sources.

## 5. Significance

The present study provided data about the ability of aphids in the application of environmental bioindicators.

## Declaration of competing interest

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

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## Ethical approval

Because aphid is a worldwide pest insect, and due to HM's negative impact on living organisms, such investigation was welcomed by farmers. We took the permit from both farms' owners to collect specimens without affecting the crop of the field. Also, the present study was approved by the Institutional Committee of Post-Graduate Studies and Research at King Saud University (Saudi Arabia)

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