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SOURCES OF RESISTANCE AND TRICHOME ANALYSIS OF PHILIPPINE INDIGENOUS *Hoya* SPECIES AGAINST THE MILKWEED APHID, *Aphis nerii* Boyer de Fonscolombe (HEMIPTERA: APHIDIDAE)

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ABSTRACT

The Philippines is one of the centers of diversity of *Hoya* in the world and an extensive collection of indigenous *Hoya* species is conserved at the Institute of Plant Breeding (IPB), University of the Philippines, Los Baños (UPLB). In maintaining these *Hoya* species inside the screenhouse, the major insect pest noted to attack the plants is the milkweed aphid, *Aphis nerii* Boyer de Fonscolombe. Thus, in this study, *Hoya* species were evaluated for resistance against this insect pest. Among the 45 *Hoya* species evaluated, 11 showed promising results wherein no aphid infestation was consistently observed during the three evaluation trials, namely *H. aurigueana*, *H. carnosa*, *H. coriacea*, *H. diversifolia*, *H. greenii*, *H. imperialis*, *H. madulidii*, *H. obscura*, *H. odorata*, *H. paziae*, and *H. pubicalyx*. As the first line of plants defense against herbivory, four resistant (*H. madulidii*, *H. pubicalyx*, *H. carnosa*, and *H. obscura*) and two susceptible (*H. buotii* and *H. meliflua*) species were sampled for analysis of their trichome traits. The longest mean trichome length was observed in *H. carnosa* (0.38 mm), followed by *H. madulidii* (0.33 mm), and lastly, *H. pubicalyx* and *H. obscura* (0.15 mm). The highest mean trichome density (at 1.64 mm^2 microscopic field) was recorded in *H. madulidii* (54.45), followed by *H. pubicalyx* (7.5), and lastly, *H. carnosa* (3.55) and *H. obscura* (3.45). In contrast, no trichome was observed in the susceptible species *H. buotii* and *H. meliflua*. Hence, trichomes are one of the important resistance traits of *Hoya* against the milkweed aphid. To our knowledge, this is the first study on resistance evaluation in *Hoya* and investigating the role of its trichomes against the milkweed aphid. The results of this study can aide in the management of milkweed aphids and in developing improved *Hoya* cultivars with resistance to insects such as milkweed aphids.

Keywords: *Hoya*, milkweed aphid, resistance, trichomes

INTRODUCTION

Hoya or wax plant is highly prized horticulturally by local and foreign plant collectors due to the unique beauty of their showy, star-shaped, and waxy flowers, as well as their exotic, succulent, and waxy leaves (Rodda *et al.* 2020; Rahayu *et al.* 2018). Their remarkable flowers are pentamerous, sympetalous, and complex in morphology (Wanntorp *et al.* 2011). It is gaining popularity in the country as evident by the burgeoning online selling and social media pages, and increasing number of commercial gardens catering to its trade, both locally and internationally.

This ornamental plant belongs to the Apocynaceae family which comprises the largest genus with around 350 - 500 identified species (Kloppenburg *et al.* 2012; Rodda 2015). The Philippines, along with Borneo and New Guinea, is recognized as one of the centers of *Hoya* diversity (Cabactulan *et al.* 2017). As of April 2022, the Co's Digital Flora of the Philippines reported more than 200 species and subspecies of *Hoya* with Philippine origin (Pelser *et al.* 2022). Most of these *Hoyas* are endemic species and distributed at all altitude levels (Kloppenburg 1991; Maranan & Diaz 2013).

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The Institute of Plant Breeding (IPB) at the University of the Philippines at Los Baños (UPLB) holds an extensive collection of various indigenous *Hoya* species discovered or acquired throughout the country. In maintaining these *Hoya* species, the major insect pest noted to attack the plants is the yellow aphid or milkweed aphid, *Aphis nerii* Boyer de Fonscolombe.

Aphis nerii is a polyphagous, phloem feeder, viviparous, and parthenogenetic insect species which is a common pest of some important ornamentals belonging to the Apocynaceae and Asclepiadaceae families (Groeters & Dingle 1989; McAuslen 2001). The milkweed aphids also have short life cycle (involving five nymphal instars) and are highly fecund (McAuslen 2001). They are sucking insects which can also act as virus vectors and their colony secretes honeydews which promote growth and development of black fungal sooty mold.

One of the well-known insect deterrent plant traits which is also considered as the first line of plant defense against herbivory is the trichome. Trichomes vary in size, shape, type, and density on different plants. Carandang *et al.* (2013) reported morphological variations in floral trichomes of different color and forms of *Hoya mindorensis*. Likewise, Basir *et al.* (2022) revealed different types of floral trichomes present in three Philippine *Hoyas*.

Studies correlating to the role of trichome traits to insect resistance have been reported in many plant species (Handley *et al.* 2005; Dalin *et al.* 2008; Xiao *et al.* 2017) with studies on biochemical and molecular bases of resistance mostly focused on the model plant *Arabidopsis thaliana*. Hence, trichomes can play an important role in host plant resistance against insects.

In this study, the Philippine indigenous *Hoya* species conserved at the IPB, UPLB and maintained in the screenhouse were evaluated for resistance against milkweed aphids under natural infestation. The trichomes of some *Hoya* species were also analyzed as one of the possible resistance mechanisms of *Hoya* against milkweed aphid.

MATERIALS AND METHODS

Evaluation of Indigenous *Hoya* Species

A total of 45 indigenous *Hoya* species (Table 1) collected/acquired throughout the country and maintained in the screenhouse of the IPB, UPLB were evaluated. Inside the screenhouse, the milkweed aphids (*Aphis nerii* Boyer de Fonscolombe) freely infest the plants and were allowed to choose for their preferred host.

These *Hoya* species were evaluated for three trials and rated according to the developed rating scale ranging from 0 to 3 based on the number of milkweed aphids infesting the plant: 0 = no aphid (resistant; R), 1 = 1 to 3 aphids (least susceptible; LS), 2 = 4 to 15 aphids (moderately susceptible; MS) and 3 = more than 15 aphids (highly susceptible; HS).

Table 1 The Philippine indigenous *Hoya* species used in this study

Species	Source	Remarks
H. albida	Polillo Island, Quezon Province	Small-whitish/yellowish flower packed with minute hairs
H. alwitriana	Mindanao	Yellow flower with white center and with unusually bigger leaves
H. aurigueana	Quezon Province	Light yellow flower with rosy tip corolla lobe
H. benitotanii	Philippines	Presence of thick leathery pointed leaves
H. bicolensis	Bicol region	Pink-flowered *Hoya* with slightly sunken corona lobe
H. bifunda	Polillo Island, Quezon Province	Small-sized pink *Hoya* flower
H. bilobata	Siniloan, Laguna	One of the smallest *Hoya* flower forming a puff ball
H. bordenii	Philippines	Orange-red flower with deeper red colored center
H. buotii	Quezon Province	Presence of whitish hair/pubescence on the flower
H. burtoniae	Montalban, Rizal	Presence of compact fuzzy leaves
H. cagayanensis	Quezon Province	All white-colored *Hoya* flower with white sap
H. camphorifolia	Siniloan, Laguna	Prominent veined leaves similar to camphor tree
H. cardiophylla	-	Prominent heart-shaped leaf base
H. carnosa	Bicol region	Known as grandmother's wax plant
H. carnosa variegated	-	Undulating creamy-white leaf edges
H. celata	Real, Quezon	White velvety flower

Table 1 (Continued)

H. concava	Real, Quezon	Concave-shaped leaves
H. coriacea	Mount Apo, Davao	Grows vigorously forming a large umbel of hairy flower
H. crassicaulis	Panay Island	Perfect ball-shaped flower umbel
H. curtisii	-	Spade-shaped leaves with variegation
H. cutis-porcelana	Biliran, Samar	Porcelain-like flower
H. davidcumingii	-	Contrasting flower color of pink and yellow with mild caramel scent
H. densifolia	-	Strong-scent *Hoya* flower
H. diversifolia	Indonesia	Velvety textured-flower
H. greenii	Mount Apo, Davao	Highly erect corona with highly reflex corolla
H. halconensis	Mindoro Province	Flower with warm violet tinge and densely hairy
H. heuschkeliana	Mount Isarog	Urn-shaped *Hoya*
H. imperialis	Palawan	Large-flower *Hoya*
H. incrassata	Siniloan, Laguna	Strong scent *Hoya* forming a ball-shaped umbel
H. juannguoana	Palawan	*Hoya* flower with strong spicy fragrance
H. lacunosa	Siniloan, Laguna	Strong-scent *Hoya* with slightly sunken leaf
H. landgrantensis	Real Quezon	Light yellow colored *Hoya* flower
H. lazaroi	Mount Banahaw, Quezon	Few example of non-twining *Hoya*
H. lucardenasiana	Siniloan, Laguna	Oval shaped leaves with reddish margin
H. madulidii	Pollilo Island, Quezon Province	Presence of minute hairs on the leaves and stem
H. marvinii	Aurora Province	Fuzzy ball-like flower
H. meliflua	-	Exudes nectar which stains the flower
H. memoria	-	Profuse bloomer with waxy, light speckled leaves
H. obscura	Laguna	Pendant-type *Hoya*
H. odorata	Mount Makiling, Laguna	Citrus and sweet-scented *Hoya*
H. paziae	Panay Island	Bushy-type *Hoya*
H. pimenteliana	Siniloan, Laguna	All white colored *Hoya* flower with transparent sap
H. pubicalyx	Polillio Island	Proliferous bloomer
H. siariae	Tayabas, Quezon	Campanulate type flower
H. surigaoensis	Mindanao	Prominent reddish colored leaves

Trichome Analysis of *Hoya* Species

Trichomes were observed under a digital dissecting microscope with attached camera (Optika microscope model SZM-LED1, Italy). Trichome measurements (length and density) and image viewing in the computer were performed using the microscope's complementary software (OptikaISview version 3.6.6).

Young shoots from selected species were used in the trichome analysis. Trichome density was determined by counting the number of trichomes from twenty (20) microscopic fields (20 random parts of the shoot) measuring 1.48 x 1.11 mm (or 1.64 mm^2) at 45x magnification. On the other hand, using 50 trichomes, trichome length (in mm) was measured from the base toward the tip of a trichome using the above-mentioned microscope software. Data analysis was performed through a one-way analysis of variance (ANOVA) followed by Tukey's test at $P < 0.05$. Data were graphed using Prism version 8.1.1 (GraphPad Software, Inc.).

RESULTS AND DISCUSSION

Hoya is an important ornamental plant species with high endemism and diversity in the Philippines. At the IPB, UPLB, the collection of *Hoya* species are maintained in a screenhouse (Fig. 1a), wherein they are naturally exposed to the milkweed aphids (Fig. 1b) which are the predominant insect pests in the germplasm.

Aphids usually prefer to feed in the plant terminal growth (Hall & Ehler 1980) due to the succulent nature of *Hoya* species (Fig. 1b). As sap-sucking insects, they tend to remain attached (through their rostrum and stylets) on their preferred hosts (Fig. 1c & 1d). However, once overcrowding and/or decrease in plant quality occur (e.g., reduced sap amount, plant senescing, etc.), the aphids usually develop wings (Fig. 1c) for migration and transfer to another host plants for survival (McAuslane 2001; Harrison & Mondor 2011). Throughout the three evaluation periods, some *Hoya* species remained infested while others were not, given the polymorphic capabilities of the insect which allows it to

choose the plants where it would like to stay (or stay away).

An evaluation was performed to identify *Hoya* species with potential resistance to milkweed aphid. Among the 45 species evaluated, no aphid infestation during the three evaluation trials was consistently observed in 11 *Hoya* species, namely, *H. aurigueana*, *H. carnosa*, *H. coriacea*, *H. diversifolia*, *H. greenii*, *H. imperialis*, *H. madulidii*, *H. obscura*, *H. odorata*, *H. paziae*, and *H. pubicalyx* (Fig. 2). Thus, these *Hoya* species are putative resistant species which may be least or not preferred by the milkweed aphids. In contrast, presence or infestation of milkweed aphids were observed in the remaining 34 *Hoya* species during the course of three evaluation trials (Fig. 2).

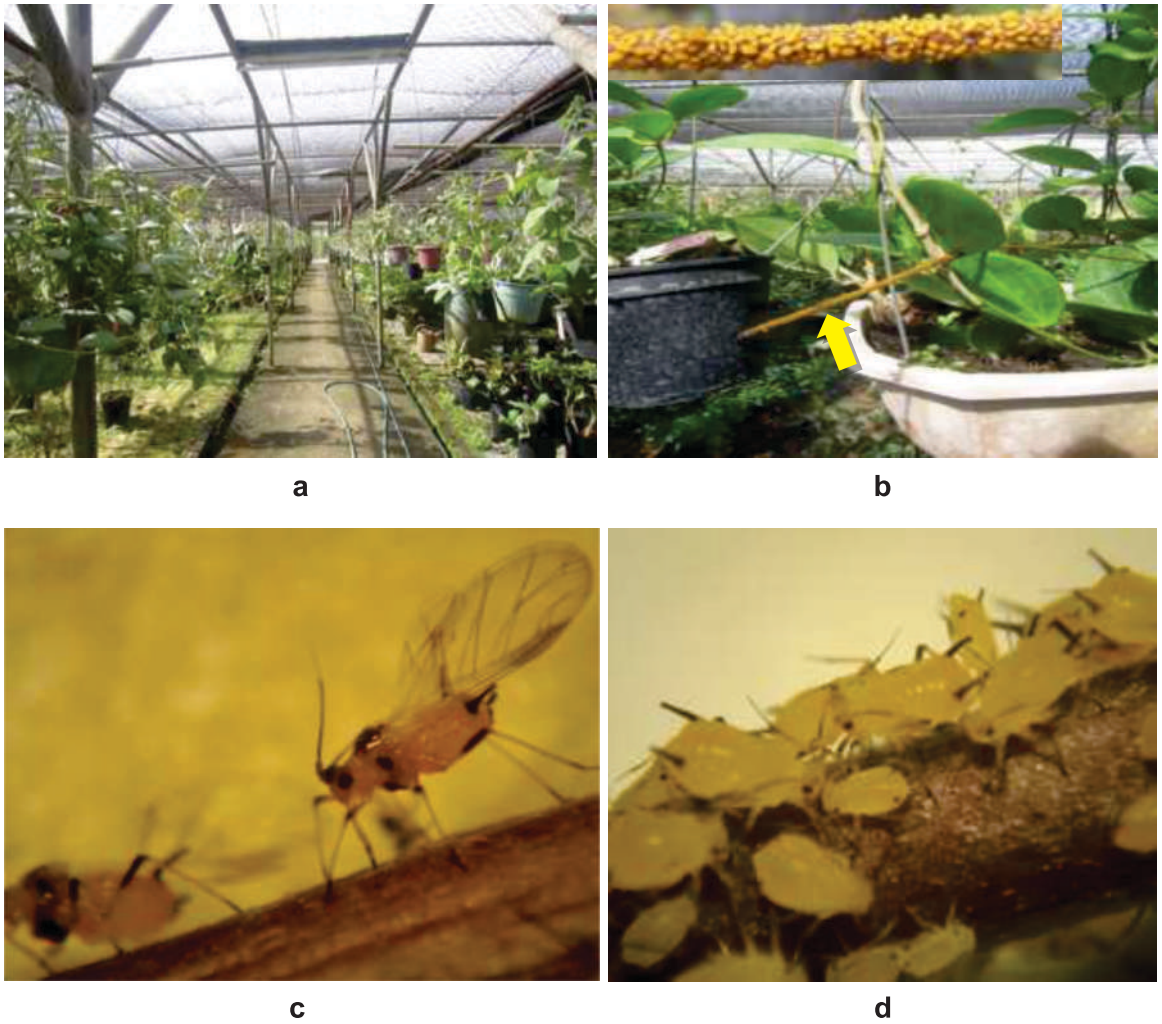

Figure 1 Indigenous *Hoya* species conserved at the IPB screenhouse
Notes: (a) exposed to milkweed aphids (*Aphis nerii* Boyer de Fonscolombe); (b) aphids infestation at the plant terminal growth (arrowhead) with a zoomed image inlaid on top; (c) feeding of sap-sucking winged; and (d) wingless aphids.

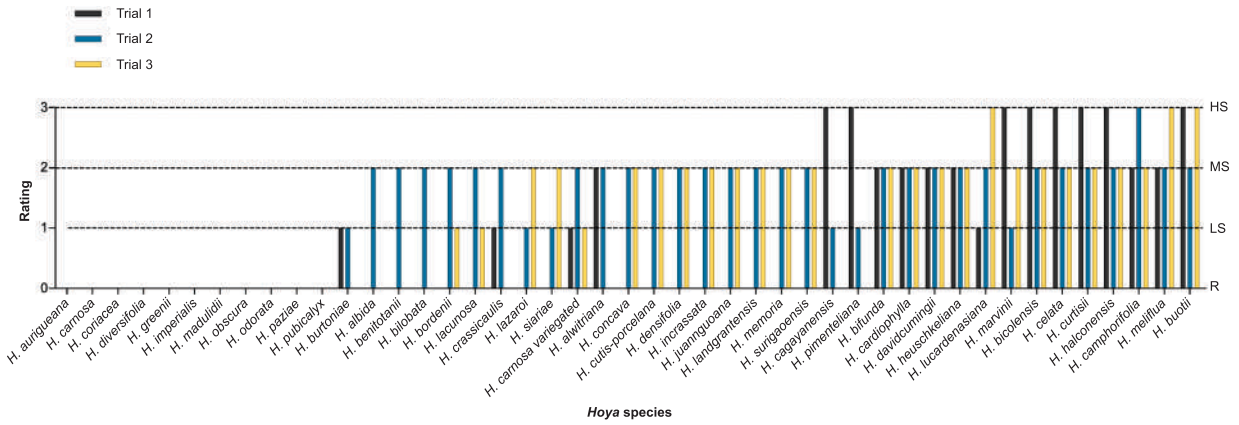

Figure 2 Evaluation results of 45 indigenous *Hoya* species against the milkweed aphids for three trials inside the IPB screenhouse
Notes: R = resistant; LS = least susceptible; MS = moderately susceptible; HS = highly susceptible.

Insect resistance mechanisms in plants are diverse which include the roles of trichomes, secondary metabolites, defensive compounds, etc. (War *et al.* 2012). Trichome-mediated plant resistance against aphids has been reported in various crops, such as cotton, wheat, alfalfa, potato, among others (Peter *et al.* 1995).

Plant trichomes can deter aphids by providing physical barriers leading to limited contact to the plant or by producing toxic trichomal chemicals (Peter *et al.* 1995). Although there might be other mechanisms of resistance in *Hoya*, we sampled four resistant species, namely, *H. madulidii*, *H. pubicalyx*, *H. carnosa*, and *H. obscura* (Fig. 3) for analysis of their trichome traits as the first line of defense against insects.

In addition, the sampled *Hoya* species appeared to be the least preferred species compared to the glabrous or trichome-less species, such as *H. buotii* and *H. meliflua* (Fig. 4), wherein infestation of milkweed aphids were consistently observed for three evaluation trials (Fig. 2).

Figure 3 Examples of milkweed aphid-resistant *Hoya* species showing inflorescence: (a) *H. madulidii*; (b) *H. pubicalyx*; (c) *H. carnosa*; and (d) *H. obscura*

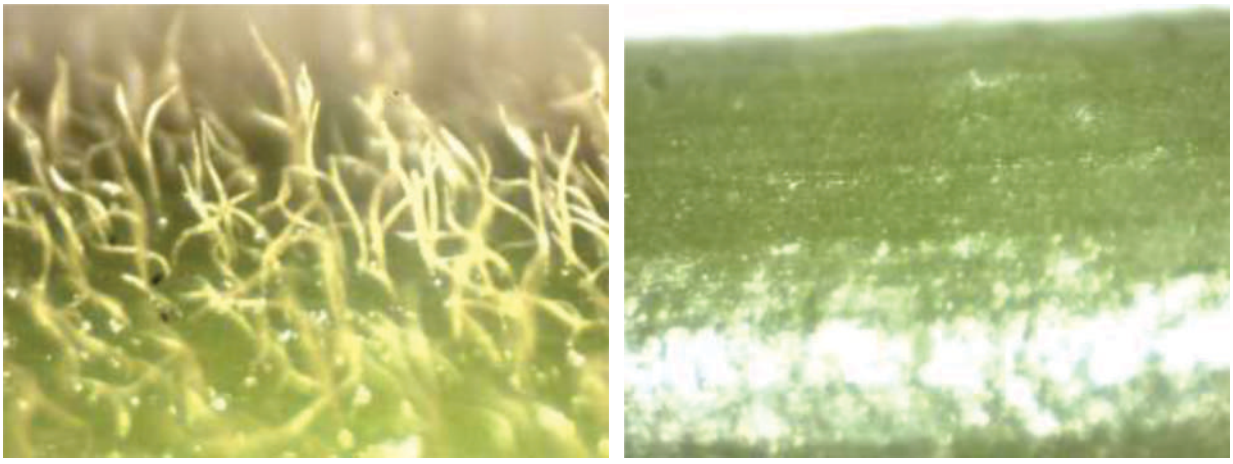

Figure 4 Shoot of *H. madulidii*: (a) showing trichomes and (b) *H. buotii* with no trichomes (glabrous type) at 30x magnification

Statistical analysis of trichome traits between these *Hoya* species with contrasting resistance to milkweed aphid showed that *H. carnosa* has the longest trichome ranging from 0.20 to 0.61 mm (mean of 0.38 mm), followed by *H. madulidii* with 0.23 to 0.43 mm (mean of 0.33 mm) trichome length, and lastly, *H. pubicalyx* and *H. obscura* with mean trichome length of 0.15 mm (Table 2).

For the trichome density, the highest number of trichomes was observed in *H. madulidii* which ranged from 43 to 65 (mean of 54.45), followed by *H. pubicalyx* which ranged from 3 to 13 (mean of 7.5), and lastly, *H. carnosa* and *H. obscura* with no significant difference on trichome density (mean of 3.55 and 3.45, respectively) (Fig. 5).

Thus, *H. obscura* has relatively fewer and shorter trichomes compared to *H. pubicalyx* and *H. carnosa*, while the latter two species have relatively higher trichome density and longer trichomes, respectively, than the former. Meanwhile, combination of both trichome traits (highly dense and long trichomes) is observed in *H. madulidii*. On the other hand, the susceptible *H. buotii* and *H. meliflua* species have no trichome.

Table 2 Trichome length of six *Hoya* species (n = 50)

Species	Length (mm)		
	Minimum	Maximum	Mean ± SEM
H. carnosa	0.20	0.61	0.38 ± 0.01 [a]
H. madulidii	0.23	0.43	0.33 ± 0.01 [b]
H. pubicalyx	0.10	0.20	0.15 ± 0.03 [c]
H. obscura	0.10	0.25	0.15 ± 0.01 [c]
H. buotii	0.00	0.00	0.00 ± 0.00 [d]
H. meliflua	0.00	0.00	0.00 ± 0.00 [d]

Note: Means in column followed by the same letters are not significantly different at 5% level of Tukey's test.

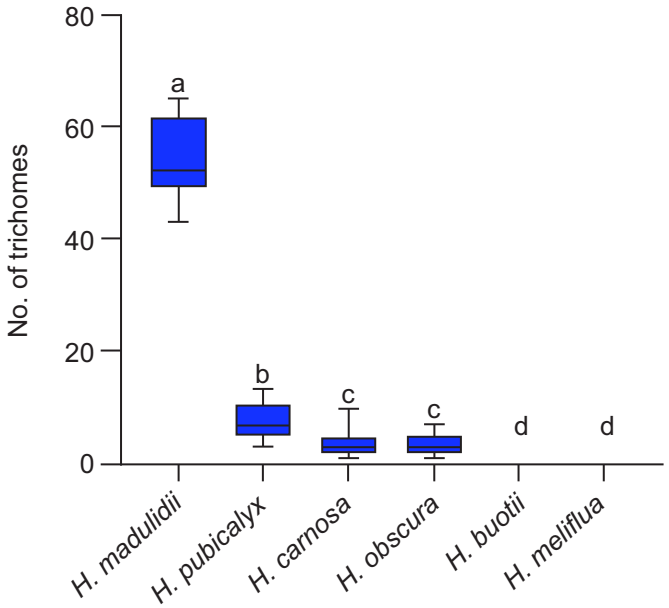

Figure 5 Trichome density of six *Hoya* species at 1.64 mm^2 microscopic field
Note: Means followed by the same letters are not significantly different at 5% level of Tukey's test.

CONCLUSION

Eleven (11) indigenous *Hoya* species exhibited promising resistance against the milkweed aphid (*Aphis nerii* Boyer de Fonscolombe). The presence of trichomes in *H. obscura*, the relatively high trichome density in *H. pubicalyx*, long trichomes of *H. carnosa*, and combination of both traits in *H. madulidii* likely provided the observed resistance. The findings suggest that susceptibility could be attributed also by lack of trichomes as observed in trichome-less *H. buotii* and *H. meliflua* species. Thus, trichome is one of the important traits and one of the possible resistance mechanisms of *Hoya* against milkweed aphid. To our knowledge, this is the first study on evaluation and resistance of *Hoya* species against the milkweed aphid and elucidating the role of *Hoya* trichomes. The results of this study can be used for managing milkweed aphids and in developing improved *Hoya* cultivars with resistance to insects, such as milkweed aphids.

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