INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
19–0844/202x/00–0–000–000
DOI: 10.17957/IJAB/15.1435
http://www.fspublishers.org

Full Length Article

Susceptibility of *Bemisia tabaci* (MEAM1) Gennadius (Hemiptera: Aleyrodidae) to Deltamethrin, Thiamethoxam and Pyriproxyfen in Oman

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Received 21 May 2019; Accepted 10 January 2020; Published _____

Abstract

Bemisia tabaci is an important insect pest of many crop plants and also transmits several viruses including *Tomato Yellow Leaf Curl Virus* (TYLCV). Intensive use of insecticides has led to the development of insecticide resistance in *B. tabaci.* This study was conducted to develop baseline susceptibility of *B. tabaci* eggs, nymphs and adults to three commonly used insecticides (deltamethrin, thiamethoxam and pyriproxyfen) on tomato, eggplant and cucurbits in Oman. Leaf-dip bioassay method was used to expose eggs, nymphs and adults of *B. tabaci* against insecticide treated leaves in laboratory studies. Lethal concentrations (LC_{10}, LC_{50} and LC_{90}) were calculated for the three life stages of SQU-1 (resistant-conventional) and Pairidaeza (susceptible-organic) strains. A very low level of resistance to deltamethrin was detected in adults (RF = 4.3) and nymphs (RF = 2.7). The field application rate of deltamethrin resulted in reduced mortality of 47.0–59.6% in the SQU-1 strain and 62.7–67.4% in the Pairidaeza strain. A very low level of resistance was also observed for thiamethoxam in adults (RF = 2.2) and nymphs (RF = 2.7). The determined baseline susceptibility levels of *B. tabaci* eggs, nymphs and adults against deltamethrin, thiamethoxam and pyriproxyfen can be used in resistance monitoring in Oman by comparing any shift from these LC_{50} values in future. These findings can be helpful to initiate resistance management programs to slow the resistance evolution in *B. tabaci* in Oman. © 2020 Friends Science Publishers

Key words: *Bemisia tabaci*; Toxicity; Deltamethrin; Thiamethoxam; Pyriproxyfen

Introduction

The tobacco/silver leaf whitefly, *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) occurs in most parts of the world; is an important agricultural pest, and causing economic damage to crops (Palumbo *et al.* 2001). It has been listed as one of the world's 100 worst invasive alien species (Lowe *et al.* 2000) and infesting more than 600 host plant species (DeBarro *et al.* 2011; Li *et al.* 2011) including cassava, cotton, sweet potatoes, tobacco, tomatoes (https://www.cabi.org/isc/datasheet/8927), and cucurbits (Dennehy *et al.* 2010; Cameron *et al.* 2014) . Adults and nymphs of *B. tabaci* damage plants by sucking nutrients and through the excretion of honeydew which reduces plant growth and yield by interfering photosynthesis (Jones 2003). *B. tabaci* also transmits more than 100 different plant viruses during feeding (Hogenhout *et al.* 2008) including Tomato Yellow Leaf Curl Virus (TYLCV) in tomato (Berlinger 1986).

B. tabaci was reported as a serious pest of tomato in 1997 from Oman (Azam *et al.* 1997) and was also recorded from eggplant, cucumber and melon (Kaakeh *et al.* 2007). The TYLCV was isolated from tomato in 2008 (Khan *et al.* 2008). The infected plants my show vein/inter-vein or leaf yellowing, yellow blotching or mosaic of leaves, leaf curling or crumpling, leaf vein thickening, leaf enations, leaf cupping, stem twisting and plant stunting (https://www.cabi.org/isc/datasheet/8927). *B. tabaci* is a cryptic species complex with more than 40 morphologically indistinguishable species (Hu *et al.* 2017). Within *B. tabaci* species complex, the Mediterranean (MED or biotype Q) and Middle East-Asia Minor 1 (MEAM1 or biotype B) species are highly invasive and cause substantial economic damage to crops (Luo *et al.* 2002; Chu *et al.* 2006; Vassiliou *et al.* 2011).

B. tabaci has been usually controlled with carbamates, OPs and pyrethroids representing 50 conventional insecticides (Horowitz *et al.* 2011). Resistance in *B. tabaci* to insecticide has developed due to the repeated applications of the same active ingredients and their use in larger quantities (Denholm *et al.* 1998; Horowitz *et al.* 2007). Repeated applications of insecticides exert high selection pressure which increases the rate of resistance development and also crop production cost (Naranjo and Ellsworth 2009).

B. tabaci has tremendous potential to develop resistance to different insecticides (Horowitz *et al.* 2007) and has been reported to develop resistance to 64 active ingredients of different groups of insecticides (https://www.pesticideresistance.org/).

Deltamethrin (pyrethroid), thiamethoxam (neonicotinoid) and pyriproxyfen (juvenile hormone analog) have been used to control several insect pests including *B. tabaci* (Dennehy and Williams 1997; Li *et al.* 2000; Dennehy *et al.* 2008; Tsagkarakou *et al.* 2009). These insecticides have different modes of action. The pyrethroids target voltage-gated sodium channels (VGSCs), neonicotinoids work on acetylcholine receptors in the insect nervous system and insect juvenile hormone (JH) analog controls metamorphosis and development (Ishaaya and Horowitz 1992; Cahill *et al.* 1995; Dhadialla *et al.* 1998; Tomizawa and Casida 2005). Both neonicotinoids and insect growth regulators (IGRs) have been successful in controlling *B. tabaci,* which also resulted in their excessive use. In a survey conducted in Oman, majority (95%) of the farmers used insecticides consisting of 29 different active ingredients (Kaakeh *et al.* 2007). Deltamethrin and esfenvalerate (pyrethroids) have been extensively used in Oman mostly in aerial sprays against dubas bug (550 tons from 1993 to 2010) (Thacker *et al.* 2003; MAF 2014).

Resistance to deltamethrin, thiamethoxam and pyriproxyfen in *B. tabaci* has been reported from other regions (Toscano *et al*. 2001; Horowitz *et al.* 2002; Nauen *et al*. 2002; Nauen and Denholm 2005). In Oman, *B. tabaci* resistance has been reported to malathion and diazinon (organophosphates) (Talukder *et al.* 2008). However, no baseline data on susceptibility to deltamethrin, thiamethoxam and pyriproxyfen are available for *B. tabaci* populations in Oman. This study was conducted to generate baseline data on susceptibility of *B. tabaci* eggs, nymphs and adults to deltamethrin, thiamethoxam and pyriproxyfen which are commonly used on vegetables in Oman.

Materials and Methods

Whiteflies collection and rearing

Two separate colonies of *B. tabaci* adults (MEAM1) were collected from Agricultural Experiment Station (AES) at the Sultan Qaboos University (SQU), Seeb (23.5910° N, 58.1730° E) and the 'Pairidaeza' organic farm at Barka (Al-Batinah governorate) (23.668854° N, 57.852961° E), Oman during March and April 2017. More than 100 adults were collected from infested tomato plants at each site using an aspirator. The collected adults were transported in cool box with ice. At the AES, different pesticides have been used in past while 'Pairidaeza' is a certified organic farm and pesticides have not been used for last three years. The SQU-1 (resistant) and Pairidaeza (susceptible) strains of *B. tabaci* colonies were reared separately inside two walk-in glass cages (3 m × 4 m × 3 m with a mesh door) in a secluded section of the glasshouse at AES. The colonies were maintained on potted eggplants in a 48 cm^3 cage which was placed inside each walk-in glass cage with temperature and relative humidity (RH) set at 28 ± 2°C and 65 ± 5%, respectively. Inside individual cages, three pots with 2–3 plants were placed in 40 × 40 cm^2 metal containers with 10 cm high edges. Pots were replaced every two weeks. Plants were irrigated with a programmed automatic drip system. Eggplant seedlings were grown in a growth chamber (25 ± 2°C and 65 ± 5% RH) at the SQU and fresh plants at 3–4 leaf stage were regularly provided to maintain the colonies.

Insecticides and concentrations tested

The formulated insecticides used in bioassays were bought from local market and included: deltamethrin 25 g L^{-1} (Delta 2.5 EC from Arab Pesticides and Veterinary Drugs Mfg. Co., Jordan), thiamethoxam 240 g L^{-1} (Actara 240 SC from Syngenta, India) and pyriproxyfen 100 g L^{-1} (Admiral 10 EC from Sumitomo Chemicals Co., Japan). Concentrations of each formulated insecticide were prepared with deionized water, by 3X serial dilutions, and 1–2 concentrations above the field recommended rates. The concentrations for deltamethrin were 0.74, 2.2, 6.7, 20, 60 and 180 μg/mL, for thiamethoxam were 0.2, 0.56, 1.70, 5, 15, 45 and 135 μg/mL, and for pyriproxyfen were 0.4, 1.2, 3.7, 11.1, 33.3 and 100 μg/mL.

Leaf-dip bioassay for adults

Six to seven concentrations of each insecticide that gave 15 to 85% mortality were selected for the bioassays (Heong *et al.* 2013). A leaf-dip bioassay method was adopted from Nauen *et al*. (2008). Leaf discs (45 mm) from eggplant leaves were dipped for 15 s in an insecticide solution separately for each concentration. Deionized water was used for control. Leaf discs were air dried for 60 min on paper towel and placed upside down on a 1.5% agar already poured in Petri dishes (55 mm). Petri-dish lids were ventilated with two rows of small holes and lids were covered with fine mesh using glue to prevent escaping of adults. *B. tabaci* adults were collected from the colonies with an aspirator into small plastic vials and immobilized by immersing the vials in the ice for 2–4 minutes. Working on a chilling pad, 20 *B. tabaci* adults (mixed sex) were transferred gently onto each treated leaf disc. Each concentration was replicated three times and a total of 360 adults were used for deltamethrin and pyriproxyfen, and 480 adults for thiamethoxam. Petri dishes were then covered with the already prepared lids and sealed with parafilm. Petri dishes were placed upside down and kept at 24 ± 2°C temperature, 60 ± 5% RH and a 12:12 h photoperiod in laboratory.

Leaf-dip (3-leaf whole plant) bioassay for eggs and nymphs

A leaf-dip (3-leaf whole plant) bioassay method was adopted from Bielza *et al*. (2019). An eggplant with three leaves each trimmed into a small rectangle (4 cm × 6 cm) were placed in the SQU-1 and Pairidaeza rearing cages for 24 h for egg laying to allow synchronization of each developing stage. For eggs and nymphs, separate bioassays were done. In egg-bioassay, after 24 h each leaf (with ~50 eggs) was completely merged in the insecticide solution for 20 sec. A single plant was used for each concentration, with each of the three infested leaves counted as three replicates. In bioassay for nymphs, the 3-leaf plants were inspected for the presence of 2nd instar nymphs 15 days after egg laying, and then treated by dipping individual leaves. Eggs and the immobile 2nd instar nymphs were counted on each leaf under stereomicroscope and a total of 900–1400 eggs and 600–1050 nymphs were used in these bioassays.

Data recording and analysis

Mortality of adults was assessed after 48 h for deltamethrin and after 72 h for thiamethoxam and pyriproxyfen. Adults not moving after gentle touch by a needle were considered dead. Number of eggs and hatched alive nymphs were recorded 7 days after treatment in the egg bioassay. In the bioassays for nymph, the number of dead nymphs and pupae were recorded 5 days after treatment. Percent mortality was computed following Abbott (1925). Lethal concentration values of each of the insecticide for the eggs, nymphs and adult stages were calculated separately using Polo Plus Version 2.0 (LeOra 1987). Resistance factors (RF) were calculated by dividing LC_{50} of SQU-1 strain (resistant) by LC_{50} of Pairidaeza strain (susceptible). An RF of <2 means no resistance while 2–10 is considered as very low, 11–20 as low, 21–50 as moderate, 51–100 as high and >100 as very high resistance (Saleem *et al*. 2008). Percent corrected mortality caused by the three insecticides applied at label recommended rate against eggs, nymphs and adults of *B. tabaci* were analyzed by single factor ANOVA using SPSS v19 and means were separated at $LSD_{0.05}$.

Results

Susceptibility of adults to insecticides

The acute contact LC_{50} for deltamethrin was 55.09 and 12.69 μg/mL for adults of SQU-1 and Pairidaeza strains, respectively. A low level of resistance (RF = 4.3) to deltamethrin was detected in SQU-1 adults compared to Pairidaeza. SQU-1 and Pairidaeza populations LC_{50s} for thiamethoxam were 4.10 and 1.85 μg/mL, respectively. The SQU-1 population was 2.2-and 1.3-fold resistant to thiamethoxam and pyriproxyfen, respectively suggesting very low to no resistance against these insecticides (Table 1).

Susceptibility of nymphs and eggs to insecticides

LC_{50} of deltamethrin for nymphs of SQU-1 and Pairidaeza strains were 28.73 μg/mL and 8.37 μg/mL, respectively and nymphs of SQU-1 strain exhibited RF value 2.7-fold as compared to Pairidaeza strain. A very high LC_{50} of 7837 µg/mL was determined for SQU-1 strain while LC_{50} for Pairidaeza strain could not be determined because of the concentration range used. Therefore, RF for eggs against deltamethrin could not be calculated. LC_{50} for thiamethoxam was 7.21 and 2.59 μg/mL for nymphs of SQU-1 and Pairidaeza strains with RF of 2.7 for former strain while RF for eggs was 2.3-fold. LC_{50} for pyriproxyfen was 5.29 and 4.67 μg/mL against nymphs of SQU-1 and Pairidaeza strains with RF 1.1. LC_{50} for pyriproxyfen was 3.39 and 2.18 μg/mL for eggs of SQU-1 and Pairidaeza strains, respectively. The RF for eggs against pyriproxyfen was 1.6 (Table 2).

Mortality by field application rate

The field application rate of deltamethrin caused 47.0 ± 6.3% and 59.6 ± 6.5% mortality in adults and nymphs of SQU-1 strain, respectively, at dose of 20 μg a.i/mL, however, egg mortality was only 14.0 ± 3.1% (Table 3). Thiamethoxam caused 82.0 ± 9.4%, 86.7 ± 8.5 and 17.3 ± 4.2% mortality in adults, nymphs and eggs of SQU-1 strain, respectively, at dose of 100 μg a.i/mL. Pyriproxyfen the field application rate at the dose of 75 μg a.i/mL caused 82.3 ± 9.8, 92.3 ± 10.5 and 82.0 ± 9.9% mortality in adults, nymphs and eggs, respectively. Pyriproxyfen in SQU-1 strain which was not significantly different than the Pairidaeza strain. Mortality in adults and nymphs caused by thiamethoxam and pyriproxyfen in both strains was significantly higher ($F= 109$, $df = 2$, $P < 0.001$; $F = 54$, $df = 2$, $P < 0.001$) than deltamethrin. Mortality in eggs of both strains caused by pyriproxyfen was significantly higher ($F= 247$, $df= 2$, $P < 0.001$) than deltamethrin and thiamethoxam. There was no significant difference in adult ($F= 1.3$, $df= 1$, $P=0.27$), nymph ($F= 0.33$, $df= 1$, $P=0.57$) and eggs ($F= 0.89$, $df, 1$, $P=0.1$) mortality between the SQU-1 and Pairidaeza strains.

Discussion

Deltamethrin, thiamethoxam and pyriproxyfen are currently used in the management of *B. tabaci* in different horticultural crops in Oman. Resistance in *B. tabaci* has developed as a result of the intensive use of these insecticides (Li *et al.* 2000; Dennehy *et al.* 2008; Tsagkarakou *et al.* 2009). A very low resistance to deltamethrin was detected in adults (4.3-fold) and nymphs (2.7-fold) of SQU-1 strain with reduced mortalities in both stages of the tested strains at field application rate. Houndete *et al*. (2010), while establishing baseline susceptibility, recorded a very low 1.6–4.7-fold resistance to deltamethrin

Table 1: Toxicity of three insecticides against adults of two *B. tabaci* strains using leaf-dip bioassay

Insecticides	Strains	Total number tested	LC_{10} (µg/mL) (95% FL)	LC_{50} (µg/mL) (95% FL)	LC_{90} (µg/mL) (95% FL)	Slope (± SE)	*het*[a]	RF[b]
Deltamethrin	SQU-1	360	0.66 (0.15-1.62)	55.09 (24.92-112.18)	1256.9 (389.1-4226.9)	0.81 ± 0.17	0.47	4.3
	Pairidaeza	360	0.46 (0.26-1.74)	12.69 (5.42-39.35)	526.7 (181.2-1229.5)	0.66 ± 0.15	0.15	
Thiamethoxam	SQU-1	480	0.27 (0.01-1.41)	4.10 (0.98-15.30)	135.64 (42.3-7141.4)	0.95 ± 0.13	1.85	2.2
	Pairidaeza	480	0.12 (0.09-1.62)	1.85 (0.08-5.20)	85.63 (22.3-1421.5)	0.64 ± 0.12	0.67	
Pyriproxyfen	SQU-1	360	0.32 (0.07-0.76)	8.39 (5.28-12.86)	218.2 (100.5-811.9)	0.91 ± 0.13	0.41	1.3
	Pairidaeza	360	0.27 (0.06-0.67)	6.59 (4.27-11.38)	182.4 (84.7-567.4)	0.84 ± 0.14	0.32	

LC_{10}, LC_{50} and LC_{90} are the concentrations (µg/mL) that will kill 10, 50 and 90% of the *B. tabaci* adults, respectively
60 adults used in control
[a]A value lower than 1 indicates that homogeneity and linearity of dose-mortality response were rejected
[b]Resistance factor (RF) = LC_{50} of SQU-1 strain divided by LC_{50} of Pairidaeza strain

Table 2: Toxicity of insecticides against nymphs and eggs of two *B. tabaci* strains using leaf-dip (3-leaf whole plant) bioassays

Insecticides	Strains	Life stage	Total number tested	LC_{10} (µg/mL)	LC_{50} (µg/mL)	LC_{90} (µg/mL)	Slope (± SE)	*het*[a]	RF[b]
Deltamethrin	SQU-1	Nymphs	600	0.82 (0.29-1.87)	28.73 (7.12-63.36)	697.8 (283.2-1963.5)	1.08 ± 0.13	1.30	2.7
		Eggs	900	15.87 (4.38-37.01)	7837 (3832-18936)	--	0.68 ± 0.13	0.07	--
Thiamethoxam	SQU-1	Nymphs	1050	0.91 (0.27-2.25)	7.21 (3.27-12.71)	157.89 (49.7-2210.9)	1.42 ± 0.19	0.78	2.7
		Eggs	1400	11.62 (3.75-22.95)	3319.5 (889.4-54602.0)	--	0.52 ± 0.10	1.01	2.3
	Pairidaeza	Nymphs	1050	0.63 (0.15-1.52)	2.59 (0.24-11.24)	102.8 (29.7-710.9)	1.51 ± 0.28	0.27	
		Eggs	1400	3.56 (1.57-12.32)	1419.5 (693.4-21361.1)	--	0.66 ± 0.19	0.58	
Pyriproxyfen	SQU-1	Nymphs	600	0.29 (0.06-0.74)	5.29 (2.59-8.38)	93.7 (49.6-267.1)	1.03 ± 0.16	0.69	1.1
		Eggs	900	0.13 (0.04-0.28)	3.39 (2.29-4.98)	86.18 (42.5-262.3)	0.91 ± 0.07	0.27	1.6
	Pairidaeza	Nymphs	600	0.33 (0.08-0.98)	4.67 (2.24-7.27)	87.6 (36.4-187.9)	1.11 ± 0.12	0.36	
		Eggs	900	0.11 (0.05-0.53)	2.18 (0.94-4.47)	68.58 (35.8-182.6)	0.73 ± 0.09	0.14	

LC_{10}, LC_{50} and LC_{90} are the concentrations (µg/mL) that will kill 10, 50 and 90% of the *B. tabaci* nymphs or eggs, respectively
[a]A value lower than 1 indicates that homogeneity and linearity of dose-mortality response were rejected
[b]Resistance factor (RF) = LC_{50} of SQU-1 strain divided by LC_{50} of Pairidaeza strain

Table 3: Efficacy of the three commonly used insecticides against *B. tabaci* applied at label recommended rate

Insecticides	Label rate	a.i. (µg/mL)	Strain	Percent corrected mortality		
				Adults	Nymphs	Eggs
Deltamethrin	80 mL per 100 L water	20	SQU-1	47.0 ± 6.3a	59.6 ± 6.5a	14.0 ± 3.1a
			Pairidaeza	62.7 ± 4.3a	67.4 ± 7.3a	16.1 ± 3.4a
Thiamethoxam	8 g per 20 L water	100	SQU-1	82.0 ± 9.4b	86.7 ± 8.5b	17.3 ± 4.2a
			Pairidaeza	94.3 ± 11.6b	87.6 ± 8.3b	25.0 ± 4.6a
Pyriproxyfen	150 mL per 200 L water	75	SQU-1	82.3 ± 9.9b	92.3 ± 10.5b	82.0 ± 9.9b
			Pairidaeza	93.7 ± 10.1b	94.4 ± 11.3b	87.6 ± 8.7b

Values sharing same letters in column don't differ significantly ($P > 0.05$)

in *B. tabaci* collected from cotton fields. The same populations, however, showed reduced susceptibility (RF = 44) to another pyrethroid, bifenthrin. Resistance factor for eggs treated with deltamethrin could not be calculated because LC_{50} was not determined for the susceptible strain. A very low level of resistance was observed for thiamethoxam in adults (2.2-fold) and nymphs (2.7-fold) of SQU-1 strain, while no resistance (<2 fold) was detected in egg stage. Adults of SQU-1 strain treated with thiamethoxam had slightly reduced but non-significant mortality at the field application rate. Pyriproxyfen treated eggs, nymphs and adults did not show any resistance. Eggs of SQU-1 strain treated with field application rate of pyriproxyfen did not show reduction in susceptibility.

Findings of very low resistance ratios to deltamethrin in the SQU-1 strain may be due to its cautious use at the experimental station. *B. tabaci* from other commercial farms where deltamethrin is repeatedly used may show reduced susceptibility levels. Very low resistance ratios to thiamethoxam and lack of resistance to pyriproxyfen show that *B. tabaci* has retained a general level of susceptibility to these insecticides. These two insecticides are relatively new to Oman. It is unlikely that they may cause any resistance problem and their effectiveness is expected to be maintained in near future. Another reason for the very levels of resistance is because of the presence of B biotype in Oman. One strain of *B. tabaci* representing the B biotype had resistance factors between 1–8 showing very low to no resistance to imidacloprid, thiamethoxam and acetamiprid (Qiong *et al.* 2012). The Q biotype of *B. tabaci* has shown stronger resistance to neonicotinoid insecticides than B biotype (Ma *et al*. 2007; Luo *et al*. 2010; Rao *et al*. 2011; Qiong *et al.* 2012).

Monitoring field populations for their susceptibility to the most used insecticide classes is crucial for early detection of resistance development (Roush and Miller 1986). While developing resistance management strategies for an insect pest, it is important to establish the baseline susceptibility levels (Prabhaker *et al.* 2008). Baseline susceptibility data provide a reference point to which subsequent susceptibility data can be compared with. Any shift in the susceptibility to a particular insecticide from the

reference would detect resistance in its early stages.

The determined baseline susceptibility levels of *B. tabaci* adults, nymphs and eggs against deltamethrin, thiamethoxam and pyriproxyfen can be used for continuous monitoring of *B. tabaci* populations. Natural variations in responses to insecticides in populations collected from various geographic regions in Oman should be expected. *B. tabaci* populations should also be tested at different time of the year, for example September to December, that could help explain the causes of variability in insecticide susceptibility. Any change in the susceptibility levels indicates development of resistance in *B. tabaci.* Once resistance is detected, resistance management strategies must be initiated and implemented before control failures occur. An extensive survey and toxicological work with several strains collected from different regions in Oman, with different history of insecticide applications, is underway which will help in broadening the susceptibility baseline data of *B. tabaci*.

Conclusion

Our results provide a baseline for future comparisons of the sensitivity of *B. tabaci* to three insecticides that represent the primary classes being used to control this pest, each with a different mode of action. The range of concentrations across which these populations responded will allow baseline sensitivity studies in other governorates in Oman to test the active range of response for these insecticides. Insecticide resistance management (IRM) is a crucial component of a successful IPM program (Foster *et al.* 2002). Preventative approaches should be implemented instead of relying on only insecticides (Timmeren *et al.* 2018). IRM strategies should include rotation of insecticides (based on IRAC classification with different modes/site of action). Thiamethoxam and pyriproxyfen did not show any reduction in susceptibility and should be included in rotation. Use of insecticides should be integrated with cultural and biological pest management tools that will provide effective management of *B. tabaci* in Oman.

Acknowledgements

The authors acknowledge the financial support provided by Sultan Qabos University, Oman through internal grant 'IG/AGR/CROP/18/02'.

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