Tropical Agriculturist, Vol.167 (2), 2019 DOI: https://doi.org/10.4038/ta.v167i2.75

Biology and management of *Harpedona marginata* Distant (Hemiptera: Miridae): A pest of *Dioscorea* species in Sri Lanka

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Abstract

***Harpedona marginata* Distant (Hemiptera: Miridae) is an emerging pest in *Dioscorea* cultivations in Sri Lanka. Pest damages were observed in July to September 2017/2018. Thirty three *Dioscorea* accessions were evaluated at Horticultural Crops Research and Development Institute (HORDI), Gannoruwa and all evaluated accessions were susceptible for this pest. The study was focused on morphological characterization, information on life cycle and chemical control measures of the pest. Male and female insects are morphologically very similar. Head and body have black, 3-4 mm in length. White colour patches were found on infested leaves. In severe infestation, leaves become brownish colour. *H. marginata* took 26.0 ± 0.47 days to complete its life cycle at 27 ^{0}C. Potential insecticides to control *H. marginata* in laboratory bioassay were 0.75% Neem formulation 5 m*l/l* (Lakgro neem), Neem seed water extract (30 g/*l*), Thiamethoxam 25% WG, 0.5 g/*l* (Actara), Imidacloprid 20% SL, 1 m*l/l* (Admire) and Thiamethoxam 25% WG + Cloranthraniliprole 20%SC 0.25 g/*l* (Vertako). Thiamethoxam 25% WG and 0.75% Neem formulations were shown positive results in controlling both adults and nymphal stages of *H. marginata* in field screening.**

Key words: Life Cycle, Morphology, Neem

Introduction

Yam (*Dioscorea* spp.) Is a tropical tuber crop cultivated for food and medicinal purpose. Genus *Dioscorea* consists of 630 identified species including *D. alata, D. esculenta, D. bulbifera, D. fenterfila, D. rotundata, D. trifida, D. composita, D. spicata*

and *D. tomentosa*. Nearly 40 *Dioscorea* accessions are grown in Sri Lanka (Senanayake *et al*., 2011) with many edible accessions belong to *D. alata*, few of *D. esculenta* and *D. bulbifera* (Abeywickrama *et al*., 2018). *Dioscorea* are grown commercially in Kegalle, Rathnapura and Mawanella due to many benefits such as high income and a food crop with health benefits. Optimum growth of *Dioscorea* spp. occurs in well drained fertile soils with pH5.5-6.5 and in temperatures ranging between 25 ^{0}C – 30 ^{0}C under sunlight or partly shaded conditions (Korada *et al*., 2010).

Dioscorea were grown in home gardens and there was no serious pest damage to the crop until the recent incidence of *H. marginata* infestation reported with severe leaf discoloration. Infested leaves are highly prone to leaf diseases and even death of infested seedlings have been reported. *H. marginata* was reported in China, Taiwan, Nepal and Thailand. In year 2000, *H. marginata* was found causing damage to *Dioscorea* in Taiwan and later believed to be a major pest in fields of *Dioscorea* (Lin, 2001). Although this pest were reported in several other countries their damage symptoms, life cycle and control measures to manage the pest population were not well documented. Therefore, present research was focused on morphological characterization of the pest, damage symptoms, information on life cycle and chemical control measures. This information will be helpful to overcome the pest incidences in commercial cultivations of *Dioscorea*.

Materials and methods

Sample collection and pest identification

Pest infested plant samples were collected in the research field of Horticultural Crop Research and Development Institute (HORDI), Gannoruwa and farmer fields in Kandy (Kundasale, Manikhinna), Kegalle (Kegalle) and Rathnapura districts. Symptoms of the leaves due to insect feeding were observed under the microscope (10x10) and the pest collected from the leaf samples was identified using the key to genera of tribe Eccritotarsini (Basnagala *et al*., 2002), and the voucher specimens were kept in insect museum HORDI, Gannoruwa. Confirmation of the damage symptoms was done by taking healthy leaves from the field and exposing them to adult and nymphal stages of already identified insect for feeding for two days. Symptoms developed leaves were then compared with infested leaves from research field and farmer fields.

Varietal Screening for infestation of *H. marginata* Distant (Hemiptera: Miridae) under field condition

Thirty three accessions of edible *Dioscorea* species belongs to *D. alata, D. esculenta* and *D. bulbifera* were used for this study. Pest incidences were observed from June to September in 2017. Top fifty leaves of each vine were observed and number of leaves with infestation was recorded. Each accession consists of four replicates and there were six vines per replicate.

Bio efficacy of selected insecticides on *H.marginata* Distant (Hemiptera: Miridae)

Commercial Neem formulations (80% Neem 400ppm, 0.75% Neem 37.5ppm) and Neem seed water extract (30 g/*l*), Thiamethoxam 25% WG 125 ppm, Bufroprezin 10% WG 60 ppm, Pymetrozin 50%WG 250 ppm , Imidacloprid 20% SL 200 ppm, and Thiamethoxam 25% WG + Cloranthraniliprole 20% SC (0.25 g/*l*) were selected for the bioassay. Uninfected cleaned leaves were dipped in insecticides solutions for 5 minutes and those leaves were dried in air in open environment for 3 minutes before exposing to the *H. marginata* adults in each cup. Leaves treated with water and dried for 3 minutes were used as the control, and all the treatments were replicated three times. Randomly selected five adults were introduced into each cup. Insect counts were obtained until 7 days (Figure 1). Corrected mortality data were analyzed using SAS 9.12 with ANOVA. Percentage corrected mortality was calculated using Abbott's formula.

$$(\text{Corrected mortality \%}) = 1 \frac{\text{No. Of insects in the control}}{\text{No. Of insects in the treatment plot after the treatment}} X\ 100$$

Figure 1. Bioassay for Harpedona marginata for the efficacy of different insecticides

Field screening

Insecticides (Thiamethoxam 25% WG) and a 0.75% Neem formulation were selected based on results of the bioassay and screened against *H. marginata* in research fields, HORDI, Gannoruwa. There were three replicates for each treatment and candidate

insecticides were sprayed for one time under natural infestation for the four months old Raja ala (*D. alata*) which showed higher infestation. *H. marginata* adults and nymphs were counted just before and 3, 7 and 21 days after the application of the insecticides in top 50 leaves in each vine. Data were analysed using SAS 9.12, ANCOVA. Pre counts were used as a covariate.

Life cycle of *H. marginata*

Sexually matured male and female couple for copulation was introduced to leaves as 1:1 ratio per cup. Water filled cups were used to maintain the freshness of the leaves. Leaf petioles and veins were checked daily for the presence of eggs after introducing *H. marginata*. Number of days taken to egg laying, location of egg laying and number of eggs laid at a time were observed under the room temperature at 27 ^{0}C. Then, the experimental units were observed for the number of days taken to nymphal stage developments, and those nymphs were fed with newly introduced leaf until growing to adulthood. Days taken to nymph stages until emergence of adults were recorded. The experiment was replicated ten times.

Results and discussion

Most of the adults and nymphs of this pest was fed on lower side of the leaves. Pest damage symptoms on leaves, produced in the laboratory and observed in open field, were similar. In both circumstances white colour patches on the upper surface of leaves were observed but there was no any distortion of the leaf. Feeding patches turned into brownish colour with the time (Figure 2).

(a) **(b)** **(c)**

Figure 2. Development of symptoms in uninfested leaves (a) and *H.marginata* damage symptoms in laboratory (b) and open field and (c)

Pest identification

H. marginata was identified using the key published by Lin (2001) and Basnagala (2002), and voucher specimens were kept at HORDI insect museum. Both male and female have head and body black in colour. Male was 3.5-3.8 mm in long, legs and rostrum were pale brownish yellow. Wing membrane is translucent and fuscous. Ventral part of the body was dark fuscous. Genitalia were a Pygophore with long, curved, and spine –like projection. Left paramere was long and curved, right paramere was enlarged and with bi lobbed apex. Female was very similar to male with about 3.4 - 4.0mm body length (Lin, 2001).

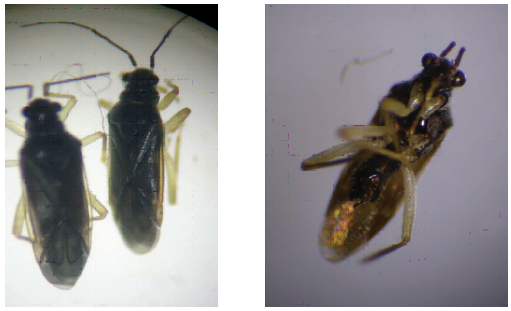


Figure 3. Dorsal view of Adults (10x10) and Ventral view (10x10) of Male insect of *H. marginata*

Screening of *Dioscorea* accessions for *H. marginata* Distant (Hemiptera: Miridae) infestation under field condition

All the accessions had damages and percentage of damage was significantly varied among the accessions (12.5% - 90%). Results revealed that least infestation of the pest were on Eshuma (*D. rotundata*), Thembala (*D. alata*) and Guru ala (*D. alata*). Out of 33 accessions, the highest infestation was found in Maha Angili ala (*D. alata*) and Angili ala (*D. alata*) (Table 1).

Table 1. Percentage of infested leaves in 33 *Dioscorea* accessions in open field in HORDI 2017 *Yala* (n=300) and Yield (t/ha)

Accession	% Infested leaves	Yield(t/ha)*
Maha Angili ala(a)	90.0[a]	40.3
Angili ala(a)	87.5[a]	20.8
Raja ala(a)	85.0[ab]	42.3
Heen Hingurala(a)	85.0[ab]	39.6
Kekulu ala(a)	85.0[ab]	38.6
Jappna Purple(a)	82.5[abc]	50.0
Kiri kodol(a)	77.5[abc]	66.0
Suta Hingurala	75.0[abc]	38.3
Kindala(a)	72.5[abcd]	49.2
Dandila(a)	67.5[abcde]	50.3
Rathnawali(a)	67.5[abcde]	58.2
Jamburala(a)	67.5[abcde]	70.8
Udala (b)	65.0[abcde]	11.9
Jaffna Rassawalli(a)	62.5[abcde]	36.7
Heen Angili ala(a)	62.5[abcde]	25.5
Suta kukula(a)	60.0[abcde]	14.2
Ledantha(a)	60.0[abcde]	45.3
Dandinu ala(a)	57.5[bcde]	20.9
Rathabala(a)	57.5[bcde]	44.2
Dam ala(a)	55.0[cde]	22.5
Ethpada ala(a)	47.5[def]	54.6
Thirunuththiwalli(a)	47.5[def]	20.3
Kombu ala(a)	45.0[efg]	38.0
Kahatangala(a)	35.0[fgh]	80.3
Murunkawalli(a)	32.5[ghi]	60.8
Java ala (e)	30.0[hi]	23.7
Rathambala(a)	27.5[hi]	44.2
Natta ala(e)	25.0[hi]	20.2
Kiri ala(a)	22.5[rt]	16.3
Thembala(a)	17.5[jk]	55.5
Ini ala(a)	17.5[jk]	45.4
Eshuma (r)	15.0[k]	23.7
Guru ala(a)	12.5[k]	69.6
CV%	6.1	

Means Percentage infested leaves followed by the same superscript letters are not significantly different at P=0.05

**Yield data only for one replicate; Accession a - D. alata, Accession e- D. esculenta, Accession b- D. bulbifera, Accession r - D. rotundata*

Bio efficacy of insecticides on *Harpedona marginata* Distant (Hemiptera: Miridae) in laboratory

Neem formulation (0.75%) and Neem seed kernel extract were significantly effective in controlling *H. marginata*, where 100% mortality was shown at three days after application insecticide by either dipping or spraying, under the laboratory condition. However, 80% Neem was not effective in controlling the pest population (Table 2).

Table 2. Percentage of corrected mortality of *H. marginata* adults after application of different Neem formulations

Treatments	Dilution rate	Percentage of corrected mortality	
		3 DAA*	7 DAA*
80% Neem	0.5 m*l*/1*l*	00.0^{c}	00.0^{c}
Neem seed extract	30 g/1l	47.2^{b}	83.3^{b}
0.75%Neem	5 m*l*/1*l*	100^{a}	-
0.75%Neem	10 m*l*/1*l*	100^{a}	-
0.75%Neem spray	5 m*l*/1*l*	83.3^{a}	100^{a}
Neem seed extract spray	30 g/1*l*	91.6^{a}	91.6^{a}
CV %		5.8	4.4

** DAA- Days After Application of Neem formulations*

Note: Means followed by the same superscript letter within the column are not significantly different at p=0.05

In addition to 0.75% Neem formulation and Neem seed extract, Thiamethoxam 25% WG 125ppm, Imidacloprid 20% SL 200 ppm and Thiamethoxam 25% WG + Cloranthraniliprole 20%SC (0.25g/l) were significantly effective to control *H. marginata* adults and nymphs in laboratory bioassay. Bufroprezin 10% WP 60ppm showed 66-78% mortality at 7 days after application whereas 50% WG Pymetrozin 250ppm was not effective in controlling the pest under the laboratory condition (Table 3).

Candidate insecticides Thiamethoxam 25% WG, Imidacloprid 20% SL and Thiamethoxam 25% WG+ Cloranthraniliprole 20% SC were highly effective for the pest. The efficacy of Imidacloprid 20% SL against *H. marginata* also had been pointed out by Yang in 2005 (Yang, 2005).

Table 3. Percentage of corrected mortality of *H.marginata* adults after application of different insecticides in laboratory bioassay

Treatments	Dilution (g/ml/l)	% of corrected mortality 3 DAA*	%of corrected mortality 7 DAA*
Thiamethoxam 25% WG	(0.5 g/*l*)	100[a]	-
Bufroprezin 10%WP	(0.6 g/*l*)	6.67[b]	66.6[a]
Pymetrozin 50% WG	(0.5 g/*l*)	0.00[b]	0.00[b]
Imidacloprid 20% SL	(1 ml/*l*)	100[a]	-
Thiamethoxam 25% WG + Cloranthraniliprole 20%SC	(0.25 g/*l*)	100[a]	-
CV %		26.4	12.8

** DAA- Days After Application of insecticides*

Note: Means followed by the same superscript letters within column are not significantly different at p=0.05

Pre-counts of the insects per leaf of 0.65, 0.57 and 0.55 respectively in T1, T2 and T3 were not significantly different. The number of insects per leaf in Thiamethoxam 25% WG treatment was significantly lower in 3DAA. Seven days after application, insect counts were further significantly reduced per leaf in Thiamethoxam 25% WG and 0.75% Neem applied treatments. Insect counts remained lower in 21 days after application of treatments (Table 4).

Table 4. Field screening of selected insecticides for *H.marginata* in 2017

Treatment	No.of insects/leaf 3 DAA*	No.of insects/leaf 7 DAA *	No.of insects leaf 21 DAA*
T1 - 0.75% Neem	0.26[ab]	0.01[b]	0.11[b]
T2 - Thiamethoxam 25% WG	0.16[b]	0.00[c]	0.0[b]
T3 - Control	0.59[a]	0.62[a]	0.78[a]
CV%	11.5	2.5	8.0

Note: Means followed by the same superscript letters within the column are not significantly different at p=0.05

**DAA- Days After Application of insecticides*

Pre-counts of the insects 0.2, 0.26 and 0.25, respectively in T1, T2 and T3 were not significantly different in insecticides screening in 2018. Data showed that 3 days after spraying, pest counts were significantly low in Thiamethoxam 25% WG applied treatment and 0.75% Neem applied treatment compared to the control. *H. marginata*

population was remained at a low average even in 21 days after application of both treatments (Table 5).

Table 5. Field screening of selected insecticides for the *H. marginata* in 2018

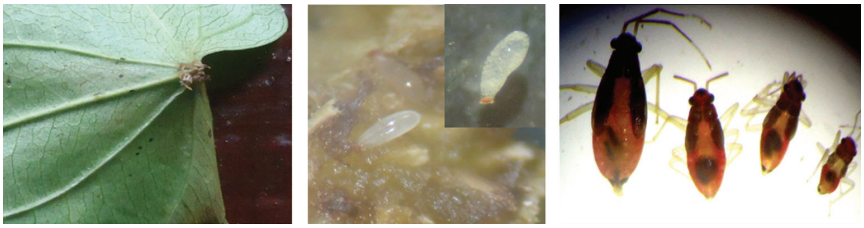
Treatment	No. of insects/ leaf 3DAA*	No. of insects/ leaf 7DAA*	No. of insects/leaf 21DAA*
T1 –0.75%Neem	0.06^{b}	0.00^{b}	0.08^{b}
T2 –25% WG Thiamethoxam	0.00^{c}	0.00^{b}	0.00^{c}
T3 – Control	0.33^{a}	0.42^{a}	0.45^{a}
CV%	1.4	2.3	3.3

** DAA Days After Application of insecticides*

Note: Means followed by the same superscript letters within the column are not significantly different at p=0.05

Life cycle of *H.marginata*

As the sign of egg deposition, leaf petiole and leaf veins turned to dark brownish black in colour. When those places were dissected eggs could be observed. They laid oval shaped single egg at a time (Figure 4). *H. marginata* takes 26±0.47 days at 27 ^{0}C to complete the life cycle (Table 6).



(a) **(b)** **(c)**

Figure 4. Location egg deposited (a) Eggs (b) and nymph stages(c) of *H. marginata* (10x10)

Table 6. Average number of days (mean ± se) taken to complete the life cycle (n=10, 27 ^{0}C)

Life stages	Mean duration (d)
Eggs	6.0d±0.27
Nymph	13.0b ±0.26
Adult	7.0 c ±0.16
whole life cycle	26.0 a±0.47
CV%	3.7

Note: Means followed by the same letters are not significantly different at p=0.05

Conclusion

Both adults and nymphs of *H. marginata* Distant (Heteroptera: Miridae) feed on leaf sap of *Dioscorea* spp. leaving white colour patches or brown colouration on leaves. The pest infestation was high in June to September in 2017 and 2018. The highest leaf damage percentage was observed in Maha Angili ala and Angili ala. Effective control measures under laboratory conditions were 0.75% Neem formulation, Neem seed water extract, Thiamethoxam 25% WG 125ppm, Imidacloprid 20% SL 200 ppm and Thiamethoxam 25% WG + Cloranthraniliprole 20% SC (0.25g/l). Thiamethoxam 25% WG 125 ppm and Neem 0.75% are the potential insecticides to manage *H. marginata* adults and nymphs. *H. marginata* takes 26.0±0.47 days to complete the life cycle of *H. marginata* at 27 ^{0}C.

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