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Dusky Cotton Bug *Oxycarenus* spp. (Hemiptera: Lygaeidae): Hibernating Sites and Management by using Plant Extracts under Laboratory Conditions

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ABSTRACT. The dusky cotton bug, *Oxycarenus* spp., has now attained the status of a major pest of cotton crops that affects lint as well as the seed quality of cotton. Surveys were conducted to explore the hibernating sites in the districts Faisalabad, Multan and Bahawalpur. The efficacies of six different plant extracts, i.e. Neem (*Azadirachta indica*), Milkweed (*Calotropis procera*), Moringa (*Moringa oleifera*), Citrus (*Citrus sinensis*), Tobacco (*Nicotiana tobacum*) and Castor **(***Ricinus communis*) were tested by using three different concentrations of each plant extract, i.e. 5, 2.5 and 1.5% under laboratory conditions at 25±2°C and 70±5% RH. The data were recorded 24, 48, 72 and 96 hours after treatment application. However, *Psidium guajava*, *Azadirachta indica*, *Eucalyptus camaldulensis* and *Mangifera indica* were graded as host plants heavily infested by *Oxycarenus* spp. Results ($\alpha \leq 0.05$) indicated that increasing the concentration of extracts also increased the mortality. *Nicotiana tobacum* and *Calotropis procera* respectively displayed maximum 72 and 71, 84 and 80, 97 and 89% mortality at all concentrations, i.e. 1.25, 2.50 and 5.00%, after 96 hours of application. Two concentrations (2.5 and 5%) are the most suitable for obtaining significant control of the dusky cotton bug.

KEY WORDS: *Oxycarenus* spp, Threat, Hibernating Sites, Plant Extracts, Management.

INTRODUCTION

Cotton is one of Pakistan’s major foreign exchange earning crops. Cotton is attacked by a number of insect pests, which not only affects the yield but also exports. The dusky cotton bug, *Oxycarenus* spp., which used to be regarded as a minor pest, has now attained the status of a major pest. In the past, dusky cotton bugs usually appeared on cotton once most of the cotton bolls had opened. The damage caused by the bugs on unripe seeds was therefore negligible. Now, however, with the introduction of Bt cotton, the crop span lasts for the whole year. On the other hand, the use of contact poisons has become limited and there is total reliance on systemic insecticides. Under such conditions, the dusky cotton bug was able to flare up, attaining the status of a major pest. It causes a continuous decrease in cotton-seed weight, germination and oil quality (HENRY 1983). After feeding, greasy spots appear on the bolls that become disfigured following the injection of toxic saliva (SCHAEFER & PANIZZI 2000). Females lay eggs in the cotton lint and feed on the seeds. At the end of the breeding season, the cotton seed bug seeks a hibernaculum. Overwintering adults are not completely inactive, but do not feed or mate until Malvaceae seeds are available again. Seeds of various host plants are available at different periods throughout the year, maintaining the cotton seed bug populations (SWEET 2000). Both adults and nymphs of the dusky cotton bug feed on seeds and may also feed on the leaves and young stems of host plants. Sometimes this leads to 6.8, 32 and 6% reductions in cotton yield, seed weight and oil content, respectively (SEWIFY & SEMEADA 1993). It also feeds on leaves and young stem/petiole tissue to obtain additional moisture (HOLTZ 2006). This pest has also been reported feeding on certain fruits and vegetables, including apple, avocado, maize, dates, figs, grapes, peach, okra, pineapple and pomegranate, as well as hibiscus (USDA 2009). It completes 6-7 generations per year. The last generation hibernates on the branches or leaves of grass and weeds. So because of this emerging risk it is necessary to devise a safe and environmentally friendly management strategy. For this purpose plant extracts have been used. Owing to the high cost of synthetic insecticides and their hazardous impact on the human and environment, biopesticides are superior for insect control (Stoll 2001) because they are non-toxic and biodegradable (DOLUI & DEBNATH 2010). Many plants have now been reported for their pesticidal properties, the most important of which are *Azadirachta indica* A. JUSS., *Cassia fistula* L., *Lantana camara* L., *Chrysanthemum coronarium* (L.) CASS. ex SPACH, *Calotropis procera* (AITON) W.T. AITON, *Punica granatum* L. and *Murraya koenigii* (L.) SPRENG. (PARKASH & RAO 2006).

The objective of this study was to investigate alternative host plants of the dusky cotton bug in order to adopt a suitably controlled strategy that prevents its migrating to the cotton crop without disturbing the environment and natural enemies. To this end different natural plant extracts were tested to find the most effective ones to use in the IPM program.

MATERIAL AND METHODS

Surveillance Studies

Alternative host plants of the dusky cotton bug were found in different parts of the districts of Faisalabad (Ayub Agricultural Research Institute i.e. Entomological, Horticulture, wheat, cotton, pulses, Biotechnology and Vegetable Research area, farmer fields), Multan (Government research fields, farmer fields) and Bahawalpur (Government research fields, farmer fields). Different plants – trees, flowers, crops and weeds – were visited in these localities: *Psidium guajava* L., *Phoenix dactylifera* L., *Citrus sinensis* (L.) OSBECK, *Azadirachta indica*, *Syzygium cumini* (L.) SKEELS, *Moringa oleifera* LAM., *Simmondsia chinensis* (LINK) C.K. SCHNEID., *Dalbergia sissoo* ROXB., *Eucalyptus camaldulensis* DEHNH., *Morus alba* L., *Punica protopunica* BALF., *Callistemon acuminatus* CHEEL, *Alstonias cholaris* L.R. BR., *Mangifera indica* L., *Pongamia pinnata* (L.) PANIGRAHI, *Ficus benjamina* L., *Jatropa curcas* L., *Ricinus communis* L. and *Helianthus annuus* L. Plants heavily infested with dusky cotton bugs were selected for collecting a population. Number of hibernating dusky cotton bug was observed with different infestation scales.

Toxicoological studies

Adults of the dusky cotton bug were collected in perforated plastic tubes for carrying out the toxicological studies in the laboratory. Six botanicals – Neem (*Azadirachta indica*), Milkweed (*Calotropis procera*), Moringa (*Moringa oleifera*), Citrus (*Citrus sinensis*), Tobacco (*Nicotiana tobacum* L.) and Castor (*Ricinus communis***)** – were used against the dusky cotton bug including a control treatment. Leaves of Neem, Tobacco, Moringa and Ak, Citrus peel and Castor seeds were collected and shade-dried for 20 to 25 days. The dried specimens were ground with an annex grinder. 50 g of each powdered botanical were mixed with one litre of water to make up a 5% solution. Two serial solutions (2.5 and 1.25%) were made from the 5% solution to perform the bioassay with 5 replicates. The botanicals were applied in the laboratory by dipping filter papers in the different plant extracts and drying them for 20 minutes before being placed in Petri dishes. Dusky cotton bugs were put in a freezer for 5 minutes to slow down their activity and to facilitate moving them. 20 adult dusky bugs were placed in each Petri dish containing filter paper dipped in plant extract. Data were collected 24, 48, 72 and 96 hours after application. The average percentage mortality in each treatment was corrected by Abbott's Formula (ABBOTT 1925).

$$\text{Percentage Mortality} = \frac{\text{Number of dead adults}}{\text{Number of adults introduced}} \times 100$$

$$\text{Corrected \%} = \left(1 - \frac{\text{Insect population in treated after treatment}}{\text{Insect population control after treatment}}\right) \text{x } 100$$

RESULTS

Oxycarenus spp. is a seed feeder, with primary hosts found within the Malvaceae family, specifically *Gossypium* spp. Studies of alternative host plants of the Dusky cotton bug *Oxycarenus* spp. were carried out during 2012-13. Different available alternative host sites were visited in the vicinity; 23 different host plants were kept under observation. Of these, 18 plants were found to be hibernating sites for the dusky cotton bug, but large populations were also found on *Psidium guajava*, *Eucalyptus camaldulensis*, *Mangifera indica*, *Azadirachta indica* and *Syzygium cumini*, as shown in Table 1. The period of occurrence was December to April.

Table 1. Intensity of the Dusky cotton bug on its alternative host plants during sedentary periods. 0-4 scale infestation: 0 = absent, 1 = <50, 2 = <100, 3 = <150, 4 = >150.

No.	Common Name	Botanical Name	Presence	Grade
1.	Guava	*Psidium guajava*	+	4
2.	Date palm	*Phoenix dactylifera*	+	1
3.	Citrus	*Citrus sinensis*	+	1
4.	Neem	*Azadirachta indica*	+	3
5.	Jaman	*Syzygium cumini*	+	2
6.	Moringa	*Moringa olefera*	–	0
7.	Jujuba	*Simmondsia chinensis*	+	1
8.	Sheesham	*Dalbergia sissoo*	–	0
10.	Eucalyptus	*Eucalyptus camaldulensis*	+	4
11.	Mulberry	*Morus alba*	+	1
13.	Pomegranate	*Punica protopunica*	–	0
15.	Bottle brush	*Callistemon acuminatus*	+	1
16.	Alastonia	*Alstonias cholaris*	–	0
17.	Mango	*Mangifera indica*	+	3
18.	Sukhchain	*Pongamia pinnata*	–	0
19.	Ficus	*Ficus benjamina*	+	1
21.	Jatropha	*Jatropha curcas*	+	1
22.	Castor	*Ricinus communis*	+	1
23.	Sun flower	*Helianthus annuus*	+	1

Table 2. Toxicological effects of plant extracts at 5% concentration.

Treatment	Percentage Reduction of Dusky Cotton Bug (Means±SE)			
	24 hrs	**48 hrs**	**72 hrs**	**96 hrs**
Azadirachta indica	58.13±0.33 b	61.67±1.20 b	61.00±1.53 b	67.00±2.52 b
Calotropis procera	74.31±2.96 a	77.33±5.24 a	81.10±3.21 a	80.13±1.76 a
Moringa oleifera	43.33±2.03 c	46.67±2.19 c	51.00±2.52 c	52.33±1.20 c
Citrus sinensis	35.66±1.20 c	45.33±1.20 c	44.57±1.86 cd	44.23±2.33 cd
Nicotiana tobacum	76.00±1.00 a	77.33±1.20 a	79.67±0.88 a	84.03±2. 33 a
Ricinus communis	06.66±3.67 d	23.23±4.82 d	33.43±3.82 d	36.67±5.81 d

Means sharing similar letters are similar with each other at (α = 0.05).

Table 3. Toxicological effects of plant extracts at 2.5% concentration.

Treatment	Percentage Reduction of Dusky Cotton Bug (Means±SE)			
	24 hrs	**48 hrs**	**72 hrs**	**96 hrs**
Azadirachta indica	70.67±3.48 ab	72.57±5.46 b	73.00±4.36 b	74.02±2.65 b
Calotropis procera	86.00±6.08 a	85.67±6.17 ab	87.33±3.48 a	89.00±4.51 a
Moringa oleifera	55.33±5.54 bc	57.10±6.25 c	58.00±5.20 c	59.33±5.49 c
Citrus sinensis	51.67±8.87 cd	52.00±4.73 c	53.11±4.36 c	53.43±5.81 c
Nicotiana tobacum	85.00±2.88 a	91.23±3.28 a	93.67±2.96 a	97.33±0.83 a
Ricinus communis	35.33±6.35 d	37.00±4.04 d	38.00±6.03 d	39.00±5.20 d

Means sharing similar letters are similar with each other at (α = 0.05).

Table 4. Toxicological effects of plant extracts at 1.25% concentration.

Treatment	Percentage Reduction of Dusky Cotton Bug (Means±SE)			
	24 hrs	**48 hrs**	**72 hrs**	**96 hrs**
Azadirachta indica	47.00±2.08 ab	56.00±6.03 bc	56.67±6.36 bc	59.33±5.36 b
Calotropis procera	50.33±2.85 bc	52.33±3.67 c	66.09±6.11 ab	71.00±6.01 ab
Moringa oleifera	23.10±7.62 a	37.32±3.18 a	46.01±7.31 ab	58.10±6.36 b
Citrus sinensis	41.12±3.06 c	45.00±6.66 c	47.11±1.15 c	50.91±6.64 b
Nicotiana tobacum	51.67±6.39 a	66.67±4.41 c	69.00±4.93 ab	72.00±3.84 b
Ricinus communis	07.80±4.33 bc	20.40±3.38 b	26.90±5.81 a	29.60±5.81 a

Means sharing similar letters are similar with each other at (α = 0.05).

Six different types of plant extracts were used at different concentrations, i.e. 5%, 2.5% and 1.25%. Table 2 shows the toxicological effects of plants extracts at 5% concentration. 24 hrs after application *Calotropis procera* and *Nicotiana tobacum* caused maximum mortality (86 and 85% respectively), followed by *Azadirachta indica*, *Moringa oleifera* and *Citrus sinensis* with 70.66, 55.33 and 51.66% mortality respectively. *Ricinus communis* showed minimum mortality (35.33%). After 48 and 72 hrs there was no significant increase in percentage mortality. However, 96 hrs after application, *Nicotiana tobacum* and *Calotropis procera* yielded maximum mortalities of 97.33 and 89% respectively, followed by *Azadirachta indica*, *Moringa oleifera* and *Citrus sinensis* with 74, 59.33 and 53.33% mortality respectively. *Ricinus communis* showed minimum mortality (39%). *Nicotiana tobacum* and *Calotropis procera* offered significant control at 5% concentration. Similarly, at 2.5% concentration *Nicotiana tobacum* and *Calotropis procera* caused maximum mortalities of 76 and 74% respectively, followed by *Azadirachta indica*, *Moringa oleifera* and *Citrus sinensis* with 58.33, 43.3 and 35.7% mortality respectively, 24 hrs after application. *Ricinus communis* caused minimum mortality (6.6%); after 48 and 72 hrs there was no significant increase in percentage mortality. However, 96 hrs post application, *Nicotiana tobacum* and *Calotropis procera* caused maximum mortality (84.33 and 80% respectively) followed by *Azadirachta indica*, *Moringa oleifera* and *Citrus sinensis* with 67, 52.33 and 44.33% mortality respectively. *Ricinus communis* showed minimum mortality (36.6%). *Nicotiana tobacum* and *Calotropis procera* gave significant control at 2.5% concentration, as shown in Table 3. At 1.25% concentration *Nicotiana tobacum* and *Calotropis procera* caused maximum mortality (51.6 and 50.4% respectively) followed by *Azadirachta indica*, *Moringa oleifera* and *Citrus sinensis* with 47, 23 and 41% mortality respectively 24 hrs post application. *Ricinus communis* showed minimum mortality (7.8%). Here also there was no significant increase in percentage mortality 48 and 72 hrs after application. But 96 hrs post application, *Nicotiana tobacum* and *Calotropis procera* caused maximum mortality (72 and 71% respectively), followed by *Azadirachta indica*, *Moringa oleifera* and *Citrus sinensis* with 59, 58 and 50% mortality respectively. *Ricinus communis* caused minimum mortality (29.6%), as shown in Table 4. *Nicotiana tobacum* and *Calotropis procera* caused maximum mortality at all concentrations but this was significant only at the 5% and 2.5% concentrations.

DISCUSSION

Oxycarenus spp. exhibits migratory behaviour, moving from one host plant to another throughout the year. Many scientists have worked on the alternative host plants of the dusky cotton bug (KIRKPATRICK 1923, ODHIAMBO 1957, AVIDOV & HARPAZ 1969, ADU-

MENSAH & KUMAR 1977, ANANTHAKRISHAN et al. 1982, RAM & CHOPRA 1984, SWEET 2000). These host plants produce seeds at different times of year, providing potential food sources for *Oxycarenus* spp. (SCHAEFER & PANIZZI 2000). *Oxycarenus* spp. does not feed during this stage but draws only a little moisture from these hosts (PEARSON 1958, HENRY 1983). 24 hrs after application *Azadirachta indica* caused 70.66 and 58.33% mortality at 5 and 2.5% concentration respectively. These findings are supported by ASLAM & NAQVI (2000), who tested the efficacy of a neem product (phytopesticide FWB) and perfekthion against sucking pests of cotton (jassid, thrips, whitefly). He concluded that the neem product was less toxic than perfekthion. The neem product caused 88.86 and 54.5% mortality at concentrations of 5 and 2.5% respectively 24 hours after application. The neem product is a much safer, non-polluting biopesticide. The neem extract controlled the dusky cotton bug (NURULAIN et al. 1989) better than malathion after 72 hrs. TIWARI et al. (2006) used neem-based insecticides against *Dysdercus koenigii* (FABRICIUS, 1775) to evaluate their effects on development. Neem caused various developmental deformities and mortality. The toxicity and morphogenetic effects of the neem-based insecticide econeem (1%) against the third nymphal instar of *D. koenigii* were evaluated (KODANDARAM et al. 2008). This resulted in delayed metamorphosis, wing growth inhibition, no moulting and finally death within 24-48 hrs of application. It effectively reduced the population of *Helicoverpa armigera* (HÜBNER, 1809) and inhibited larval development on a chick pea crop (NELSON et al. 1996). Mortality of the dusky cotton bug increased with higher concentrations of the extracts used in the experiment. These findings are supported by ANITA et al. (2012), who tested the larvicidal effects of *Annona squamosal* L., *Moringa oleifera* and *Eucalyptus globulus* LABILL. Where mortality of insects increased with higher concentrations, the time taken to achieve 100% mortality decreased. So in a short period of time a higher percentage mortality was achieved. They also confirmed the insecticidal action of *Eucalyptus* sp., *Bougainvillea glabra* CHOISY, *Azadirachta indica*, *Saraca indica* L. and *Ricinus communis* against *Tribolium castenum* (HERBST, 1797). In our findings *Ricinus communis* showed minimum control at all concentrations, which is not in agreement with KODJO et al. (2011), who found that an oily emulsion of *R. communis* resulted in 100% mortality against the diamond back moth (*Plutella xylostella* (LINNAEUS, 1758)). This difference may be due to the difference in the biology of dusky cotton bug and *P. xylostella*. But this was confirmed by DEGRI et al. (2013), who investigated the effects of *Azadirachta indica*, *Chromolena odorata* (L.) KING & H. E. ROBINS. and *Ricinus communis* on pod-sucking bugs, where *A. indica* and *C. odorata* performed better than *Ricinus communis*. These two species can replace insecticides. Not all the products used against the dusky cotton bug in this experiment are referred to in the literature; only a few of them have been used against this pest. However, all of these products have been used on different pests on agricultural crops. In the past *Citrus sinensis* peel oil was used against the

Callosobruchus maculatus (FABRICIUS, 1775) and *Musca domestica* LINNAEUS, 1758 (PALACIOS et al. 2009, EKEH et al. 2013), *Calotropis procera* against *Musca domestica*, *Tribolium castenum* and *Plutella xylostella* (BEGUM et al. 2010, GANDHI et al. 2010), *Nicotiana tobacum* leaf powder against *Sitophilus zeamais* (MOTSCHULSKY, 1855) and *Callosobruchus maculatus* (IDOKO & ADEBAYO 2011, MUSA & OPADIRAN 2013), *Moringa oleifera* leaves against *Tribolium castenum* and *Anopheles stephensi* LISTON, 1901 (ANITA et al. 2012, PRASAD & SHARMA 2014), *Rocinus communis* against the snout weevil *Scyphophorus acupunctatus* GYLLENHAAL, 1838 and the leaf miner, *Eupterote mollifera* WALKER, 1865 (RAMAMURTHY et al. 2012).

CONCLUSION

Psidium guajava, *Eucalyptus camaldulensis*, *Mangifera indica*, *Azadirachta indica* and *Syzygium cumini* were found to be major hibernating sites of the dusky cotton bug, so for its proper management it is necessary to prevent its moving from alternative host plants to the cotton crop by using suitable spraying material, especially the biopesticides used in the experiment, in order to keep the environment safe and unpolluted. *Nicotiana tobacum* and *Calotropis procera* at 2.5 and 5% concentrations proved to be the best. On increasing these concentration, mortality will increase, too, within a short time.

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