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COMPARATIVE STUDIES ON THE LARVICIDAL EFFICACY OF OCIMUM SANCTUM AND ANNONA SQUAMOSA AN AGAINST RED COTTON BUG, *DYSDERCUS SINGULATUS*

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ABSTRACTS

The red cotton bug, *Dysdercuscingulatus* (Fab). is an important pest of cotton and okra. Although synthetic chemical insecticides can control it, the side effects are enormous. *Dysdercuscingulatus* (Fab.) is a serious pest of cotton and distributed all the cotton growing region of India. The application of easily degradable plant compounds is considered to be one of the safest methods to control insect pests and vectors as an alternative source for the synthetic pesticides. The study indicated that essential compounds were the only chemical used for the control of mosquito larvae while extract was used as the control of adult mosquitoes. The essential compounds were extracted by steam distillation and their chemical composition determined by Gas-chromatography coupled to mass spectroscopy. A study was made to monitor the effect of plant extracts on different instars of larvae and pupae of mosquito vector *D.cingulatus*. Bio-assay was made using the solvent acetone to find out the median lethal concentration. Plants, like *Ocimum sanctum* and *Annonasquamosa* which possess insecticidal properties are seemed to be better vector control agents than the synthetic xenobiotics. These results suggest a potential utilization of the extracts of these two plant species for the control of *Dysdercuscingulatus*.

KEYWORDS: *Dysdercuscingulatus*, insecticide, larvicidal activity, mosquito control, phyto-compounds, GC-MS analysis, *Ocimum sanctum* and *Annonasquamosa.*

INTRODUCTION

The red cotton bug (*Dysdercuscingulatus*) has wide distribution; it is a major pest in cotton growing regions of northern India particularly Punjab and Uttar Pradesh. This pest also occurs throughout the Maharashtra state but is of minor importance. It is commonly known as 'cotton stainer'. Host plants of cotton stain rare cotton, bhendi, ambadi, hollyhock and several other malvaceous plants. The adult bug measures about 12-15 mm in length. The females are longer (15 mm) than the males (12mm). It is blood red in color except eyes, scutellum, anal style and antennae which are black colored. Besides, there is black spot on each of the membranous forewings. Cotton stainer feeds both on immature and mature seeds. Their penetrations into the developing cotton boils transmit fungi on the immature lint and seed, which latter on stain the lint with typical yellow color, hence the name "cotton strainers". Heavy infestations on the seeds affect the crop mass, oil content and the marketability of the crop. The cotton stainer*Dysdercuscingulatus* (Fab.), commonly known as red cotton bug causes serious damage by feeding on developing bolls and ripe cotton seeds (Natarajan and Rajendren, 2005). It is distributed all over the cotton producing regions of India (Sahayaraj and Illayaraja, 2008). In India cotton production is about 295 million bales (H" 480 1b bales) during 2009-2010 against 113.9 million bales in the rest of the world. Also, India has the largest area under cotton cultivation (10.31million ha) and yield was 486 kg ha-1 during 2009-2010 (Cotcorp, 2010). Due to hazards associated with the increased use of synthetic pesticides the use of biopesticides especially from marine algae has gained considerable attention on the eco-friendly approaches for the management of insect pest.

*Dysdercuscingulatus*is a serious pest of cotton and distributed all the cotton growing region of India (Chari, 1998; Venugopal, 1994; David andAnanthakrishnan, 2004). It is difficult to control by insecticidebecause itis highly mobile, Polyphagous (Iwata, 1975) and Polymorphic (Sahayaraj and llayaraja, 2008) pest of many Malvaceae crops. Terrestrial plants like *Catharanthusroseus* G. Don, *Paratheniumhysterophorus* Don and Nephrolepsextracts were have insecticidal activity against red cotton bug (Rajendran andGopalan, 1980; Gahukan, 1995; Gawande and Burkhade, 1998).Moreover, neem based pesticide like neem gold (Abraham and Ambika, 1979) also shows nymphicidal activity against this pest. Ovicidal

activity of *Pedalium murex* (Linn.)(Sahayaraj*et al*., 2006) on *D.cingulatus* was reported earlier.

In India, herbs have long been used for promotion of health, prevention and treatment of diseases. *Ocimum sanctum* L., commonly known as 'Tulasi' in Tamil and holy basil in English, has been claimed to be valuable against a wide variety of diseases. Indian Materia Medica describes the use of the plant in the treatment of a number of ailments like bronchitis, rheumatism and pyrexia (Nadkarni, 1976). Studies on the immunomodulatory effect of *O. sanctum* have been reported for various animal species (Singh *et al*., 1996; Singh and Majumdar, 1997).

Tulasi (Holy Basil) is a traditional plant considered sacred by the Hindus. This religion links the plant with the Goddess figure as described in the Puranas. Hindus regard it as an earthly manifestation of goddess Vrindavani, who is dear to Lord Vishnu. The name "Tulasi" in Sanskrit means "the incomparable one." The Shyama Tulasi or Krishna Tulsi (*Ocimum sanctum* L. syn.*Ocimumtenuiflorum*) possesses great medicinal value as mentioned in CharakSamhita, an ancient Indian literature.

It is a most common household plant in India and grows wild in tropics. Native to India, it is a short lived perennial herb or small shrub of Mint family Labiatae (Lamiaceae). It has small leaves with a strong smell and purple flowers. The foliage is green or purple, strongly scented. Oil extracted from leaves of this plant possesses significant insecticidal properties (Nanasombat and Lohasupthawee, 2005). *Ocimum sanctum* has been extensively studied for therapeutic potentials in various areas like immuno-stimulation, anticancer antioxidant, as adjuvant to radiotherapy, antiulcer, analgesic and antidiabetic (Hammer *et al*., 1999).

Annonasquamosa Linn, belonging to family Annonaceae is commonly found in India & cultivated in Thailand & originates from the West Indies & South America. It is mainly grown in gardens for its fruits & ornamental value. *Annonasquamosa* L. (Annonaceae), commonly known as the custard apple tree is a native of West Indies. But the cultivation is present throughout India, because of its edible nature. It is a fruit tree considered as a native of Central America also and hence has a wider cultivation throughout the regions of tropics. The taste of the pulp of the fruit is really sweet because of its higher sugar content of about 58% of dry mass, and hence it is found clear that the fruit pulp possess a high calorie value. This plant was reputed to contain several medicinal properties (Gajalakshmi*et al*., 2011).

One of the major obstacles hindering cotton cultivation is insect pest manifestations. In particular, the sucking bugs such as *Dysdercus* Spp., (Hemiptera) cause severe injury or losses by feeding on developing cotton balls and ripe cotton seeds. These pests are difficult to control by synthetic insecticide application because the nymphs and adults of *Dysdercus* Spp., are highly mobile and have many alternate host plants such as castor, lady's finger, turnip, cabbage (Sahayaraj and Majesh Tomson, 2010). In the present work alcoholic extract of *Ocimum sanctum* and *Annonasquamosa*were investigated for potential larvicidal activity. To identify and characterized the compounds of therapeutic value extracted from *O.sanctum* and *A. squamosa*. The analytical methods chosen are Gas Chromatography/Mass Spectrometry (GC/MS). The methods were applied to characterize the infusion prepared from this plant and to make a comparison between the alcoholic extracts of the leaves.

MATERIALS AND METHODS

Plant material and oil distillation

The medicinal plant of *Ocimum sanctum* and *Annonasquamosa*were collected from in and around area of Pattukkottai, Thanjavur District, Tamil Nadu and South India. The plant was identified with the help of flora presidency, Tamil Nadu and Karnatic flora (Gamble 1967; Matthew 1983) and standard references (Krtikarand Basu 1935). A voucher specimen has been preserved in our laboratory. The plants leaf were dried and powdered of 50 g powdered sample were extracted with ethanol using soxhlet apparatus and concentrated *in-vacuo*. Approximately, 5 g of extract was obtained from 100 g of dried powder material. The extracts were dried in an air conditioned room at 25°C, milled and submitted to hydrodistillation in a Clevenger-type apparatus for 4 hours. The extractwere dried in anhydrous sodium sulphate, filtered, stored in amber glass bottles in a refrigerator (4°C) for investigation of chemical constituents and larvicidal activity.

GC-MS Analysis

The extracts of *Ocimum sanctum* and *Annonasquamosa* was hed with sterile distilled water, and they were shade dried and powdered by using Pestle and Mortar and for the alcoholic extracts (96% alcoholic solution) roots and leaves. The tincture was prepared by mixing all parts of the plant with a 50% alcoholic solution for 30 days. The infusion was also prepared by mixing parts of the plant with hot water for 20 min and the alcoholic extracts by mixing the fresh parts of the plant with a 96% alcoholic solution for 12 days.

The dry fractions (20g) were dissolved in 75ml of alcohol and than soaking for 24 hrs. After soaking, collect a filtrate and evaporate under liquid nitrogen. Then concentrate the filtrate for GC-MS analysis.

For the GC-MS analysis a 30m x 0.25mm I.D x 1.0 μmdf fused Elite-1 (100% Dimethyl Poly Siloxane) column; GC Clarus 500 Perkin Elmer gas chromatograph with Mass detector- Turbo mass gold- Perkin Elmer, Software- Turbo mass 5.1. The samples (1μl) were introduced *via* an all – glass injector working in the split mode (10:1), with Helium as the carrier gas.

Oven temperature programme: 110 deg-2min hold, upto 280 deg at the rate of 5 deg/9min hold. Injector temperature: 250 deg C. GC time – 45 mins.

MS Programme: Inlet line temperature: 200^{o}C, Source temperature: 200^{o}C, Electron energy: 70eV, Mass scan: (m/z) 45-450. MS time – 46 mins.

The identification of the constituents was performed by computer library search, retention indices and visual interpretation of the mass spectra. Compounds were identified by comparing their mass spectrum to those of the database of the GC-MS (NIST 62.lib), literature (McLafferty and Stauffer 1989) and retention indices (Adams 2007).

Collection and storage of experimental animals

Larvae of *Dysdercuscingulatus*were obtained from a permanent colony. The larvae were cultured and maintained in the laboratory at 27 ± 2^{0}C and 50 - 75% of relative humidity. Larval forms were maintained in tray by providing dog biscuit and yeast powder in the 3:1 ratio.

Test for Larvicidal activity (WHO, 1996)

The laboratory colonies of *Dysdercuscingulatus*were used for the larvicidal activity. The instarII and instarIV larvae and pupae of the selected mosquito species were kept in 1 litreglass beaker and different concentration of selected plant extract was added to find out LC_{50}.

Larvicidal bioassay

Different concentrations of extract (300, 250, 100, 50 and 25 μg/ml) were prepared using distilled water. The mosquito larvae were treated with extract by using the method of WHO

(1981). Ten larvae of *Dysdercuscingulatus*were introduced in different test concentration of both plant extracts along with a set of control containing distilled water without any test solution. After adding the larvae, the glass dishes were kept in laboratory at room temperature. By counting the number of dead larvae at 24hrs of exposure, the mortality rate and the median lethal concentration were obtained. Three replications were maintained for each concentration. Dead larvae were removed as soon as possible in order to prevent decomposition which may cause rapid death of the remaining larvae. The water used for the study was analyzed by usingthe method of APHA (1996). Mortality was recorded after 24 h of exposure during which no nutritional supplement was added.

The experiments were carried out 27 ± 2^0C. Each test comprised of three replicates with four concentrations (300, 250, 100, 50 and 25 µg/ml). Data were evaluated through regression analysis. From the regression line, the LC_{50} values were read representing the lethal concentration for 50% larval mortality of *Dysdercuscingulatus*.

RESULTS AND DISCUSSION

Physical and chemical characteristics of water used for the study, like temperature 27 ±0.5^{0}C, pH 7.3 ± 0.5, dissolved oxygen 3.6 ±0.5 mg/l, dissolved carbondioxide 1.2 ±0.5 mg/l, salinity 1.5 ±0.5 ppt and alkalinity 125 ±0.5 mg/l were within the permissible limits throughout the study periods.

The 24h bioassay is a major tool for evaluating the toxicity of phytotoxins, and a number of researchers have been applying this method to assess the toxiceffect of different plant extractionagainst mosquitoes (Sakthivadivel and Daniel 1999). The mosquito larvae exposed under plant extracts showed significant behavioral changes. The changes were observed within 30 minutes of exposure. The most obvious sign of behavioral changes observed in *Dysdercuscingulatus*was inability tocome onthe surface. The larvae also showedrestlessness, loss of equlibrium andfinally led to death. Remia and Logaswamy(2010) reported that these behavioral effects weremore pronounced in case of *Catharanthusroseus* than *Lantanacamara* extracts after exposures. These effects may be due the presence of neurotoxic compounds in both the plants. In the present study the behavioral effects were more pronounced in case of *Ocimum sanctum* and *Annonasquamosa*extracts after exposures. These effects may be due the presence of neurotoxic compounds in both the plants. No such behavioral changes were obtained in control groups.

Results of the experiment conducted for evaluating the larvicidal efficacy of both plants showed that they are toxic to the *D.cingulatus*larvae. Three replicates of each extract and control were performed in order to ascertain the consistency of the results (Tables 1- 3). The corrected percent mortalities were analyzed using Abbott's formula (Abbott 1925). The mortality data were analyzed using Prism Version 3 from which lethal concentration (LC_{50}) values (24 h) and 95% confidence intervals (CI) were determined. The LC_{50} value of the test extract was compared with that of *Ocimum sanctum*reflecting the potencies of the two; the one with a lower LC_{50} value being more potent of the two.

The crude extract of *Annonasquamosa*was found to be active on the IV th instar larvae of *D.cingulatus*. The larvicidal activity varied with the concentration and exposure. The larvicidal activity of *Annonasquamosa* was comparable to that of *Ocimum sanctum*. The exact active principles in *Ocimum sanctum*responsible for the larvicidal effect have been reported to contain sufficient amount of tetranortriterpenoids (Pegeland Rogers 1990). The observed mosquito larvicidal effects could possibly be due to these compounds (Siddiqui*et al*. 2000).

The results from the *D.cingulatus*larvicidal assay using two different plants are shown in Table 4. The most active essential compounds against third instar larvae of *D. cingulatus* were those of *Ocimum sanctum* and *Annonasquamosa*. Sukumar et al. (1991) reported that *C. citratus* causes significant growth inhibition and mortality in later developmental stages of *A. aegypti*. The analysis of the essential oil of this plant from the state of Ceará, showed that its major components are geranial (60.3%) and neral (39.7%).

Lippiasidoides essential oil and its main constituent thymol were shown to be very active against *A. aegypti* larvae. Sukumar et al. (1991) studied of *Ocimum americanum* showed that solvent extracts from the whole plant have ovipositional deterrence against *A. aegypti*. Matos (2000) reported that *Ocimumgratissimum* essential oil displays antifungal (*Aspergillus* and *Trichoderma*) and antibacterial (S*taphylococcus*) activities. *O. gratissimum* oil presented antihelmintic activity against *Haemonchus contortus*, the main nematode of ovines and caprines in Northeastern Brazil (Pessoa et al. 2002). The citrus oils, although they have insecticidal activities (Ezeonu et al. 2001) and *Hyptissuaveolens* that is used as mosquito repellent (Palssonand Jaenson 1999) were not effective in the larvicidal test. Supavarnet al. (1974) tested 36 vegetable extracts on *A. aegypti* and found that 11.1% were capable of

producing mortality at a concentration of 500 ppm but only 2.8% produced the same effect at a concentration of 100 ppm.

The use of vegetable oil presents a better option in comparison to chemical pesticides for the larval mosquito control, as chemicals may cause environmental hazardsand proved troublesome in the long run (Ranapukar*et al.* 2001). Extensive research has been carried out on the effect of botanical derivatives of the neem tree and its derivatives (Mullaand Su 1999).

Methanolicextract of the leaves of *Ocimum sanctum* and *Annonasquamosa* were evaluated for mosquitocidal activity against the immature stages of mosquitoes, *Culex quinquefasciatus, Anophelesstephensis* and *D.cingulatus*in the laboratory (Sivagnaname and Kalyanasundaram, 2004). A survey of literature on control of different species of mosquito revealed that assessment of the efficacy of different phytochemicals obtained from various plants has been carried out by a number of researches on the field of vector control *Ageratinaadenophora* (Spreng.) showed toxic effects on the mosquito species of *D.cingulatus*and *Culexquinquefasciatus* (Rajmohan and Ramaswamy 2007). *Albiziaamara*and *Ocimum sanctum* showed larvicidal andrepellant properties against *Aedesaegypti* and neem seed kernel extracts showed higher larvicidal activity of *Aedesaegypti* (Palsson and Janeson 1999; Sakthivadivel and Daniel 1999). A detailed laboratory study on extracts of fruit of *Piper nigrum* against larvae of *Culexpipines, Aedesaegypti* and *Aedestogoi* was carried out (Park *et al*. 2002). The authors determined the LC_{50} and observed the behavioural changes and mortality in the larvae. Similar observations were noticed in the present study and support the potential applications of these herbs in mosquito control measures.

Molluscicidal and mosquito larvicidal efficacy of *Ocimum sanctum*and mosquitolarvicidal property of *Momordicacharantia* have already been reported (Manisha Srivastava *et al.* 2007 and Singhet al. 2006) and observed them safe for humanhealth. In conclusion the leaf extract of *Ocimum sanctum* and *Annonasquamosa*are highly toxic even at low doses these plants may eventually prove to be useful larvicide. Further analysis is required to isolate the active principles and optimum dosages, responsible for larvicidal and adult emergence inhibition activity in *D.cingulatus*. The product of these plants canbe well utilized for preparing phytochemicals from which all the non-target organisms can be rescued from harmful vectors. These plants would be eco-friendly and mayserve as suitable alternative to synthetic

insecticides as they are relatively safe, inexpensive and are readily available in many areas of the world.

Table 1: Larvicidal effects of ethanolic extracts of *Ocimum sanctum* and *Annona squamosa* on larvae of *Dysdercus cingulatus* after a 24 h treatment at room temperature.

S. No	Concentration of the extract (mg/ml)	No. of larvae Dead/No. exposed (*O. sanctum*)	No. of larvae Dead/No. exposed (*A. squamosa*)	Mortality
1	Control	0/30	0/30	0
2	0.025	3/30	3/30	10
3	0.050	6/30	6/30	20
4	0.075	9/30	9/30	30
5	0.100	12/30	12/30	40
6	0.150	15/30	15/30	50
7	0.200	18/30	18/30	60
8	0.200	24/30	24/30	70
9	0.250	30/30	30/30	80
10	0.300	30/30	30/30	100

Table 2: Phyto-components of extract of *O. sanctum* identified by GC-MS study.

S.No	Components	Formula
1	Benzene acetaldehyde	C_8H_8O
2	5H-1-Pyrindine	C_8H_7N
3	2-Furan carboxaldehyde, 5-(Hydroxymethyl)-	$C_6H_6O_3$
4	Benzene acetic acid	$C_8H_8O_2$
5	Dodecanoic acid	$C_{12}H_{24}O_2$
6	Phenol, 3-Isopropoxy-5-Methyl-	$C_{10}H_{14}O_2$
7	3`-Acetyllycopsamine	$C_{17}H_{27}NO_6$
8	Squalene	$C_{30}H_{50}$
9	Octanoic acid, Ethyl ester	$C_{10}H_{20}O_2$
10	Benzaldehyde, 3-Hydroxy-4-Methoxy-	$C_8H_8O_3$
11	Benzaldehyde, 4-Hydroxy-3, 5-Dimethoxy-	$C_9H_{10}O_4$
12	4-((1E)-3-Hydroxy-1-propenyl)-2-Methoxy Phenol	$C_{10}H_{12}O_3$
13	Benzaldehyde, 4-Hydroxy-	$C_7H_6O_2$
14	Butanoic acid, 2-Methyl-	$C_5H_{10}O_2$
15	Nonanoic acid	$C_9H_{18}O_2$
16	Benzene acetic acid, 2,5-Dihydroxy-	$C_8H_8O_4$
17	3,7,11,15-Tetramethyl-2-Hexadecen-1-ol	$C_{20}H_{40}O$
18	Phytol	$C_{20}H_{40}O$
19	(Z)6,(Z)9-Pentadecadien-1-ol	$C_{15}H_{28}O$
20	1-(+)-Ascorbic acid 2,6-Dihexadeconate	$C_{38}H_{68}O_8$
21	Phytol	$C_{20}H_{40}O$
22	9,12-Octadecadienoic acid (Z, Z)-	$C_{18}H_{32}O_2$
23	9,12-Octadecadienoic acid, Ethyl Ester	$C_{20}H_{36}O_2$
24	Squalene	$C_{30}H_{50}$
25	Methyl Salicylate	$C_8H_8O_3$
26	1-(+)-Ascorbic acid 2,6-Dihexadeconate	$C_{38}H_{68}O_8$

+: Present; -: Absence

Table 3: Phyto-components of extract of *A. squamosa* identified by GC-MS study.

S. No	RT	Name of the Compound	Molecular Formula	Name of the compound
1	3.95	Benzene, 1,2,3-trimethyl-	C_9H_{12}	Aromatic compound
2	11.32	Undecanoic acid	$C_{11}H_{22}O_2$	Fatty acid
3	13.03	E-7-Tetradecenol	$C_{14}H_{28}O$	Alkene compound
4	13.79	Tetradecanoic acid	$C_{14}H_{28}O_2$	Myristic acid
5	16.61	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	Palmitic acid
6	16.89	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	Fatty acid ester
7	18.89	Phytol	$C_{20}H_{40}O$	Diterpene
8	19.31	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	Linoleic acid
9	19.61	Oleic Acid	$C_{18}H_{34}O_2$	Oleic acid
10	23.12	Eicosane, 2-methyl-	$C_{21}H_{44}$	Alkane compound
11	23.48	Oxirane, tetradecyl-	$C_{16}H_{32}O$	Oxirane compound
12	25.18	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	Plasticizer compound
13	27.41	Heptacosane	$C_{27}H_{56}$	Alkane
14	29.51	Squalene	$C_{30}H_{50}$	Diterpene

**Source: Dr. Duke's Phytochemical and Ethnobotanical Databases

Table 4: Percentage larval and pupal mortality of *Dysdercus cingulatus* for different concentrations of extract of *O. sanctum* and *A. squamosa* for 24 h exposure.

Plants used	Stages of exposure	Parameters	Effective concentration in µg/ml					
Annona squamosa	II instar	Larval mortality (%)	Control	50	100	150	200	250
			0	5	24	37	50	64
	IV instar	Larval mortality (%)	Control	160	180	200	220	240
			0	16	24	32	48	55
	Pupae	Pupal mortality (%)	Control	200	225	250	275	300
			0	10	19	27	53	66
Ocimum sanctum	II instar	Larval mortality (%)	Control	25	50	75	100	125
			0	15	22	31	42	55
	IV instar	Larval mortality (%)	Control	50	100	150	200	250
			0	14	35	52	63	75
	Pupae	Pupal mortality (%)	Control	200	225	250	275	300
			0	29	42	53	66	81

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