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Research Article

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Study of gonadosomatic index of 2nd - 8th days essential oil treated female *Euschistus servus*.

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Abstract

In the present investigation experiments were conducted to study the essential oils effect on female adult *Euschistus servus* (Brown stink bug). GSI effects of leaf and flower essential oils of *Cassia fistula* on the female adult *Euschistus servus*. Treatment showed significant decrease in GSI against female adult *Euschistus servus*, when compared to the control groups. The *Cassia fistula* leaf essential oils treatment decreases the GSI in female adults *Euschistus servus* more in comparison to that of Cassia fistula flower essential oils and was in the order: *Cassia fistula* leaf > *Cassia fistula* flower. Dose dependent effects were observed in the case of GSI of insects treated with essential oils. It is thus inferred that *Cassia fistula* leaf extract exhibited the lowest percent of GSI in female adult *Euschistus servus.* GSI plays a very important role in reproductive potency of insects. Increase in GSI indicated hyperactivity of gonads whereas decrease in GSI indicates poor development or malformation of gametes which results in disfunctioning of gonads.

Keywords: *Euschistus servus, Cassia fistula*, GSI.

Introduction

Soybean being a very popular crop in terms of economic returns infested by *Euschistus servus* (Brown stink bug). So, as to protect it from these pests, it becomes essentially necessary to control the rate of fertility by increase in the rate of mortality and decline the rate of fertility of *Euschistus servus*. Stink bugs belong to the order Hemiptera, the true bugs. Hemiptera, with 100,428 described species as of 2009 (Foottit and Adler 2009), with more still being discovered, are distinguished by the presence of piercing and sucking mouthparts and wings that are thin and membranous for much of the length, but are thick and hard at the base. The impression is given that the insects have only half-length wings, hence the name of the order. The stink bug family Pentatomidae has 4,123 described species (Panizzi and Schaefer 2000) and gets its scientific name from the five-sided shield-like body. Also prominent are the three-segmented antennae with prominent flagellomeres, and the triangular scutellum on the thorax (Triplehorn and Johnson 2005). While present on almost all insects, the scutellum is particularly large and often brightly colored in stink bugs.

Present investigation was an approach to control the fertility of these pests by using essential oils. It is well known that insect pests play a major role in damaging the crops and the need continues for efficacious control agents. The use of pesticides and insecticides is one way of preventing or controlling losses from insects. The control includes all the measures which make life hard for insects and kill or repel them by

disrupting their vital processes. Insecticide pollution and their off target effects along with development of resistance in insects have stimulated the search for the target specific methods of plant protection. Plant products are the greatest gift of nature and recognized source of effective insecticide. Plant products have higher specificity, little or no toxicity to non-target organism and biodegradability combine to make natural compounds more suitable for insect control. The objective of this research was to calculate GSI, which is an index to show the decline in rate of fertility of *Euschistus servus* and provide the protection for crops.

Materials and Methods

Procurement of insects: Female adults of *Euschistus servus* were procured locally from the fields of soybean and reared on normal laboratory conditions.

Procurement of experimental plant and extraction: *Cassia fistula* leaves and flowers were procured locally from the corresponding plant. *Cassia fistula* belongs to Family -Fabaceae /Caesalpiniaceae.

Isolation of the essential oil: Samples of fresh leaves (400 gm) and flowers were triturated and submitted to hydro distillation process in a Clevenger-type apparatus for 4 hours according to the method used in British Pharmacopoeia (1980). The collected essential oil was subsequently dried by anhydrous sodium sulfate (Na_2SO_4) and stored under refrigeration at 4 ºC until be tested. The amount of oil obtained was measured and the oil percentage was calculated based on the fresh weight (v/w %).

Analysis of the essential oil: The isolation, identification, and quantification of the essential oil compounds were performed with a gas chromatograph Shimadzu GC-17A (Shimadzu Corporation, Kyoto, Japan) coupled with a Shimadzu mass spectrometer detector GC/MS QP-5050A. Analyses GC/MS were carried out using helium as carrier gas at a flow rate of 0.9 mL min-1 in a split ratio of 1:20 on DB-5 column (30 m × 0.25 mm i.d., 0.25 µm film thickness) and the following temperature program: (a) 80 ºC for 0 min; (b) rate of 3 ºC min-1 from 80 to 250ºC; (c) rate of 25 ºC min-1 from 250 to 300 ºC and hold for 5 min. Injector and detector temperatures were 200 and 300 ºC, respectively.

Identification of phytocompounds: Interpretation on mass-spectrum of GC-MS-MS was conducted using the database of National Institute Standard and Technology (NIST) having more 62,000 patterns. The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library. The name, molecular weight, molecular formula, retention time and retention indices of the components of the test materials were ascertained, identified and confirmed by matching their retention times (authentic standards), retention indices (RI) and NIST mass spectral library collection (NIST, 2014). Analyses were run in triplicate.

Results in Table 1 summarize the chemical composition and retention indices (RI) found using GC/MS, where the constituents are listed in order of their elution from the column. Essential oils and components were kept under freezing until used. Series of aqueous concentrations of each essential oil were prepared with Triton X-100 as surfactant at a rate of 0.1 %. The stock solutions of different concentrations of essential oils were used at room temperature for the experimentation. The experiments were done in triplicate.

Studies on the GSI in *Euschistus servus*: GSI is a significant aspect of this research investigation concerning with the fertility rate of *Euschistus servus.* The deviation (increase or decrease) in GSI in comparison to control was related with the efficiency of gonad (in increase in GSI) or dependent on the malformation (in case of decrease in GSI) of gonadial tissue as evident in table 2.

Female Fertility Index (FFI) in adult females: Mean weight of each (control or experimental) groups of 10 adult females of *Euschistus servus* ovaries of the same adult insects have been recorded and FFI was calculated by the following formula:

FFI (in adults) =

$$\frac{\text{Wet weight of ovaries}}{\text{Wet weight of female insects}} \times 100$$

Table 1 : Components of *Cassia fistula* essential oil.

S. No.	Component*	RI_{exp}	RI_{thr}	Leaf (%)	Flower (%)
1	α-terpineol	1078	1185	tr	-
2	Methyl salicylate	1176	1188	4.3	-
3	Tridecane	1300	1300	1.7	-
4	Eugenol	1337	1355	-	tr
5	Tetradecene	1400	1386	2.7	-
6	Methyl eugenol	1400	1402	-	7.3
7	Tetradecane	1413	1400	10.5	-
8	(z)-β-farnesene	1438	1439	-	tr
9	Neryl acetone	1438	1432	tr	tr
10	Cabreuva oxide A	1442	1443	-	tr
11	(E)-α-ionone	1454	1427	tr	-
12	(E)-β-ionone	1454	1485	3.2	tr
13	Cabreuva oxide B	1462	1464	-	tr
14	2-tridecanone	1481	1491	-	tr
15	β-bisabolene	1500	1502	-	tr
16	pentadecane	1501	1500	4.4	-
17	elemicin	1550	1552	-	tr
18	Isoelemicin	1564	1566	-	tr
19	1-hexadecene	1584	1586	3.8	-
20	(E)-nerolidol	1611	1558	2.2	23.8
21	hexadecane	1612	1600	8.7	tr
22	Nerol	1670	1226	tr	-
23	heptadecane	1700	1700	5.0	-
24	2-hexadecanone	1776	1776	-	17.0
25	1-octadecene	1785	1786	2.8	-
26	octadecane	1800	1800	2.0	-
27	hexahydrofarnesylacetone	1820	1823	4.0	tr
28	Benzyl salicylate	1858	1860	7.0	-
30	2-heptadecanone	1881	1881	-	tr
31	(E, E)-farnesylacetone	1898	1898	-	tr
32	nonadecane	1900	1900	1.3	-
33	Methyl hexadecanoate	1908	1910	-	tr
34	Hexadecanoic acid	1990	1991	-	tr
35	Eicosene	1990	1990	1.8	-
36	eicosane	2023	2000	tr	-
37	Methyl linoleate	2072	2074	-	6.3
38	Methyl linolenate	2084	2085	-	tr
39	(E)-phytol	2102	2106	16.1	-
40	docosane	2200	2200	tr	-
41	Docosene	2208	2189	tr	-
42	tricosane	2300	2300	tr	5.9
43	pentacosane	2450	2500	1.7	6.1
44	heptacosane	2664	2700	2.8	12.8
45	nonacosane	2896	2900	4.0	6.5
46	Esters			10.0	-
47	Fatty acid and fatty acids esters			tr	6.3

Where: * = Components listed in order of elution, (-) = absence, tr = trace (<0.1%), RI = Retention Index, exp = experimental, thr = theoretical.

Results and Discussion

GSI (Gonado Somatic Index) plays a significant role in reproductive potency of insects as observed by Rai (2005) in *Bagrada cruciferarum*, Agrawal (2006) in *Dysdercus similis* and Tomar (2010) in *Spodoptera exigua* after treatment with plant extracts. In the present investigation the adult female of *Euschistus servus* of normal and control groups did not show any remarkable change in FFI (Female Fertility Index), while experimental groups treated with *Cassia fistula* leaf and flower essential oils showed reduction in FFI (Table 2).

Table 2: Effect of Essential oils Treatment on FFI of *Euschistus servus*.

S. No.	Name of Plant	Type of Experimental Groups	Average Body Weight		Decrease or Increase in Body Weight	Average Weight of Ovaries	FFI
			IW	FW			
1.	*Cassia fistula* leaf	Control	476 mg	476 mg	No Change	85 mg	17.85
		Treated Groups					
		2 day old	476 mg	460 mg	16 mg	80 mg	16.80
		4 day old	476 mg	452 mg	24 mg	75 mg	15.75
		6 day old	476 mg	444 mg	32 mg	70 mg	14.70
		8 day old	476 mg	426 mg	50 mg	65 mg	13.65
2.	*Cassia fistula* flower	Control	492 mg	492 mg	No Change	90 mg	18.29
		Treated Groups					
		2 day old	492 mg	482 mg	10 mg	85 mg	17.27
		4 day old	492 mg	474 mg	18 mg	80 mg	16.26
		6 day old	492 mg	462 mg	30 mg	75 mg	15.24
		8 day old	492 mg	437 mg	55 mg	70 mg	14.22

IW = Initial Weight
FW = Final Weight
Average weight of ovaries = Average weight of ovaries of 10 female insects in each group.

In the present investigation the female adults of *Euschistus servus* of normal and control groups did not show any remarkable change in FFI while experimental groups treated with *Cassia fistula* leaf and flower essential oils showed reduction in FFI (Table 2) as observed by Rai (2005) in *Bagrada cruciferarum* after treatment with abrin, Agrawal (2006) in *Dysdercus similis* after treatment with *Delonix regia* and *Dhatura alba* seed extracts and Tomar (2010) in *Spodoptera exigua* after treatment with plant glycosides. The *Cassia fistula* leaf and flower essential oils treatment decreases the GSI in female adults of *Euschistus servus*. The results on the GSI in the present investigation indicate that toxic activity of essential oil was in the order: *Cassia fistula* leaf > *Cassia fistula* flower.

Similarly Rai (2005) reported reduced GSI in adult insects of *Bagrada cruciferarum* by the treatment of glycosides extracted from the seed of *Abrus precatorius* and seed kernel of *Cerbera thevetia* and suggested that abrin was more toxic than cerberin. Agrawal (2006) reported reduced GSI in nymphs as well as in adults of *Dysdercus similis* by the treatment of *Delonix regia* and *Dhatura alba* seed extracts and suggested that *Delonix regia* seed extract was more toxic than *Dhatura alba* seed extract. Tomar (2010) reported reduced GSI in adults of *Spodoptera exigua* by the treatment of *Abrus precatorius* and *Trigonella foenum-graecum* seed extracts and suggested that *Abrus precatorius* was more toxic than *Trigonella foenum-graecum* glycoside.

GSI plays a very important role in reproductive potency of insects. Increase in GSI indicated hyperactivity of gonads whereas decrease in GSI indicates poor development or malformation of gametes which results in disfunctioning of gonads.

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