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Study of gonado somatic index of 2nd-8th days essential oil treated male *Euschistus servus*

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ABSTRACT

Euschistus servus (Hemiptera: Pentatomidae) are important insect pests of crops. Stink bug feeding reduces yields, lowers crop quality, induces delayed maturity and wounds tissues allowing for pathogen entry. Present investigation showed effective control of brown stink bugs with essential oils as evaluated by GSI parameter. The *Cassia fistula* leaf and flower essential oils treatment decreases the GSI in male 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.

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

INTRODUCTION

Euschistus servus (Brown stink bug) are significant economic pests of many agricultural crops and are frequently one of the most difficult pest groups to control in soybean. Identification of the species complex and efficient sampling techniques for stink bugs will provide valuable information that can be used in pest management. Insecticides in the organophosphate and pyrethroid classes are the most commonly used to control *Euschistus servus* in most crops. However, these insecticides are broad spectrum toxicants that may have environmental and human health risks and frequently destroy important natural enemies in the agro-ecosystem. Newer bio-insecticide such as the essential oils may offer control options that are less toxic and disruptive to natural enemies and may fit better with IPM programs.

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Stink bugs are strong fliers and this allows them to move quickly between their wide array of host crops making them difficult to detect (McPherson and McPherson 2000). For these reasons and due to a multiple-species complex, stink bugs can be extremely difficult to manage. *Euschistus servus* also overwinters as an adult, but usually under crop residues and weeds, especially common mullein, *Verbascum thapsus* (L.). Following emergence in the spring in mid to late April, they are typically found on their main host plant (Munyaneza and McPherson 1993). However, Buntin and Greene (2004) have reported an abundance of brown stink bugs in winter wheat. Brown stink bugs may complete a generation in winter wheat before moving into later maturing nearby crops as they become attractive. Every soybean production area in the world is associated with at least one economically damaging stink bug (Todd and Herzog 1980). *A. hilare*, along with *N. viridula* and *E. servus*, is one of the top three stink bug pests to attack soybeans in the Mid-Atlantic region (McPherson 1982). Stink bug feeding preference varies with the developmental progress of the crop. The insects typically move into a soybean field after flowering, remain through maturity, and migrate to more nutritious plants once the fruit is no longer succulent. Stink bug feeding can cause green stem syndrome, a plant response that results in green stems past maturity. Feeding also reduces the seed quality, slows the maturity of the plant and decreases bean germination with the introduction of pathogens, and decreases soybean yield (Underhill 1934, Emfinger *et al*. 2001, Medrano *et al*. 2007). Seeds may be rejected by importers if stink bug damage is present (Chyen *et al*. 1992), resulting in economic loss to the growers. Laboratory bioassays were conducted on soybean to evaluate the efficacy of essential oils on male *Euschistus servus* GSI. The objective of this research was to calculate GSI, which is an index to show the decline in rate of fertility of *Euschistus servus*. Understanding the overall effects of essential oils should lead to improved *Euschistus servus* management by bioinsecticides.

MATERIAL AND METHOD

Procurement of insects:

Male 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.

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	hexahydrofarnesy lacetone	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

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 male

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.

Male Fertility Index (MFI) in adult males:

Mean weight of each (control or experimental) groups of 10 adult males of *Euschistus servus* and *Ceratoma trifurcata* and testes of the same adult insects have been recorded and MFI was calculated by the following formula:

$$\text{MFI (inadults)} = \frac{\text{Wet weight of testes}}{\text{Wet weight of male insects}} \times 100$$

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 male of *Euschistus servus* of normal and control groups did not show any remarkable change in MFI (Male Fertility Index), while experimental groups treated with *Cassia fistula* leaf and flower essential oils respectively showed reduction in MFI (Table-2).

In the present investigation the male adults of *Euschistus servus* of normal and control groups did not show any remarkable change in MFI, while experimental groups treated with *Cassia fistula* leaf and flower essential oils respectively showed reduction in MFI (Table 2) as observed by Rai (2005) in *Bagrada cruciferarum* after treatment with seed extracts of *Abrus precatorius* and *Cerebra thevetia*, Agrawal (2006) in *Dysdercus similis* after treatment with *Delonix regia* and *Dhatura alba* seed extracts and Tomar (2010) in *Spodoptera exigua* after treatment with Flavonol Glycoside 3,4'-dihydroxy-7,3',5'-trimethoxy,flavone-3-O β-D-galactopyranosyl (1→4)-O-α-L-xylopyranoside and Quercitrin glycoside from *Trigonella foenum-graecum*.

The *Cassia fistula* leaf and flower essential oils treatment decreases the GSI in male 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

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

S. No.	Name of Plant	Type of Experimental Groups	Average Body Weight		Decrease or Increase in Body Weight	Average Weight of Testes	MFI
			IW	FW			
1.	*Cassia fistula* leaf	Control	411 mg	411 mg	No Change	70 mg	17.03
		Treated Groups					
		2 day old	411 mg	399 mg	12 mg	65 mg	15.81
		4 day old	411 mg	393 mg	18 mg	60 mg	14.59
		6 day old	411 mg	381 mg	30 mg	55 mg	13.38
		8 day old	411 mg	351 mg	60 mg	50 mg	12.16
2.	*Cassia fistula* flower	Control	432 mg	432 mg	No Change	75 mg	17.36
		Treated Groups					
		2 day old	432 mg	416 mg	16 mg	70 mg	16.20
		4 day old	432 mg	408 mg	24 mg	65 mg	15.04
		6 day old	432 mg	400 mg	32 mg	60 mg	13.88
		8 day old	432 mg	382 mg	50 mg	55 mg	12.73

IW = Initial Weight; FW = Final Weight; Average weight of testes = Average weight of testes of 10 male insects in each group.

hyperactivity of gonads whereas decrease in GSI indicates poor development or malformation of gametes which results in disfunctioning of gonads

CONCLUSION

The results exhibited that this new insecticide is of great economic importance from the agronomic point of view. In view of environmental and health concerns, determined efforts were made in the last years to reduce and rationalize the use of chemicals worldwide in order to manage pests more effectively. Moreover, the basic concern of using plant extracts is with designing and implementing pest management practices that meet the goals of farmers in reducing pest losses by reducing the numbers of the noxious agents (pests) while at the same time safeguarding against the longer term risks of environmental pollution, hazard to human health and reduced agricultural sustainability.

The essential oils treatment mainly affected the functioning of gonads and decrease in GSI. The decline in GSI could be correlated with quantitative depletion of protein metabolites in the gonads of the treated insects, large number of immature pathological oocytes which did not attain such maturity which is required for egg laying. The antifertility activity was in the order: *Cassia fistula* leaf > *Cassia fistula* flower.

Conflict of Interests

Authors declare that there is no conflict of interests regarding the publication of this paper.

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