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# Study of Knock down Toxicity of *Cassia fistula* extract treated *Euschitus servus*

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**ABSTRACT:**
*Euschistus servus* (Hemiptera: Pentatomidae) commonly known as brown stink bug are serious insect pests of soybean crop. This pests feeding reduces crop yields, decline crop quality, induces delayed crop maturity and increases wounds tissues allowing easy pathogen entry. Recent investigation showed effective control of *Euschistus servus* with *Cassia fistula* leaf and flower essential oils as evaluated by KD toxicity. The values of $KD_{100}$, $KD_{50}$, $KD_0$ and sub-lethal concentration were found out and data was tabulated in table 1-2. Results showed that *Cassia fistula* plant extract had a higher toxicity on all nymphal instar stage of brown stink bug.

**Keywords:**
KD toxicity, *Cassia fistula* and *Euschistus servus*.

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## 1. INTRODUCTION

Brown stink bug are important economic pests of several agricultural field crops and one of the most serious pest groups to control in soybean crop. Organophosphate insecticides and pyrethroid are mostly used to control *Euschistus servus* in various crops. Above insecticides are toxicants of broad spectrum that have health and environmental risks. Newer bio-insecticide extracts from plant offer excellent options that are better options than traditional insecticides.

Stink bugs move quickly between their wide array of host crops making them difficult to detect (McPherson and McPherson 2000). It also overwinters as an adult. 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. Every soybean production area in the world is associated with at least one economically damaging stinkbug (Toddand 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. Pest infestation reduces the seed quality, delays the maturity of the crop and reduces bean germination with easy introduction of pathogens, hence reduction in soybean yield (Underhill 1934, Emfinger *et al*. 2001, Medrano *et*

*al*. 2007). Seeds may be rejected by importers if pest damage is present (Chyen *et al*. 1992), resulting in great economic harm to the farmer.

Insect pest damages the crop and new alternative ways are regularly found to control them. Most common way to control insects from times is insecticides. Insecticides have serious environmental issues worldwide. The scientists throughout the world focused for plant extracts as environmentally safe alternatives for pest control with less impact on ecosystem and health risks. Plants products have active compounds in them working as an excellent source of bio-insecticides in future. Recent work focuses on controlling brown stink bug with leaf and flower essential oils of *Cassia fistula* plant.

## 2. MATERIAL AND METHODS

Methodology used in this experimentation are as follows:

**Collection of insects:** 1st – 5th nymphal instars of brown stink bug were collected from local soybean crop. Pests were provided water and food ad libitum on normal laboratory protocol.

**Extraction of plant products by Soxhlet method**: Experimental plant flowers and leaves were collected from local area. Plant parts were cleaned, powdered into coarse pieces mechanically and following Soxhlet method extracted along with petroleum ether. The plant extract were filtered after 35 cycles was done and extracted for 20 hrs with 200 ml alcohol and again filtered.

About 5.5 gm experimental plant leaf and flower extract were weighed. With distilled water 1% stock solution was prepared and stored in dark glass bottle at 4°C. For KD toxicity experimentation further dilution was done and calculated the $KD_{100}$, $KD_{50}$ (method adopted after Finney 1971), $KD_0$ and sublethal concentrations. All protocols were done in triplicate.

**Essential oils isolation:** Samples of plant extracts were triturated and submitted to hydro distillation process in a Clevenger-type apparatus for 4 hours as per the method used in British Pharmacopoeia (1980). The essential oil collected was subsequently dried by anhydrous sodium sulfate ($Na_2SO_4$) and kept at 4 °C until used. The amount of essential oil obtained was measured and the oil percentage was calculated based on the fresh weight (v/w %).

**Essential oils analysis:** The identification, isolation and quantification of the *Cassia fistula* leaf and flower essential oil active compounds were done with a gas chromatograph Shimadzu GC-17A (Shimadzu Corporation, Kyoto, Japan) coupled with a Shimadzu mass spectrometer detector GC/MS QP-5050A. GC/MS analyses were done 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 °C for 0 min; (b) rate of 3 °C min-1 from 80 to 250°C; (c) rate of 25 °C min-1 from 250 to 300 °C and hold for 5 min. Injector and detector temperatures were 200 and 300 °C respectively.

**Phytocompound identification:** Mass-spectrum of GC-MS-MS interpretation was done using the database of National Institute Standard and Technology having above 62,000 patterns. The spectrum of the unknown active components was compared with the spectrum of known active components stored in the library of NIST. The molecular weight, name, molecular formula, retention indices and retention time of the active components of the plant materials were identified, ascertained and reconfirmed by matching their retention indices (RI) and retention times (authentic standards) and mass spectral library collection of NIST (NIST,2014). Protocols were run in triplicate.

Essential oils of *Cassia fistula* leaf and flower and active components were kept under refrigeration until used for experimentation. Different series of aqueous concentrations of *Cassia fistula* leaf and flower essential oil were prepared with Triton X-100 as surfactant at a rate of 0.1 %. The stock solutions of *Cassia fistula* leaf and flower essential oils were used at room temperature for the experiments. The protocols were done in triplicate.

**Experimental contact bioassay:** 10 nymphs/per nymphal instar stage of brown stink bug control

group and experimental groups were used for experiments. $KD_{100}$, $KD_{50}$ and $KD_0$ and sublethal concentrations were detected out for different groups of control and experimental group separately with various concentrations of essential oils of *Cassia fistula* leaf and flower and tabulated in table 1-2. Knock down concentration ($KD_{50}$) as the hrs needed to produce 50% mortality in experimental stages was determined by Probit analysis method (Finney, 1971). Normal / control groups were provided with sufficient food and water ad libitum and were also treated with same concentration of experimental essential oils of *Cassia fistula* as used for experimental nymphal groups.

## 3. RESULT AND DISCUSSION

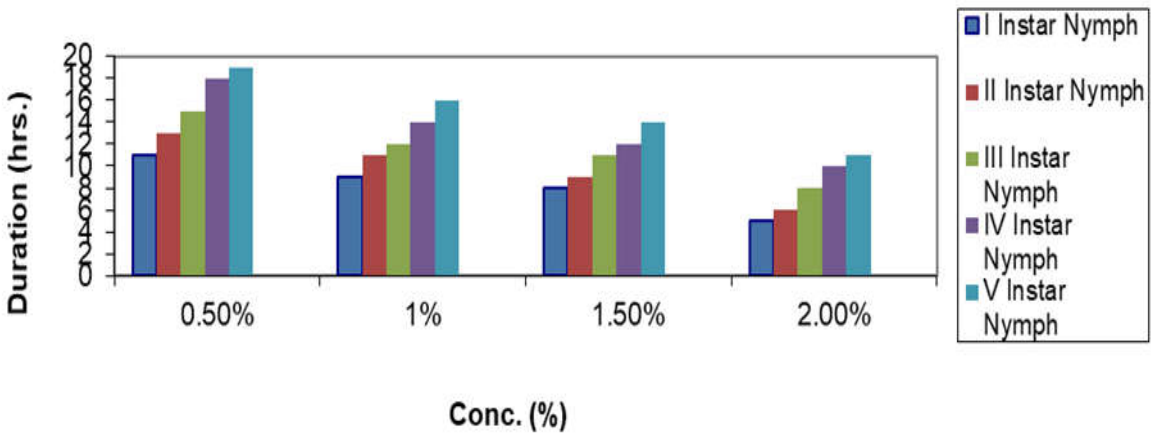
*Euschistus servus* is a common pest of Order – Hemiptera, Family - Pentatomidae with high rate of fertility and their nymphal and adult stages causes a lot of damage to the standing crop of soybean. So, the present investigation was done to know more about the KD toxicity of essential oils from *Cassia fistula* flower, leaf respectively in $1^{st}$-$5^{th}$ instar nymphal stages of *Euschistus servus*.

The experiments were done separately for the KD toxicity of *Cassia fistula* leaf, flower essential oils and data for $KD_{100}$, $KD_{50}$, $KD_0$ and experimental concentration was recorded and summarized in Table 1-2 and Graph 1-2. The normal and control groups $1^{st}$-$5^{th}$ instar nymphal stages of *Euschistus servus* were provided with normal food and water ad libitum in laboratory and show insignificant mortality after treatment.

**Table 1: Showing KD Toxicity of** *Cassia fistula* **leaf essential oil on** *Euschistus servus***.**

| Name of Plant | Concentration (%) | I Instar Nymph Duration (hrs.) | II Instar Nymph Duration (hrs.) | III Instar Nymph Duration (hrs.) | IV Instar Nymph Duration (hrs.) | V Instar Nymph Duration (hrs.) | Mortality (%) | KD Toxicity Values |
|---|---|---|---|---|---|---|---|---|
| Essential oil of *Cassia fistula* leaf | 0.50 | 19<br>11<br>8<br>6 | 21<br>13<br>09<br>07 | 24<br>15<br>10<br>07 | 28<br>18<br>12<br>09 | 32<br>19<br>14<br>09 | 100%<br>50%<br>Nil<br>Nil | $KD_{100}$<br>$KD_{50}$<br>$KD_0$<br>Exp Conc. |
| | 1.0 | 17<br>09<br>07<br>04 | 20<br>11<br>07<br>05 | 22<br>12<br>09<br>08 | 27<br>14<br>10<br>09 | 30<br>16<br>12<br>11 | 100%<br>50%<br>Nil<br>Nil | $KD_{100}$<br>$KD_{50}$<br>$KD_0$<br>Exp Conc. |
| | 1.5 | 15<br>08<br>06<br>03 | 19<br>09<br>06<br>05 | 21<br>11<br>08<br>07 | 26<br>12<br>08<br>07 | 29<br>14<br>10<br>09 | 100%<br>50%<br>Nil<br>Nil | $KD_{100}$<br>$KD_{50}$<br>$KD_0$<br>Exp Conc. |
| | 2.0 | 13<br>05<br>03<br>02 | 16<br>06<br>04<br>02 | 18<br>08<br>06<br>03 | 23<br>10<br>06<br>05 | 27<br>11<br>09<br>06 | 100%<br>50%<br>Nil<br>Nil | $KD_{100}$<br>$KD_{50}$<br>$KD_0$<br>Exp Conc. |

Graph 1: Showing $KD_{50}$ Toxicity of *Cassia fistula* leaf essential oil on *Euschistus servus*.




Graph 2: Showing $KD_{50}$ Toxicity of *Cassia fistula* flower essential oil on *Euschistus servus*.

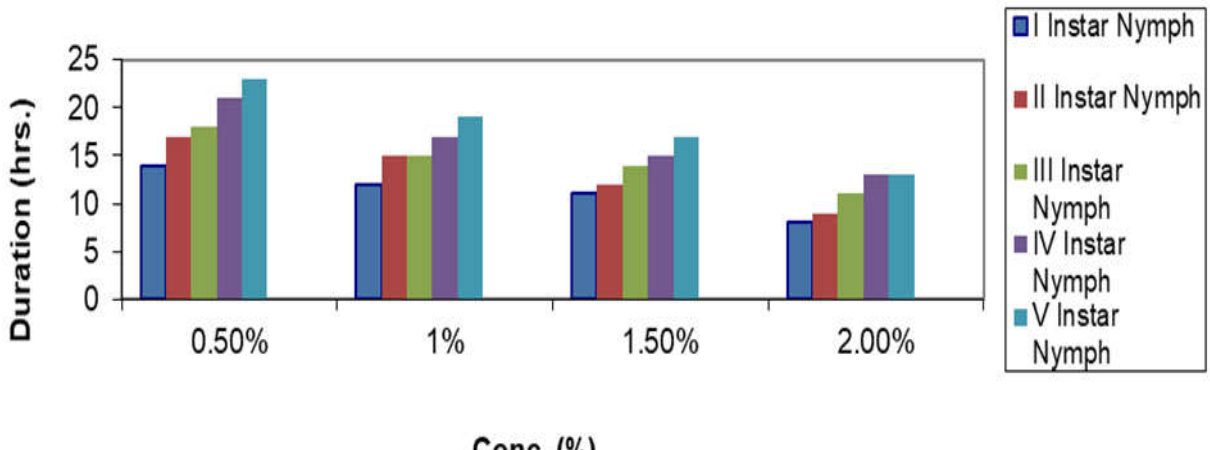


**Table 2: Showing KD Toxicity of** *Cassia fistula* **flower essential oil on** *Euschistus servus***.**

| Name of Plant | Concentration (%) | I Instar Nymph Duration (hrs.) | II Instar Nymph Duration (hrs.) | III Instar Nymph Duration (hrs.) | IV Instar Nymph Duration (hrs.) | V Instar Nymph Duration (hrs.) | Mortality (%) | KD Toxicity Values |
|---|---|---|---|---|---|---|---|---|
| Essential oil of *Cassia fistula* flower | 0.50 | 22<br>14<br>11<br>9 | 24<br>17<br>12<br>10 | 27<br>18<br>13<br>10 | 31<br>21<br>15<br>12 | 35<br>23<br>17<br>13 | 100%<br>50%<br>Nil<br>Nil | $KD_{100}$<br>$KD_{50}$<br>$KD_0$<br>Exp Conc. |
| | 1.0 | 20<br>12<br>10<br>07 | 24<br>15<br>11<br>08 | 25<br>15<br>12<br>11 | 30<br>17<br>13<br>12 | 33<br>19<br>14<br>14 | 100%<br>50%<br>Nil<br>Nil | $KD_{100}$<br>$KD_{50}$<br>$KD_0$<br>Exp Conc. |
| | 1.5 | 18<br>11<br>09<br>06 | 22<br>12<br>09<br>08 | 24<br>14<br>11<br>10 | 29<br>15<br>11<br>10 | 31<br>17<br>13<br>12 | 100%<br>50%<br>Nil<br>Nil | $KD_{100}$<br>$KD_{50}$<br>$KD_0$<br>Exp Conc. |
| | 2.0 | 16<br>08<br>05<br>03 | 18<br>09<br>07<br>03 | 21<br>11<br>09<br>06 | 26<br>13<br>09<br>06 | 30<br>13<br>11<br>08 | 100%<br>50%<br>Nil<br>Nil | $KD_{100}$<br>$KD_{50}$<br>$KD_0$<br>Exp Conc. |

The essential oils from *Cassia fistula* leaf, flower treatment on 1st-5th instar nymphal and adult stages of *Euschistus servus* showed nymphicidal and activity at 0.50%, 1.0%, 1.5% and 2.0% concentration and the $KD_{50}$ value were detected as follows:

**1. Essential oil of** *Cassia fistula* **leaf:-**

For 0.50%
- 11 hrs in I Instar Nymph
- 13 hrs in II Instar Nymph
- 15 hrs in III Instar Nymph
- 18 hrs in IV Instar Nymph
- 19 hrs in V Instar Nymph

For 1.0%
- 9 hrs in I Instar Nymph
- 11 hrs in II Instar Nymph
- 12 hrs in III Instar Nymph
- 14 hrs in IV Instar Nymph
- 16 hrs in V Instar Nymph

For 1.5%
- 8 hrs in I Instar Nymph
- 9 hrs in II Instar Nymph
- 11 hrs in III Instar Nymph
- 12 hrs in IV Instar Nymph
- 14 hrs in V Instar Nymph

For 2.0%
- 5 hrs in I Instar Nymph
- 6 hrs in II Instar Nymph
- 8 hrs in III Instar Nymph
- 10 hrs in IV Instar Nymph
- 11 hrs in V Instar Nymph

**2. Essential oil of** *Cassia fistula* **flower:-**

For 0.50%
- 14 hrs in I Instar Nymph
- 17 hrs in II Instar Nymph
- 18 hrs in III Instar Nymph
- 21 hrs in IV Instar Nymph
- 23 hrs in V Instar Nymph

For 1.0%
- 12 hrs in I Instar Nymph
- 15 hrs in II Instar Nymph
- 15 hrs in III Instar Nymph
- 17 hrs in IV Instar Nymph
- 19 hrs in V Instar Nymph

For 1.5%
- 11 hrs in I Instar Nymph
- 12 hrs in II Instar Nymph
- 14 hrs in III Instar Nymph
- 15 hrs in IV Instar Nymph

- 17 hrs in V Instar Nymph

For 2.0%

- 8 hrs in I Instar Nymph
- 9 hrs in II Instar Nymph
- 11 hrs in III Instar Nymph
- 13 hrs in IV Instar Nymph
- 13 hrs in V Instar Nymph

The data was recorded in table 1 and 2 by the method adopted after Finney (1971).

The recent experimental findings proved the efficacy of essential oils from *Cassia fistula* leaf, flower while normal / control groups showed the great survival rate of *Euschistus servus* 1st-5th instar nymphal stages. The *Cassia fistula* leaf, flower essential oils were evaluated for its nymphicidal activity against the 1st-5th instar nymphal stages of *Euschistus servus* respectively.

The experimental groups showed the decline in viability percentage and the experiments suggested that 1st-5th instar nymphal stage were more prone to the experimental different concentration. The 1st-5th instar nymphal stage never transforms to next life stages and never ever grows into healthy full grown adults.

The *Cassia fistula* leaf, flower essential oils proved to be nymphicidal agents in present investigation.

A lot of research work has been done on the nymphicidal, larvicidal and adulticidal (insecticidal) property of plant products by Isman *et al.* (1990), Dixit *et al*. (1991), Bhattacharya *et al*. (1993), Pandji *et al*. (1993), Luthria *et al*. (1993), Girach *et al*. (1994), Chang *et al*. (1995), Atal *et al.* (1998), Kumar *et al*. (1999), Jaswanth *et al*. (2002), Kumar (2003) and Bhide *et al*. (2004).

In the present work *Cassia fistula* leaf, flower essential oils and *Lantana camara* leaf, flower and fruit essential oils were evaluated for KD toxicity in all nymphal stages of brown stink bug i.e. *Euschistus servus*.

Bhide and Rai (2005) worked on Knock Down Toxicity of *Cerbera thevetia* seed kernel extract in different nymphal stages in *Bagrada cruciferarum* and they observed that the extract of *Cerbera thevetia* being a contact poison for insect, penetrate the body wall and tracheal system resulted into death probably lending the extract the insecticidal activity against *Bagrada cruciferarum*.

Agarwal (2006) worked on Knock Down Toxicity of *Delonix regia* and *Dhatura alba* seed extract in different nymphal stages in *Dysdercus similis* and they observed that the extract of *Delonix regia* and *Dhatura alba* showed decline in viability percentage and 1st nymphal stage were more susceptible to test solution in comparison to other nymphal stage while last nymphal stage were less affected and never hatched into healthy adults. Most of the nymphs developed into small sized, morphological malformed weakened adults with less developed gonads and the oocytes never attain such maturity, which is required for oviposition. The seed extract of *Delonix regia* and *Dhatura alba* were proved to be nymphicidal as well as antiovipositional agents.

Tomar (2010) worked on Knock Down Toxicity of Flavonol Glycoside 3,4′-dihydroxy-7,3′,5′-trimethoxy,flavone-3-O-β-D-galactopyranosyl (1→4)-O-α-L-xylopyranoside from *Abrus precatorius* and Quercitrin glycoside from *Trigonella foenum-graceum* seed extract in 4th instar larval stage of *Oberea brevis* and 3rd-5th instar larval stage and adults of *Spodoptera exigua* and observed that Flavonol Glycoside 3,4′-dihydroxy-7,3′,5′-trimethoxy,flavone-3-O-β-D-galactopyranosyl (1→4)-O-α-L-xylopyranoside and Quercitrin glycoside showed decline in viability percentage and were more susceptible to the test solution.

In the present investigation toxicity levels of essential oil is as follows: *Cassia fistula* leaf > *Cassia fistula* flower suggesting the nymphicidal action in 1st-5th instar nymphal stages of *Euschistus servus*. So, *Cassia fistula* leaf, flower essential oils was more effective in present investigation due to creating some obstructions in hatching or moulting in experimental insects.

The result shows that natural insecticides were of great economic point of view. The reason for

using new natural pesticides is that, these are active at highly acceptable level as well as these plant products are non-persistent type, biodegradable and their residues not accumulated in the food chains, toxic to only small targeted insects pests and it could be quite significant after formulation in large scale to use these natural insecticides of plant origin to control crops and to increase the productivity of the developing country like India.

## 4. CONCLUSION

Present work depicts that plant products are an excellent source of alternative bio-insecticide from being eco-friendly with less environmental hazards and reduced human health risks from their exposure. The use of these bio-insecticides make a great way to manage insect pest that to with reduce harmful effects. In future can serve on broad spectrum worldwide.

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