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EFFECT OF *CASSIA FISTULA* ON *CERATOMA TRIFURCATA*

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ABSTRACT

Bean leaf beetle (BLB) is one of the pests of concern of soybean. BLB larvae feed on soybean roots, particularly nodules and reduce leaf and pod nitrogen content. Adults feed on cotyledon, leaves and pods and reduce foliage. Foliage reduction showed a delay in canopy development and consequently, yield reduction. During pod fill, BLB may feed on the surface of pods. While larval feeding on root and defoliation due to adult beetle feeding affect soybean yield indirectly, pod feeding inflicts direct injury on yield. The feeding injury on the pod surface facilitates an entry point of bacterial and fungal infection. Additionally, the feeding injury to soybean pods exposes the seeds to excess moisture. The seeds in scarred pods may become shrunken, discolored and moldy. Thus pod surface feeding by BLB directly reduce yield weight and quality. Plant–derived extracts have long been a subject of research in an effort to develop alternatives to conventional insecticides and will continue to be an important source model of insecticides. So, to control the fecundity and viability the larvae and adults of *Ceratoma trifurcata* were treated with the seed extract of *Cassia fistula* which proved to be larvicidal and adulticidal in action. The potential application of this extract affects insect fertility and appeared to be an extremely promising approach in bio-insecticides direction while at the same time reduction of adverse environmental and health impacts associated with conventional insecticides/pesticides.

Key Words: Bean leaf beetle, foliage, injury, infection.

1. INTRODUCTION

Soybean has many insect pests limiting its production, including, the bean leaf beetle. Damage often occurs as the plants are emerging and if the bean leaf beetles are present in large enough numbers, stand reduction can occur. (Rajeshwar, 2013). Large populations are also capable of causing extensive defoliation to older stands. Bean leaf beetle larvae develop through six instars before pupating in the soil. The larvae lived underground to the base of the stem or roots, feeding on soybean roots, particularly nodules and feed there for three to six weeks. High larvae infestation (19 larvae per plant) may cause significantly smaller root nodules and reduce leaf and pod nitrogen content. Bean leaf beetle adults feed on the above ground part of soybean such as cotyledon, leaves and pods. Adult beetle feeding on soybean leaves and cotyledons in vegetative stages is estimated to reduce up to 2.15 $inch^2$ (13.93 cm^2) of foliage. Foliage reduction on cotyledons and seedlings showed a delay in canopy development and consequently, yield reduction as a result of cumulative foliage loss between VC and V_3 stages. Nevertheless, it

has been noted that a soybean plant's ability to tolerate defoliation, either by delayed leaf senescence or compensatory leaf regrowth, can offset the foliage loss due to bean leaf beetle feeding. During pod fill, bean leaf beetles may feed on the surface of pods. While larval feeding on root and defoliation due to adult beetle feeding affect soybean yield indirectly, pod feeding inflicts direct injury on yield. The feeding injury on the pod surface facilitates an entry point of bacterial and fungal infection. Additionally, the feeding injury to soybean pods exposes the seeds to excess moisture. The seeds in scarred pods may become shrunken, discolored and moldy. Thus pod surface feeding by bean leaf beetles directly reduce yield weight and quality. Particularly under dry conditions, bean leaf beetles feed on the pedicles, clipping the pod in the process. It has been estimated that an adult beetle may cause as much as 0.125 pods lost per day.

The bean leaf beetle (BLB), *Cerotoma trifurcata* (Forster) (Coleoptera: Chrysomelidae), is endemic to North America and it has been historically regarded in the mid-western United States as a relatively minor pest which can occasionally cause economic losses in soybeans, *Glycine max*. Hunt *et al.* (1994) determined that leaf defoliation of 68% in seedling soybeans caused 12% reduction in yield. Bean leaf beetle feeding also can cause up to 40% soybean pod damage (Witkowski and Echtenkamp, 1996). This insect has been reported to be a sporadic pest of soybean in Nebraska, however, its pest status, abundance and significance has increased in the recent years primarily due to the rapid increase in soybean acreage (Srinivas *et al.*, 2001). There are many factors which can affect the beetle population increase and these include environmental conditions, overwintering success, cultural practices, natural enemies, cultivar planted and the synchronization of soybean and beetles development (planting date). The BLB adults overwinter in field crop residue and in forest leaf litter (Lam *et al*., 2002). In the spring the adults exit the overwintering sites and move to available hosts such as alfalfa. The adults colonize soybeans as soon as the first seedlings emerge and throughout their early vegetative stages. The insect has two generations per year in Nebraska (Witkowski and Echtenkamp, 1996). Bean leaf beetle is the pest most associated with pod injury on a regular basis in the Midwest. This insect chews smooth-edged, round holes in newly expanded leaves and pods reducing yield and seed quality. It may feed on blossoms and stems. Its larvae feed on roots, nodules and the underground stem. The beetle is capable of transmitting diseases such as bean pod mottle virus (BPMV). Adult bean leaf beetles feed mostly on leaves and pods, with the most serious losses caused by pod feeding. Pod lesions caused by the beetles allow excess moisture and secondary plant disease organisms to enter the pod (Obopile and Hammond, 2001), causing seed shriveling and discoloration. As a result, the seed quantity and quality are reduced. The first increase in bean leaf beetle, population for the first generation is associated with foliar feeding and the second generation with seed and pod feeding (Hammond *et al*., 1991).

BLB is one of the pests of concerns of soybeans (Koch *et al*., 2012). Hence, there is a need to develop and evaluate integrated pest management tactics for this pest (Carrillo *et al*., 2005). Effective alternative means of soybean pest management are needed to reduce insecticide applications and the ensuing economic and environmental costs in soybean production systems (Hesler *et al*., 2012). An understanding of the phenology of this pest is essential in the development of a successful pest management program. Important applications of this information include improved timing of scouting procedures and proper implementation of management tactics (Smelser and Pedigo, 1991). Host plant phenology also affects the distribution and abundance of herbivores by producing a temporal variation in host quality. Insect herbivores often adapt to this temporal variation in resources by synchronizing their life cycles with the phenologies of the host plants. Dawn *et al*. (1999) reported that modification of planting dates has been widely used to disrupt synchrony between crops and pests. Although the population fluctuations and biology of bean leaf beetle on grain soybean has been studied

(Lam *et al*., 2001), there is lack of information about their behavior and possible management practices on soybeans. The knowledge of the infestation times of bean leaf beetle on these soybeans can help to manage them more especially in avoiding seed damage at harvestable stages. 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. Poisoning by pesticides is a serious problem throughout the world. Due to the problems associated with the indiscriminate use of synthetic insecticides like insect resistance and impact on non-target organisms, many scientists throughout the world have concentrated on the search for active natural products derived from plants as ecologically safe alternatives, because globally there is growing awareness and desire to utilize natural and environment-friendly compounds for pest control.

The efficacy of plant products has often been shown to be due to an active principle contained therein. This could be the best alternative in this direction. Further investigations to control this serious pest can be done by plant-derived products. So, the present investigation has been taken to study the lethal toxicity of *Cassia fistula* seed extract on *Ceratoma trifurcata*.

2. MATERIAL AND METHODS

The material and methods used in this investigation are as follows:

2.1 Procurement of experimental plant seeds and extraction by soxhlet method:

Seeds of *Cassia fistula* were procured from M/s "Shidh Seeds Sales Corporation", Dehradun (U.P.) India. The seeds were washed, shadow dried, mechanically powdered into coarse pieces weighed 100 gm and then extracted with petroleum ether (B. P. 60 – 80^{o}C, procured from B.D.H.) by Soxhlet method. After completion of 35 cycles in the Soxhlet the extracts were filtered and defatted plant material was again extracted with 200 ml alcohol for 20 hrs then filtered. After evaporation of solvent the extract were weighed 5.5 gm in *Cassia fistula* seed extraction. Yellowish color semi-liquid was extracted from *Cassia fistula* seeds. 1% stock solution was prepared in distilled water, kept in dark colored tightly closed glass bottles and stored in refrigerator (at 4^{o}C). The stock solution was further diluted and used at room temperature for the detection of LC doses and calculated the LC_{100}, LC_{50} (method adopted after Finney 1971), LC_0 and sublethal concentrations. The experiments were done in triplicate.

2.2 Procurement and rearing of *Ceratoma trifurcate:*

4^{th} – 6^{th} larval instar stages and adult insects of both sexes of *Ceratoma trifurcata* were procured locally from the fields of soybean and reared in the laboratory on normal laboratory conditions by providing food and water ad libitum to avoid the stress of dehydration.

2.3 Contact bioassay:

Insects were divided into different groups. Each control or experimental groups contain 10 larvae/per larval instar stage, 10 male or female insects and experiments were performed with different concentrations of extract from the seeds of *Cassia fistula* and the values of LC_{100}, LC_{50} and LC_0 and sublethal concentrations were detected out for each group separately and the data was summarized in table 1 – 5. Lethal concentration (LC_{50}) as the hrs needed to produce 50% mortality in experimental stages was determined by Probit analysis method (Finney, 1971). Control groups of *Ceratoma trifurcata* were applied topically with the same concentration of solvent as used in experimental groups. All the experimental and control groups were provided with sufficient food and water to avoid the stress of starvation and dehydration respectively.

RESULTS AND DISCUSSION

The extract of *Cassia fistula* seed was tested for lethal toxicity. The control and experimental groups were treated with extract of *Cassia fistula*

seed. The values of LC_{100}, LC_{50}, LC_0 and sub-lethal concentration for each separate group were recorded and data was summarized in table 1-5 and graphs 1-5. The 4th - 6th larval instar stage and adults of both sexes of *Ceratoma trifurcata* of normal and control groups did not show significant mortality percentage because they have been provided all normal environmental conditions with food and water ad libitum in the laboratory. The extract of *Cassia fistula* treatment on experimental 4th - 6th larval instar stage of *Ceratoma trifurcata* showed larvicidal activity whereas adulticidal activity on adults of both sexes and the respective sublethal concentration values were detected as follows:

0.02 % at 48 hrs in 4th larval instar stage.
0.2 % at 72 hrs in 5th larval instar stage.
0.3 % at 96 hrs in 6th larval instar stage.
0.5 % at 96 hrs in adult male.
0.8 % at 96 hrs in adult female.

Tomar (2009) worked on lethal toxicity of Flavonol glycoside 3,4'-dihydroxy-7,3',5'-trimethoxy,flavone-3-O-β-D-galactopyranosyl (1→4)-O-α-L-xylopyranoside extracted from *Abrus precatorius* seed and Quercitrin glycoside from *Trigonella foenum-graceum* seed on *Oberea brevis* and *Spodoptera exigua*. The 4th larval instar stage of *Oberea brevis* and 3rd – 5th larval instar stage and adults of *Spodoptera exigua* were more susceptible to the test solution. The larval stages never undergo next stages, small sized, morphologically malformed adults with less developed gonads were observed.

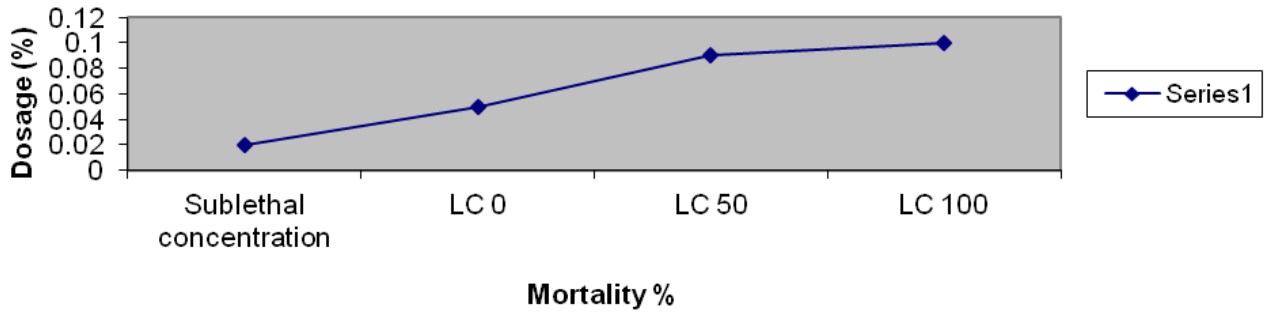
The probability of intoxication in both treated groups showed that Flavonol glycoside 3,4'-dihydroxy-7,3',5-trimethoxy,flavone-3-O-β-D galactopyranosyl (1→4)-O-α-L-xylopyranoside was more toxic than Quercitrin glycoside. These extracts proved to be larvicidal as well as adulticidal (insecticidal) agents.

In the present investigation the experimental findings proved the efficacy of extract showed decline in viability percentage, while control groups showed the high viability rate of 4th - 6th larval instar stage and adults of *Ceratoma trifurcata*. The *Cassia fistula* seed extract were evaluated for its larvicidal activity against 4th –

Table-1: Showing Lethal Toxicity of *Cassia fistula* Seed Extract in 4th Larval Instar Stage of *Ceratoma trifurcata*.

S. No.	Name of the plant seed extract	Concentration of the seed extract (%)	Duration (hrs.)	Mortality (%)	Lethal Conc. Value
1.	*Cassia fistula*	0.1 %	48	100 %	LC_{100}
		0.09 %	48	50 %	LC_{50}
		0.05 %	48	Nil	LC_0
		0.02 %	48	Nil	Sublethal concentration

Figure-1. Showing lethal concentration values, dosages and mortality rate of *Ceratoma trifurcate* in 4th larval instar stage after *Cassia fistula* seed extract treatment



6th larval instar stage and for its adulticidal activity against adult male and female of *Ceratoma trifurcata*. The experiments suggested that the 6th larval instar stage never undergoes further stages i.e. never hatched into healthy adults. Adults developed with less developed gonads and the oocytes never attain such maturity which is required for oviposition.

Table-2: Showing Lethal Toxicity of *Cassia fistula* Seed Extract in 5th Larval Instar Stage of *Ceratoma trifurcata*.

S. No.	Name of the plant seed extract	Concentration of the seed extract (%)	Duration (hrs.)	Mortality (%)	Lethal Conc. Value
1.	*Cassia fistula*	0.4 %	72	100 %	LC_{100}
		0.35 %	72	50 %	LC_{50}
		0.25 %	72	Nil	LC_{0}
		0.2 %	72	Nil	Sublethal concentration

Figure-2. Showing lethal concentration values, dosages and mortality rate of *Ceratoma trifurcate* in 5th larval instar stage after *Cassia fistula* seed extract treatment

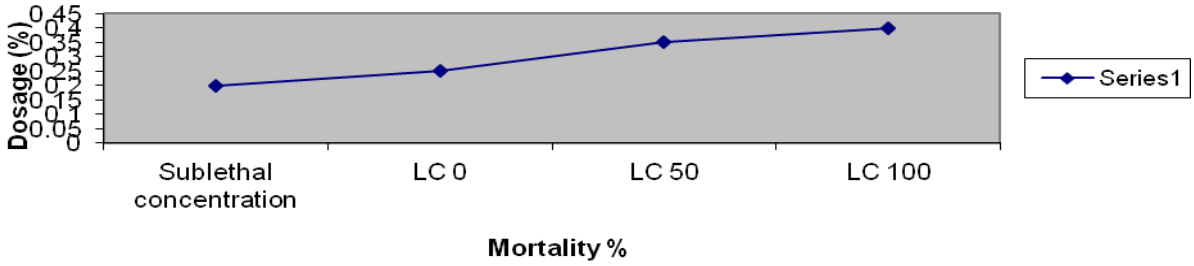


Table-3: Showing Lethal Toxicity of *Cassia fistula* Seed extract in 6th Larval Instar Stage of *Ceratoma trifurcata*.

S. No.	Name of the plant seed extract	Concentration of the seed extract (%)	Duration (hrs.)	Mortality (%)	Lethal Conc. Value
1.	*Cassia fistula*	0.8 %	96	100 %	LC_{100}
		0.6 %	96	50 %	LC_{50}
		0.4 %	96	Nil	LC_{0}
		0.3 %	96	Nil	Sublethal concentration

Figure-3. Showing lethal concentration values, dosages and mortality rate of *Ceratoma trifurcate* in 6th larval instar stage after *Cassia fistula* seed extract treatment

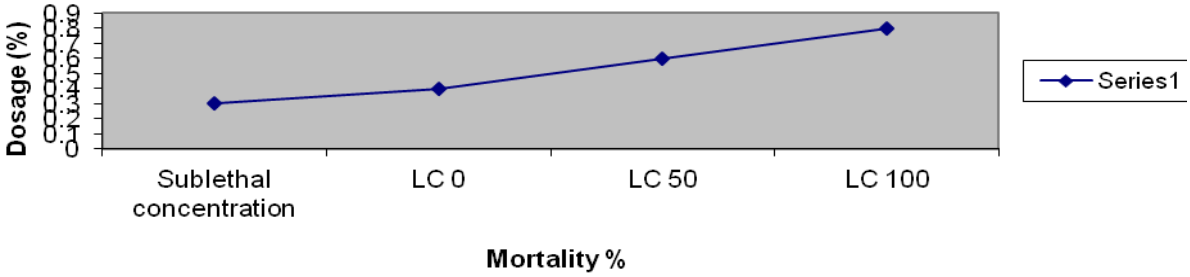


Table 4: Showing Lethal Toxicity of *Cassia fistula* Seed Extract in Adult Male of *Ceratoma trifurcata*.

S. No.	Name of the plant seed extract	Concentration of the seed extract (%)	Duration (hrs.)	Mortality (%)	Lethal Conc. Value
1.	*Cassia fistula*	3.0%	96	100 %	LC_{100}
		2.0%	96	50 %	LC_{50}
		1.0%	96	Nil	LC_{0}
		0.5%	96	Nil	Sublethal concentration

Figure-4. Showing lethal concentration values, dosages and mortality rate of Adult male of *Ceratoma trifurcate* after *Cassia fistula* seed extract treatment

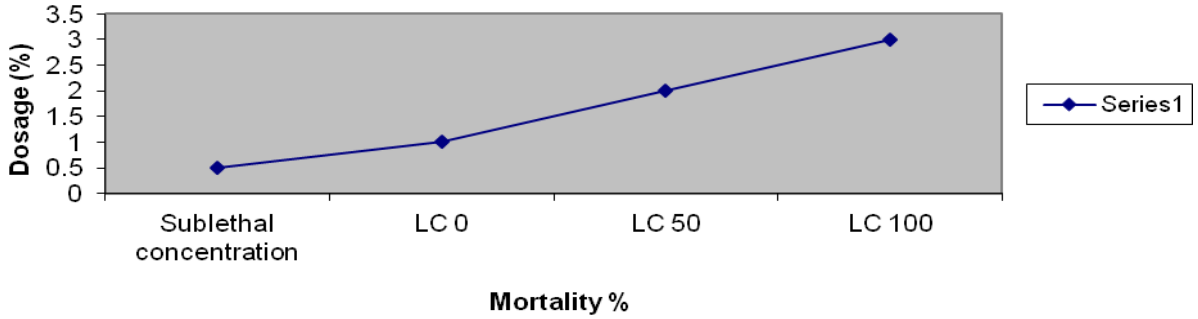
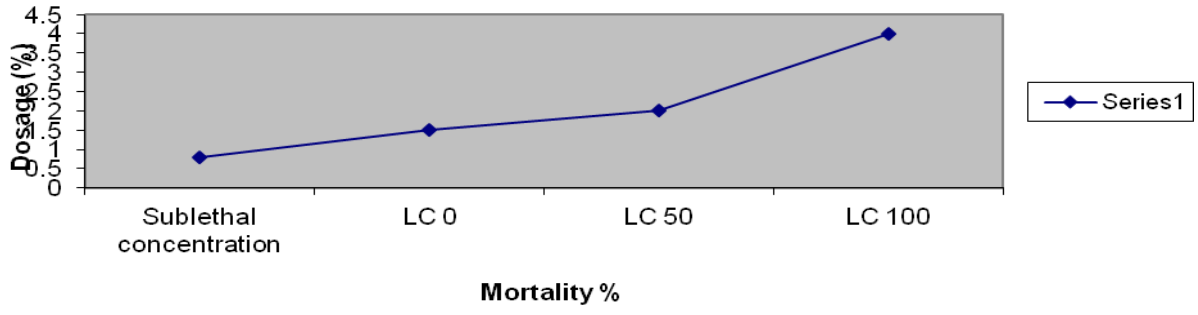


Table 5: Showing Lethal Toxicity of *Cassia fistula* Seed Extract in Adult Female of *Ceratoma trifurcata*.

S. No.	Name of the plant seed extract	Concentration of the seed extract (%)	Duration (hrs.)	Mortality (%)	Lethal Conc. Value
1.	*Cassia fistula*	4.0%	96	100 %	LC_{100}
		2.0%	96	50 %	LC_{50}
		1.0%	96	Nil	LC_{0}
		0.8%	96	Nil	Sublethal concentration

Figure-5. Showing lethal concentration values, dosages and mortality rate of Adult female of *Ceratoma trifurcate* after *Cassia fistula* seed extract treatment



In this way it is quite significant to control the fertility of *Ceratoma trifurcata* a serious pest of soybean.

The results showed that this new insecticide is of great economic importance from the agronomic point of view. In view of environmental and health concerns, a determined effort was made in the last years to reduce and rationalize the use of chemicals worldwide, in order to manage pests more effectively. By using plant products as an insecticidal agent, it concentrates on reducing the numbers of the noxious agents (pests) with agrochemicals.

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