Bio-insecticidal Activity of Endophytic Fungal Extract of Delonix regia on Callosobruchus chinensis Linn. (Coleoptera: Bruchidae)

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***Abstract*—Endophytic fungi are microorganisms that live inside the plant without causing any overt negative effect on plant tissues; rather they protect the host plant from pests and diseases. The insecticidal activity of endophytic fungal extract of leaf and seed of *Delonix regia* against pulse beetle, *Callosobruchus chinensis*, were studied. Endophytic fungi were isolated from the leaf and seed of *Delonix regia.* The various concentrations of methanol and ethyl acetate crude endophytic fungal extracts were tested against *Callosobruchus chinensis.* The percent mortality was recorded after 96h. The insecticidal activity of the endophytic fungi isolated from leaf of *Delonix regia* were (LD_{10}= 19.81mg/kg, LD_{50}= 84.63mg/kg) in methanol and (LD_{10}= 39.17mg/kg, LD_{50}= 110.3mg/kg) in ethyl acetate respectively. The fungi isolated from seed of *Delonix regia* were (LD_{10}= 12.25mg/kg, LD_{50}= 39.80mg/kg) in methanol and (LD_{10}= 28.97mg/kg, LD_{50}= 60.20mg/kg) in ethyl acetate respectively. The mortality increases with increase in concentration of endophytic fungi. The methanol solvent extract showed more insecticidal property against *C. chinensis* due to the secondary metabolites of endophytic fungi. Statistical variance, 95% confidence limits and regression equations are presented.**

***Index Terms*—Bioinsecticide, *Callosobruchus chinensis, Delonix regia,* Endophytic fungi, Mortality.**

I.INTRODUCTION

The crop and store grain pest problems are nearly as old as the beginning of crop cultivation. With a greater awareness of hazards associated with the use of synthetic organic insecticide there has been an increase need to explore suitable alternative methods of pest control. Farmers use different plant materials to protect store grain pest from pest infestation. Natural products in their crude form or plant extract provide unlimited opportunities as biopesticide. Heavy qualitative and quantitative losses occur due to heavy infestation of pulse beetle, *Callosobruchus chinensis* in the store grains. [1]-[4]. Therefore, various plants endophytic fungal extracts used for controlling the *Callosobruchus chinensis*. [5]-[8].
Delonix regia, commonly known as the flame tree, is renowned not only for its ornamental value but also for its production of a varity of secondary metabolites with significant bioactive properties. Recent studies highlighted the potential of plant-derived compounds as ecofriendly alternatives to synthetic pesticides.[9]. Extracts from seeds and leaves of *Delonix regia* demonstrated notable insecticidal activity against several economically important pest species, such as termites, ticks, cockroaches, and lepidopteran larvae. These effects shows both the direct toxicity and the inhibition of digestive enzymes critical to insect development and survival, suggesting multiple modes of action for *Delonix regia* extracts as biopesticides. [10].

Endophytes are microorganisms that grow within plants without causing any obvious symptoms of infection or disease. [11], [12]. Some of the endophyte microorganisms are thought to protect their host from attack by fungi, insect and mammals by producing secondary metabolites. Therefore plant associated microbes has explore the potential in pest control. The endophytic fungal metabolites showed pesticidal activity against major groundnut defoliator *S. litura.* [13]. In several ryegrasses that high fungi infection is correlated with a decrease in the attack frequency of the Argentine stem weevil, *Listronotus bonariensis*. [14]. Several authors studied that the role of endophytic fungi in the control of insects.

[15]-[20]. The present study was directed to assess the endophytic fungi isolated from leaf and seed of *Delonix regia* for the control of *Callosobruchus chinensis*.

II. MATERIAL AND METHODS

Insect culture

Infected seeds were obtained from the local market and culture the insect, *Callosobruchus chinensis* in laboratory. The glass jars were cleaned and dried in oven at 60 to 70 ^{0}C. Fresh non-infected grains of *Vigna unguiculata* were purchased from the market and were manually screened to remove the infected or hollow grains. The disinfected grains were then washed and dried in the oven at 60 ^{0}C to kill the stages of life cycle of pests if any. Ten males and ten females were released in 500 grams of these grains and allowed to maintain as stock culture.

Isolation of endophytic fungi

The plant parts of *Delonix regia* were collected from ACS College, Nandgaon campus and brought to the laboratory. Collected plant parts (leaf, seed) were gently rinsed in running tap water to remove adhered dust and debris. The plant parts were surface sterilized with 0.1% $HgCl_2$ for two minutes followed by washing in 70% ethanol, after that plant parts were washed with distilled water. Small pieces up to 1 cm. were cut and transferred into petridishes containing Potato Dextrose Agar medium (PDA) supplemented with Chloramphenicol antibiotic and incubated at 25 ± 2^{0}C for 3-5 days. The fungus grown out from the plates were subculture in PDA slants. The fungal mycelia growing out of the sample plates were continuously subcultured and maintained in PDA plates. [21].

Preparation of fungal extract

The 100 mg. of mycelium and spores of obtained endophytic fungi were collected separately from leaf and seed and extracted in 100 ml. of methanol and ethyl acetate solvent as stock solution.

Insecticidal bioassay

The methanol and ethyl acetate extract of endophytic fungi were screened for insecticidal activity against *Callosobruchus chinensis*. For screening 30gm seeds were shaken thoroughly with various concentration of methanol and ethyl acetate endophytic fungal extracts of leaf and seed in each jar. The dose was prepared by mixing the isolated endophytic fungi with respective solvent and was applied to grains. One jar of control containing seeds treated with only respective solvent was maintained. The treated seeds were allowed to evaporate the solvent for 48 hours. 5 male and 5 females emerged in a batch were released in each experimental and control jar containing 30gm. seeds and mortality was recorded after 24h up to 96h of treatment. The percent mortality was calculated after 96h and the observed data was subjected to probit analysis. [22], [23].

III. RESULTS AND DISCUSSION

The toxic effect of isolated endophytic fungal extract from leaf and seed of *Delonix regia* were evaluated against pulse beetle, *Callosobruchus chinensis*. The numbers of dead *Callosobruchus chinensis* were counted after 24, 48, 72 and 96h at different doses of methanol and ethyl acetate crude extract of endophytic fungi. The total percent mortality was observed after 96h. Then the corrected mortality was calculated by using Abbott's formula and the results are presented. The results showed that the mortality increases with increase in concentration at all doses (Table and Figure).

The results of the probit analysis for the estimation of LD_{10}, LD_{50}, variance, 95% confidence limits and regression equation at 96h for the mortality of pulse beetle, *Callosobruchus chinensis* are presented in table-2. In bioassay of methanol endophytic fungal extract of leaf and seed were LD10= 19.81mg/kg, LD50=84.63mg/kg and LD10= 12.25mg/kg, LD50=39.80mg/kg respectively, and in ethyl acetate leaf and seed endophytic fungal extract were LD_{10}= 39.17mg/kg, LD_{50}= 110.3mg/kg and LD_{10}= 28.97mg/kg, LD_{50}= 60.20mg/kg respectively. The insignificant χ^2 values for the regression coefficients suggest no heterogeneity to the data.

Table-1
Mortality percentage of *Callosobruchus chinensis* treated with endophytic fungal extracts isolated from leaf and seed of *Delonix regia.*

Plant parts	Solvent	Dose (mg/30gm)	No of insect	Mortality after 96h.	Percent mortality	corrected mortality
	Control	-	10	-	-	-
Leaf	Methanol	1.0	10	3	30	30
		2.0	10	4	40	40
		3.0	10	6	60	60
		4.0	10	8	80	80
		5.0	10	10	100	100
	Ethyl acetate	1.5	10	2	20	20
		2.5	10	3	30	30
		3.5	10	5	50	50
		4.5	10	7	70	70
		5.5	10	10	100	100
Seed	Control	-	10	-	-	-
	Methanol	0.5	10	2	20	20
		1.0	10	4	40	40
		1.5	10	5	50	50
		2.0	10	8	80	80
		2.5	10	10	100	100
	Ethyl acetate	1.0	10	2	20	20
		1.5	10	3	30	30
		2.0	10	5	50	50
		2.5	10	8	80	80
		3.0	10	10	100	100

Table-2
LD_{10}, LD_{50}values with variance, 95% confidence limits and probit analysis parameters for adult of *Callosobruchus chinensis* after 96h of treatment.

Plant parts	Solvent	LD_{10} mg/kg	LD_{50} mg/kg	Variance	95% CL		Regression equations	χ^2 (degree of freedom)
					Lower	Upper		
Leaf	Methanol	19.81	84.63	0.0097	1.7345	2.1205	Y = 1.0833 + 2.0320x	0.7486 (2)
	Ethyl acetate	39.17	110.3	0.0055	1.8967	2.1883	Y = -0.8235 + 2.8511x	0.4343 (2)
Seed	Methanol	12.25	39.80	0.0070	1.4352	1.7646	Y = 0.9939 + 2.5039x	0.8372 (2)
	Ethyl acetate	28.97	60.20	0.0028	1.6757	1.8835	Y = -2.1794 + 4.0344x	0.9875 (2)

Fig. 1
Regression and provisinal lines for *Callosobruchus chinensis* exposed to methanol extract of endophytic fungi isolated from leaf and seed of from leaf and seed of *Delonix regia* after 96h

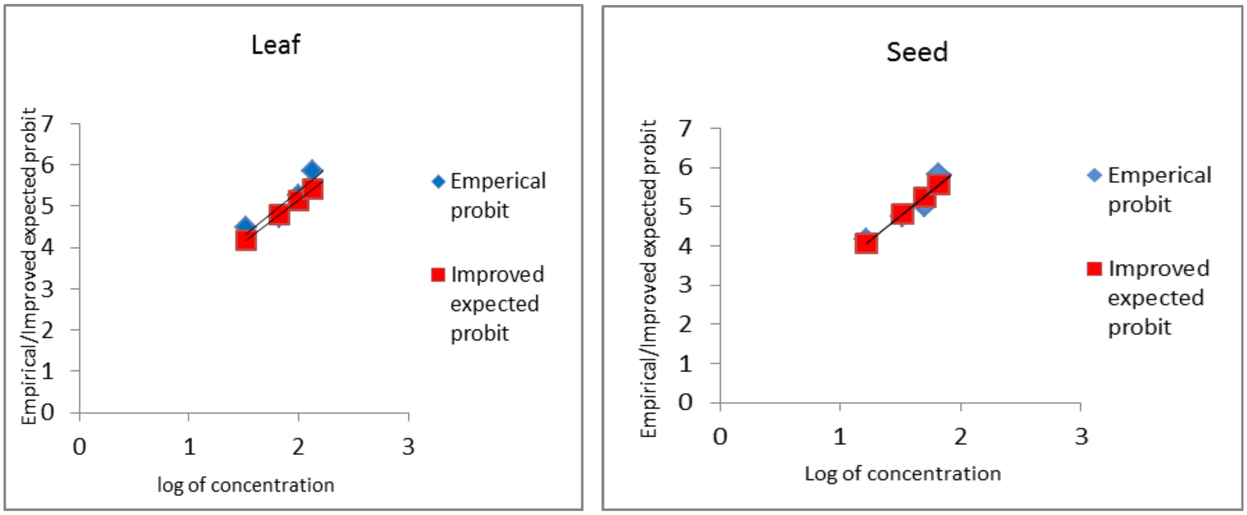
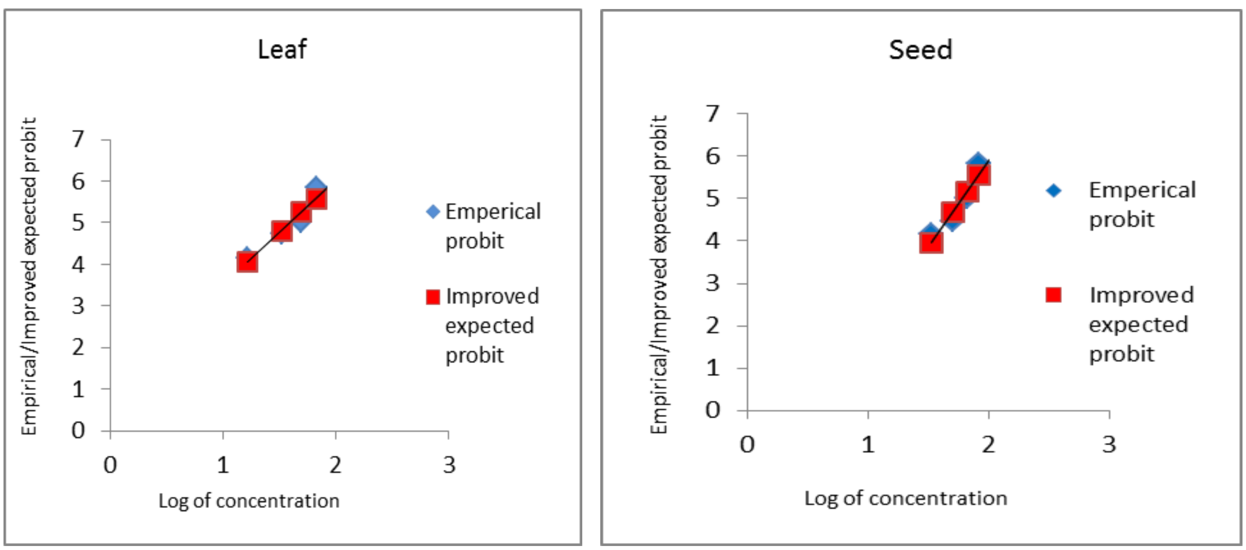


Fig. 2
Regression and provisinal lines for *Callosobruchus chinensis* exposed to ethyl acetatel extract of endophytic fungi isolated from leaf and seed of *Delonix regia* after 96 hours



The seed oil extracts of *Delonix regia* are effective in controlling maize weevil *Sitophilus zeamais* and could serve as alternative to the controversial convectional chemical insecticides. [24].

The insecticidal activity of *Delonix regia* extracts, especially from seeds and leaves, have demonstrated potent effect against a range of insect pests, including beetles, with significant mortality observed even at moderate concentrations.[9]. *Delonix regia* offers the advantage of biodegradability, lower environmental persistence, and reduced risk of resistance development in targeted pest populations as compared to synthetic pesticides.[10]. Ability of the oil rich fraction obtained from seeds of *Delonix regia* to inhibit growth of almost all the tested pesticide species revealed a broad spectrum biopesticidal property of the extracts that oils are used by the plants in defense mechanism against pathogens. [25].

The endophytic microorganisms are those that inhabit the interior of plants, especially leaves and branches and stems, showing no apparently harm to the hosts. [26]. Endophytic fungi have received considerable attention in the last 20 years because of their capacity to protect hosts against insects pests and pathogens. Toxic metabolites produced by endophytic microorganisms in many plants can greatly reduce the population of associated insects. The first reported example of plant protection to elm trees by an endophytic fungus, *Phomopsis oblonga* against the beetle, *Physocnemum brevilineum*. [27].

In our study the mortality increases with increase in concentration at all doses. Similarly, it is reported that the mortality is depends on concentration. [28], [29]. Such a dose dependent mortality with spores of fungi was observed in *Sitophilus zeamais* [30] and *S. litura* [31]. The mortality at 96h had shown that all beetles died in the treatment using 5mg. and 2.5mg of methanol endophytic fungal extract of leaf and seed respectively while in ethyl acetate endophytic fungal extract of leaf 100% mortality was recorded at 96h of exposure in 5.5mg. and 3mg. of ethyl acetate endophytic fungal extract of seed. Similarly, the ethyl acetate extract of endophytic *Alternaria alternata* induced significant inhibitory effects on survival and reproductive potential of *Spodoptera litura.* [32].

The extracts of foliar fungal endophytes isolated from *Picea rubens* Sarg. (red spruce) needles were toxic to the forest pest *Choristoneura fumiferana* Clem. (eastern spruce budworm) in dietary bioassays. [33], [34]. Toxic metabolites produced by endophytic fungi (*Epichloe* and *Neotyphodium* species) in fescue grasses greatly reduce the populations of associated herbivorous insects. These fungi produce various alkaloids that affect herbivore growth. [35].

The finding of the present investigation revealed that the endophytic fungal extract isolated from leaf and seed of *Delonix regia* possess remarkable biopesticidal property against *Callosobruchus chinensis*. The study needs further investigation to find out active ingredients responsible for insecticidal properties against wide range of store grain pest and to reach any final recommendations.

IV. CONCLUSION

The result of the study have confirmed that the endophytic fungi have explore the potential of biopesticide and grain protecting activity against store grain pest.

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