Euphorbiaceae plant extracts as ovipositional deterrent against *Callosobruchus chinensis* Linn. (Coleoptera:Bruchidae)

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ABSTRACT

The present work was carried out to screen certain formulations against the pulse beetle *Callosobruchus chinensis* Linn. raised on grains of *Vigna radiata*. The plants selected for the study included *Euphorbia hirta*, *Phyllanthus amarus* and *Jatropha gossypiifolia.* The investigation was carried out to study the efficacy of the select three plants and recording the egg laying percent by the pest insect. Different formulations using leaf of the plants were employed in the form of crude extract, aqueous suspension, aqueous extract, ethanol extract and diethyl ether extract. The treatments were made using different dose concentrations viz., 1%, 5%, 10% and 25%. The number of eggs laid by the pest insect was noted and ovipositional deterrence was adjudged. Lowest mean egg laying (No./ pair) by *C. chinensis* was observed in experimental sets treated with 25 % DEE extract of *Jatropha gossypiifolia.* Overall, DEE and ethanol extract of *J. gossypiifolia* were found to significantly reduce oviposition by the pest insect.

Key words: *Callosobruchus chinensis* Linn., Euphorbiaceae, Extracts, Egg laying
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INTRODUCTION

Insects play a very important role in the ecosystem due to their vast diversity of form, function and life-style their considerable biomass and their interaction with plant life, other organisms and the environment. They have adapted to a broad range of habitats, successfully finding their own niche, because they will consume almost any substance that has nutritional value. Insects have a direct impact on agricultural food production as, on one hand they act as pollinators while on the other, as pests.

In India, where the population is predominantly vegetarian, pulses are the most important and rich source of protein and several amino acids. Besides, they also provide energy, minerals and certain vitamins. Grain legumes are grown on some 180 million ha which is 12 to 15% of the Earth's arable land and they account for 27% of the world's primary crop production, with grain legumes alone contributing 33% of the dietary protein Nitrogen (N) needs of humans (Vance, 2001). India has an annual production potential of 17.21 million tonnes of pulses recorded in year 2011-12 (Anonymous, 2012). Agarwal *et al.* (1988) reported that about 8.5 % of total annual pulse production is lost during post harvest handling and storage.

The genus *Callosobruchus* attacks grain legumes during both pre and post harvest stages all over the world and it has been reported from the Philippines, Japan, Sri Lanka, Burma and India. It is a primary and most destructive pest of stored pulses. The bruchid infestation also affects seed quality, market value and can reduce cowpea seed viability to 2% after months of storage (Caswell, 1980).

Throughout the world synthetic chemical pesticides have been used for many years to control stored grain pests (Salem *et al.*, 2007). Even today, pest control measures in storage rely on the use of synthetic insecticides and fumigants, which is the fastest and surest method of pest control (Shaheen and Khaliq, 2005). However, the persistent use of these insecticides in granaries of small-scale farmers has led to a number of problems such as killing of non-target species, user hazards, toxic residues in food, development of genetic resistance in the treated pest, increased cost of application and the destruction of the balance of the ecosystem (Shaheen and Khaliq, 2005; Boateng and Kusi, 2008).

Plants contain a large number of secondary metabolites and those categorized under terpenoids, alkaloids, glycosides, phenols, tannin, flavanoids, etc. play a major role in plant defense and cause behavioural and physiological effect on insects. Various works screening different plants against stored grain pests include those by Negi *et al.* (1997), Srivastava and Mann (2002a, b), Kaur and Srivastava (2004), Srivastava and Gupta (2007), Srivastava and Ghei (2007), Kiradoo and Srivastava (2010), Kiradoo and Srivastava (2011), Rawat and Srivastava (2011), Rawat and Srivastava (2012), Mann and Srivastava (2013), Kosar and Srivastava (2013), Mann and Srivastava (2014).

Pulse beetle causes not only quantitative but also qualitative losses like nutritive loss and germination loss. The insect spends its entire immature stage in individual legume seeds, where it causes weight loss, decrease in germination potential and diminishes the market as well as nutritional value of the commodity. This notorious pest attacks the stored pulses and has dispersed throughout the tropics and subtropics through the medium of commerce and now has become a real menace.

The plant family Euphorbiaceae is a large family of flowering plants with 300 genera and around 7,500 species. This family occurs mainly in the tropics, with majority of the species. Some members of Euphorbiaceae have medicinal properties and are also reported to have insecticidal activity. Among the many plant species that have been used to control stored product pests is the physic nut, *Jatropha curcas* L. plant. The efficacy of *Jatropha* seed oil against insect has been reported by Huis (1991), Adabie- Gomez *et al.* (2006) and Henning (2007). Aqueous leaf extract of *Ricinus communis* L. showed excellent insecticidal activity against *Callosobruchus chinensis* L. as documented by Upasani *et al.* (2003). They isolated and tested flavonoids as insecticidal and antimicrobial agents. The isolated flavonoids showed potential insecticidal, ovicidal and oviposition deterrent activities against *C. chinensis* L. Experiments were conducted by Hossain and Haque (2010) to study the efficacy of some indigenous leaf and seed extracts including *Jatropha curcas* against pulse beetle, *Callosobruchus chinensis* (L.) on chickpea seeds. The botanicals were extracted by using acetone, ethanol, n-hexane, petroleum ether and water. The efficacy was evaluated by considering oviposition, adult emergence, seed infestation and weight loss caused by the insect. Repellency of hydroethanolic extracts of *Ricinus communis* to *Scyphophorus acupunctatus* in the laboratory was studied by Cinthia *et al.* (2012).

MATERIALS AND METHOD

The present work was, therefore carried out to screen certain formulations against the pulse beetle *Callosobruchus chinensis* Linn. raised on grains of *Vigna radiata*. The plants selected for the study included *Euphorbia hirta*, *Phyllanthus amarus* and *Jatropha gossypiifolia* all belonging to family Euphorbiaceae. The investigation was carried out to study the efficacy of the three selected plants and recording the egg laying percent by the pest insect. Different formulations using leaf of the plants were employed in the form of crude extract, aqueous suspension, aqueous extract, ethanol extract and diethyl ether extract. The treatments were made using different dose concentrations viz., 1%, 5%, 10% and 25%. The number of eggs laid by the pest insect was noted and ovipositional deterrence adjudged.

RESULTS AND DISCUSSION

The mean egg laying (No./ pair) by the bruchid *C. chinensis* under different

Table 1. Mean egg laying (no./ pair) by *C. chinensis* under different formulations of leaves of select three plants. Values given are mean ±SD

Treatments	*Plants* **Conc.**	**Euphorbia hirta**	***Phyllanthus amarus***	***Jatropha gossypiifolia***
Crude extract	Normal	13.33 ± 0.00	13.33 ± 0.00	13.33 ± 0.00
	Control	10.40 ± 0.00	10.40 ± 0.00	10.40 ± 0.00
	1%	7.38 ± 1.50	7.83 ± 0.42	7.32 ± 0.69
	5%	6.95 ± 0.95	7.58 ± 0.51	7.46 ± 0.82
	10%	7.01 ± 1.26	6.97 ± 0.62	7.65 ± 0.80
	25%	5.86 ± 1.52	6.10 ± 0.65	7.58 ± 1.06
Aqueous suspension	Normal	13.33 ± 0.00	13.33 ± 0.00	13.33 ± 0.00
	Control	10.40 ± 0.00	10.40 ± 0.00	10.40 ± 0.00
	1%	7.24 ± 0.98	7.70 ± 0.27	7.76 ± 0.45
	5%	7.06 ± 0.97	7.71 ± 0.28	7.81 ± 0.50
	10%	6.73 ± 0.99	7.52 ± 0.13	7.31 ± 0.69
	25%	6.40 ± 1.19	7.05 ± 0.33	7.73 ± 0.37
Aqueous extract	Normal	13.33 ± 0.00	13.33 ± 0.00	13.33 ± 0.00
	Control	10.40 ± 0.00	10.40 ± 0.00	10.40 ± 0.00
	1%	6.90 ± 0.54	7.24 ± 0.48	7.36 ± 0.92
	5%	6.44 ± 1.27	7.58 ± 0.49	7.14 ± 0.76
	10%	7.40 ± 0.10	7.35 ± 0.45	6.92 ± 0.75
	25%	5.91 ± 1.70	7.10 ± 0.22	6.76 ± 0.78
Ethanol extract	Normal	13.33 ± 0.00	13.33 ± 0.00	13.33 ± 0.00
	Control	9.50 ± 0.00	9.50 ± 0.00	9.50 ± 0.00
	1%	7.95 ± 0.62	7.45 ± 0.27	6.00 ± 0.87
	5%	7.83 ± 0.92	7.55 ± 0.33	5.65 ± 0.60
	10%	7.59 ± 1.31	7.40 ± 0.52	5.45 ± 1.49
	25%	6.95 ± 1.13	7.05 ± 0.87	5.65 ± 1.50
Di-ethyl **ether extract**	Normal	13.33 ± 0.00	13.33 ± 0.00	13.33 ± 0.00
	Control	9.43 ± 0.05	9.43 ± 0.05	9.43 ± 0.05
	1%	6.95 ± 1.13	7.20 ± 0.45	4.82 ± 1.07
	5%	7.23 ± 0.73	7.25 ± 0.50	4.94 ± 1.07
	10%	7.43 ± 0.63	6.85 ± 0.29	4.55 ± 1.27
	25%	7.46 ± 0.53	6.50 ± 0.25	4.10 ± 1.13

treatments of various plants studied has been presented in Table 1. ANOVA has been presented in Table 2. During the present study mean egg laying (No./ pair) by *C. chinensis* in normal sets was observed to be 13.33, while, in control sets treated with GDW it was noted to be 10.40 and in sets treated with ethanol extract it was observed to be 9.50 and in those treated with DEE it was documented as 9.43. The lowest egg laying of 4.10/ pair was observed in experimental sets treated with 25 % DEE extract of *Jatropha gossypiifolia.*

Overall, DEE and ethanol extract of *J. gossypiifolia* were found to significantly reduce oviposition by the pest insect.

For comparing the effect of different formulations ANOVA was applied. Further, based on this analysis and perusal of the results it could be concluded that the number of eggs laid by the pest insect was significantly reduced in various experimental sets ($p<0.05$). egg laying by *C. chinensis* pertaining to effect of extracts (B), treatments (D) and plant and extracts (AXB) , extracts and treatment (BXD) were highly significant ($p<0.01$), while those related to plants (A) and plants and treatments (AXD) were slightly less significant ($p<0.05$) and the rest were non-significant which included concentration (C), plants and concentration (AXC), extracts and concentrations (BXC), concentrations and treatments (CXD), plants, extracts and concentrations (AXBXC) plants, extracts and treatments (AXBXD), plants, concentrations and treatments (AXCXD), extracts, concentrations and treatments (BXCXD) and plants, extracts, concentrations and treatments (AXBXCXD).

While comparing the effects of treatments of leaves of the three select plants on egg laying performance of the beetle, it was documented that *J. gossypiifolia* significantly ($p<0.01$) reduced egg laying as compared to other two plants, *P. amarus* and *E. hirta* . When over all analysis was made to compare the effect of various extracts using ANOVA on egg laying it was found that DEE extract was observed to be most effective followed by ethanol extract, aqueous extract and crude extract, while aqueous suspension was least effective ($p<0.01$).

When comparisons were made to observe the effect of plants and their extracts it was found that egg laying was reduced in experimental sets treated with ethanol extract of *J. gossypiifolia* ($p<0.05$). While comparing the effect of plants and their concentrations it was observed that 10 and 25% concentrations of *J. gossypiifolia* leaves significantly ($p<0.01$) reduced egg laying as compared to other concentrations viz., 5%, and 1%.

When comparisons were made to see the effect of extracts and treatments on oviposition by the bruchid, it was noted that DEE extracts of all concentrations significantly ($p<0.01$) reduced egg laying. When overall analysis was made to compare the effect of plants their extract, concentrations and treatments, it was found that 25% concentrations of DEE extract of *J. gossypiifolia* significantly ($p<0.01$) reduced the number of eggs laid by the bruchid.

During the present study mean egg laying by *C. chinensis* was observed to be the lowest 4.10/ pair in experimental sets treated with 25 % DEE extract of leaves of *Jatropha gossypiifolia*. Overall, DEE and ethanol extract of *J. gossypiifolia* were found to significantly reduce oviposition by the pest insect. That *Jatropha* oil causes oviposition deterence in potato tuber moth has been reported by Shelke *et al.* (1987). The leaf extracts of *Acalypha* species (Euphorbiaceae) were reported to act as ovipositional deterrent against pulse beetle as observed by Sathyseelan *et al.* (2008). The highest larval mortality of *S. litura* was found by Kamraj *et al.* (2008) when treated with extracts of *O. canum*, *R. nasutus* and *O. sanctum*. Guerra *et al.* (2007) documented that shoot and essential oils of *Minthostachya* sp. (Lamiaceae) deterred potato tuber moth to oviposit. They also tested essential oil of *M. spicata* and *M. glabresecens* and found that this impaired oviposition by the moth, reducing the number of eggs laid by about 80% as compared to control treatment. According to Roger and Hamraoui (1994) Lamiaceae plants viz., *M. piperita*, *Origanum vulgare*, *Rosmarinus officinalis*, *Thymus vulgaris*, *Satureia hortensis* act as ovipositional deterrent against *A. obtectus*. Repellent properties of *O. kilimandscharicum* and *O. suave* have also been reported by Seyoum *et al.* (2003) and Odalo *et al.* (2005). According to Marderosian (2001), *M. pulegium* has been traditionally used as insect repellent in Iran.

Table 2.ANOVA for egg laying showing different interactions and level of significance

Source of variations	df	SS	MSS	F-cal	S/NS	S.Em.	CD 5%	CD 1%
A	2	18.67	9.33	4.44	*	0.08	0.23	0.31
B	4	139.38	34.84	16.57	**	0.11	0.30	0.39
C	3	5.29	1.76	0.84		0.10	0.27	0.35
D	3	6195.59	2065.20	981.89	**	0.08	0.23	0.31
A x B	8	74.03	9.25	4.40	**	0.19	0.52	0.68
A x C	6	1.49	0.25	0.12		0.17	0.47	0.61
A x D	6	27.63	4.61	2.19	*	0.15	0.40	0.53
B x C	12	1.17	0.10	0.05		0.22	0.60	0.79
B x D	12	119.98	10.00	4.75	**	0.19	0.52	0.68
C x D	9	10.58	1.18	0.56		0.17	0.47	0.61
A x B x C	24	4.44	0.18	0.09		0.37	1.04	1.37
A x B x D	24	69.21	2.88	1.37		0.32	0.90	1.18
A x C x D	18	2.99	0.17	0.08		0.29	0.81	1.06
B x C x D	36	2.34	0.07	0.03		0.37	1.04	1.37
A x B x C x D	72	8.88	0.12	0.06		0.65	1.80	2.37
Error	660	1388.18	2.10					
Mean	899	8069.85						

* 5% level of significance; ** 1% level of significance S.Em.- standard error of mean
C.D.- Critical difference MSS- Mean sum of square SS- Sum of square
A-Plants B- Extracts C- Concentrations ; D- All Treatments

The present findings also get support from the earlier works of Kamakshi *et al.* (2000), who reported significant reduction in the number of eggs laid by *C. maculatus* when treated with *M. arvensis* and *O. sanctum* as compared to control. Raja *et al.* (2001) also concluded that egg laying by *C. maculatus* was significantly influenced by treatments of volatile oils derived from *Mentha* species in the order of potency as *M. spicata* > *M. piperita* > *M. arvensis*. A complete prevention of egg laying by *C. analis* was observed by Juneja and Patel (1994), when the grains were treated with different plant products including mint leaves. Weaver *et al.* (1992) found the ovipositional pattern in two species of bruchids was influenced by the treatment of dried leaves of *Tetradenia riparia*, a perenial mint that suppressed population size. Oviposition inhibition by *C. maculatus* was observed by Ogunwolu *et al.* (1998), when treated *Z. zanthoxyloides*, a plant belonging to family Lamiaceae. When leaves of *V. negundo* were admixed with grains of black gram, reduction in oviposition by *C. chinensis* was observed by Prakash and Rao (1998). Roots of *Cyperus rotundas*, leaves of *Hyptis suaveolens*, bark of *Erythropheleum suaveolens*, scales of *Allium cepa*, flowers of *Eugenia aromatica*, seeds of *Lepianthes peltata* and *Aframomum melegueta* were found to be effective against *C. maculatus* as ovipositional deterrents by Adedire and Lajide (2004). Similar results were also documented by Miah *et al.* (1993) on chickpea. Dwivedi and Kumari (2000) noted reduced oviposition by *C. chinensis* when the grains were treated with *L. palmata* leaf extracts. Minimum egg laying by *C. maculatus* was recorded by Enchendu *et al.* (1988) when cowpea seeds were treated with dried ginger root and dried neem fruit powders. Bowry *et al.* (1984) suggested powdered neem cake to be more effective in reducing the number of eggs laid by *S. oryzae* infesting maize seeds. Delobel and Mollonga (1987) also observed no eggs or very few eggs being laid by *C. serratus* when treated with *N. tabacum*. Seeds of *Pachyrhizus erosus* and *A. squamosa* were found to cause significant decrease in oviposition by *C. analis* by

Kardinan *et al.* (1997). Adebayo and Gbolade (1994) observed that leaves of *L. adoensis* and *E. uniflora* were effective against oviposition of *C. maculatus*. While investigating various indigenous plant materials against *L. trifolii* on castor, Pitlehra and Borad (2001) found neem seeds kernel extract, ardusi leaf extract and kaner leaf extract to be effective in reducing oviposition. Javaid and Mpotokwane (1997) suggested powder of leaves of *N. oleander*, wood of *Combretum imbretum* bark and leaves of *C. apiculatum* and *Terminalia sericea*, leaves of *C. gratissimus*, bark of *Spiraostachys africana* and *Peltophorum africanum*, leaves of *Aloe murlothii*, fruit and bark of *M. azedarach*, fruit and leaves of *Eucalyptus* species to inhibit oviposition by *C. maculatus* and therefore support the present findings. A significant decrease in egg laying by *C. chinensis* has also been observed by Gupta (2004) when grains were treated with extract of S. surattense, *S. nigrum*, *W. somnifera*. Mathur *et al.* (1985) found neem to impair oviposition by *C. chinensis*. A reduced oviposition by *C. chinensis* was also recorded by Srivastava and Ghei (2007) when the grains were treated with plants of *T. faenum–graecum*, *T. purpurea* and *C. burhia*. Rouf *et al.* (1996) suggested extracts of leaves of *A. indica*, *V. negundo*, *Polygonum hydropiper* to decrease the oviposition by pulse beetle *C. maculatus*. Mbata *et al.* (1995) noted total inhibition of oviposition by *C. maculatus* when treated with seeds of brown pepper *P. guineense*. Subramanya *et al.* (1994) observed extract of *E.* c*itrodora* to be effective against oviposition by *C. chinensis*. Tinzaara *et al.* (2006), while testing the potential of certain botanicals found that oviposition by *Cosmopolites sordidus* was significantly low when treated with *M. azederach*, *Tagetes* and *R. communis*. Sharma and Saxena (2001) found that the egg laying by *T. castaneum* was significantly reduced when treated with extract of flowers of *Origanum majorana*. All these works support the present findings suggesting that botanicals play a significant role in deterring the insect to oviposit.

Among formulations, DEE and ethanol extract were found to significantly reduce the number of eggs laid by the pulse beetle during the present study. Earlier, Dover (1985) also observed alcohol extract of hyssop, rosemary, sage, thyme, white clove to reduce oviposition by *P. xylostella* which support the present findings. Ethanol extracts of different plants / parts were also suggested by Adedire and Lajide (1994) to reduce egg laying by *C. maculatus*. The present results are also in conformation with the works of Dwivedi and Maheshwari (1997), who reported that acetone extract of *Croton*, petroleum ether extract of *V. enceliodes* and *Occidentalis* exhibited ovipositional deterrent activity against *C. chinensis*; Pandey *et al.* (1986), who observed various plants diluted in benzene and mixed with green gram seeds to be very repulsive and a potent oviposition inhibitor for *C. chinensis*; Dwivedi and Garg (2000), who reported that acetone leaf extracts of *Tagetes*, *Ipomea* and *Acacia* exhibited 50% reduction in oviposition by *C. cephalonica*; Mann (1997), who documented powder suspension of *Aerva* to be effective in reducing egg laying remarkably by *C. chinensis*; Adedire and Akinneye (2004), who reported powder suspension and ethanol extract of *T. diversifolia* leaves were found to reduce egg laying by *C. maculatus*.

The extract concentration was also found to have a considerable effect on the number of eggs laid by *C. chinensis*, which was found to decrease significantly with the increase in concentration of the formulation during the present study, 25% being the best. These results are in agreement with the work of Olaifa and Erhun (1988), who observed a complete suppression of oviposition by *C. maculatus* when treated with 42% powder of *P. guineense*. Elhag (2000) studied the oviposition deterrence of nine plant materials on *C. maculatus* and found seed treatment with 0.1% crude extract resulted in significant reduction in egg laying by the bruchid. A decrease in the no. of eggs laid by *C. maculatus* with an increase in the amount of *E. balsamifera* plant powder was noted by Suleiman and Suleiman (2014). These reports are in accordance with the present findings.

Treatments of 10% powder suspension of roots and leaves of *Tephrosia* were found to reduce the average number of eggs laid by *C. chinensis* (Ghei, 2001). Savitri and Subbarao (1976), observed powdered neem kernel mixed directly with paddy at 1 and 2% was effective in reducing oviposition by *R. dominica* and *S. cerealella* respectively. Treatments of 10% powder suspension and aqueous extract of bark of Prosopis sp. were found to reduce egg laying by *C. chinensis* (Negi, 2007). Al Lawati *et al.* (2002) suggested that the number of eggs laid by *C. chinensis* when treated with ethanol extract were significantly less than, from those treated with methanol extract. Prijona *et al.* (1997) found extracts of seeds of *A. glabra*, *A. inuricata*, *A. squamosa*, *Stelechocarpus cauliflorus*, *Aglaia elliptica* and *Dysoxylum cauliflorum* to result in significant decrease in oviposition by *C. maculatus* at 0.5% concentration. According to Adedire and Lajide (2004) dose concentrations of 1.25 to 10% were found to inhibit oviposition by *C. maculatus*. Tebkew and Mekashe (2002), while evaluating botanicals found that *Mellettia ferruginea* when mixed with grains at 5% (w/w), deterred egg laying by *C. chinensis*; Adedire and Akinney (2004) also found that mean number of eggs laid by *C. maculatus* was reduced to 4.7 at 2% extract concentration of leaf extracts of *T. diversifolia*. They documented that egg laying reduced from 4.13 in the untreated to 17.3 in 2% powder treatments, while, Pitlehra and Borad (2001) suggested *Bougainvillea* and *Naffatia* leaf extract at 3% concentrations to be less effective in reducing oviposition by *L. trifolii*. Prasad *et al.* (1998) observed the extracts of *L. camara* in all the used concentrations to check egg laying by *S. oryzae*.

Overall, DEE and ethanol extract of leaves of *J. gossypiifolia* at 10 and 25% were found to significantly (p<0.01) reduce oviposition by the pest insect suggesting that Euphorbiaceae plant extracts do provide ovipositional deterrent action against *Callosobruchus chinensis* Linn. (Coleoptera:Bruchidae).

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