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Entomotoxicity of *Chromolaena odorata*, *Tagetes minuta* and *Reichardia tingitana* in suppressing oviposition and adult emergence of *Callasobruchus chinensis* (L) infesting stored chickpea seeds in U.P.

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Abstract
In the present investigation experiments were conducted in the Biopesticide and Toxicological plant product research laboratory, Department of Zoology, D.B.S. College, Kanpur, U.P., India. Extracts of ten asteraceous plant parts have been used for their biopotency against pulse beetle, *Callosobruchus chinensis* L. (Coleoptera: Bruchidae) on chickpea *Cicer arietinum* L. (Fabales: Fabaceae) Kabuli variety Mexican white. Estimation of their toxicity, oviposition deterrence and adult emergence against *C. chinensis* and treated chickpea seed weight loss and their germination was also assessed. Three concentrations viz. 0.5 per cent, 1.0 per cent and 2.0 per cent of each plant extract were selected and tested by dry film technique for early emerge beetles of *C. chinensis*. The data depicted from results maximum mean mortality of early emerged adults of *C. chinensis* was observed in *Chromolaena odorata* (83.08%) followed by *Chrysanthemum cinerariaefolium* (74.26) and *Tagetes minutia* (68.83), respectively. The maximum number of eggs laid per hundred extract treated grains was found in *Mantisalca duriaei* (257.00) followed by *Inula racemosa* (69.00) and *Scorzonera undulate* (51.33). Similarly, highest number of eggs bearing seeds per hundred seeds was observed in *Inula racemosa (*55.67) followed by *Mantisalca duriaei (*43.67) and *Scorzonera undulate (*42.00), respectively. It is seen that maximum number adults emergence was recorded in *I. racemosa (*143.00) followed by *M. duriaei (*134.33) > *S. undulate (*107.00) and promising percentage of seed infestation was recorded in *I. racemosa* (45.85) followed by *M. duriaei (*42.68) > *S. undulate (*35.07) while, maximum per cent seed weight loss was observed in *Mantisalca duriaei* (2.98) and *Inula racemosa* (2.90). It is also seen that maximum percent treated (2.0%) extracts of *C. odarata* treated showed 92 % seed germination followed by *T. minuta* 82 per cent and 84% in control.

Keywords: *chromolaena odorata, callosobruchus chinensis, mantisalca duriaei*

Introduction
Pulse beetle, *Callosobruchus chinensis* L. (Coleoptera: Bruchidae) also known as dhora beetle is the most destructive and serious pest of stored gram and cause both qualitative and quantitative losses in legumes. Ahmed and Din [2]; Righi Assia *et al.*, [24], Upadhyay *et al*., [31] and Chandel *et al*. [8, 9, 10, 11]. The *C. chinensis* is infested on leguminous grains such as chickpea, cowpea, green gram, lentil and black gram [17, 19, 23]. It is a cosmopolitan pest of Asia and Africa (Schmutterer) [25]. *Callosobruchus* spp. cause 12-13% loss by feeding the protein contents of grains (FAO) [13, 24]. *C. chinensis* causes up to 10% damage to stored chickpea [5, 20] and up to 90% loss to stored gram [2]. In order to manage the deterioration, more and more synthetic hazardous chemicals are being used, but unfortunately they posed various side ill effects on human health like carcinogenicity and hormonal imbalance etc. [3]. With the limitations on the use of current pest control methods, there is scope for the discovery of safe, non-polluting, ecifriendlly, bio-rational and biodegradable pest management technologies for stored products. In view to combat the above problem of ill effect of synthetic insecticides on pulses in storage under the varied ecological conditions, an attempt has therefore, been made to evaluate relative toxicity, oviposition deterrence and adult emergence effect of different formulations of ten asteraceous extract against early emerged adults of pulse beetle, *C. chinensis*.

Materials and method
Procurement of raw plant materials
For the experimental purposes asteraceous botanical parts were collected from wasteland and cultivated fields of the farmers. Among them, only ten asteraceous plants viz., aerial parts of *Cichorium intybus* (L.), *Chromolaena odorata* Linn., *Chrysanthemum cinerariaefolium* (trev.) Vis., *Inula racem*osa Hook. F., *Mantisalca duriaeri* Birq. Et Cavill., *Rechardia tingitana* (L.) Roth, *Rhaponticum acaule* (L.) DC, *Scorzonera undulate* Vahl, *Spilanthes paniculata* Well ex DC and *Tagetes minuta* Linn. Were collected from different vicinity of from U.P. and India.

Preparation of powder
Fresh collected green plant parts (leaves, Flowers and seeds, rhizomes etc.) were washed with distilled water and kept in the laboratory for 7 days for air drying followed by one day sun drying before making powder. Electric grinder was used to have coarse powder then these were passed through a 30-mesh sieve to get fine powder. Powders were kept in polythene bags at room temperature and properly sealed to prevent quality loss (Chandel and Singh) [10].

Preparation of botanical extracts
For the extraction, Soxhlet Apparatus was used; about 100g powder of each category of powder were extracted with 1500

ml of different solvents (n-hexane, acetone, methanol, petroleum ether and distilled water). Extraction of each category of powder was done in about 12 hrs. After soxhlet extraction, the material was run on rotary evaporator. The extracts were concentrated on rotary evaporator by removing the excess solvent under vacuum. After evaporation of solvent with rotary evaporator the remaining extracted material was kept on water bath for removing remaining solvent from the extracts. The extracts were stored at 4°C prior to application.

Apparatus used for experiment

Small plastic jars (capacity 50 ml) were used for the experiment, there was one set of two jars joined by clear plastic pipe of 1cm. diameter at an angle of 180 degree for each replication. One jar of each set was provided with 10 g of grains given the name ‘A’ while the other jar was kept empty and given the name **‘**B**’**. In jar ‘A**’**, the grains treated with extracts were placed, while the jar B remained empty. The jars used for experiment were disinfected with alcohol.

Preparation of Stock Solution

For stock solution, 50ml. extract in each case was taken into reagent bottles and 50ml. benzene was added in it to dissolve the constituents of the materials. The mouth of the bottles were stopper with airtight corks after which, these bottles containing the solutions were kept in refrigerator.

The Insecticidal Formulations

Five concentrations (0.25, 0.5, 1.0, 1.5 and 2.0 %) were used for experiments on insecticidal and repellent tests in the laboratory conditions. However, only three concentrations (0.5, 1.0 and 2.0 percent) were used for relative toxicity, oviposition deterrence and adult emergence test in the laboratory experiment. The different concentrations of the herbal extracts were prepared from the stock solution using benzene as solvent and Triton X-100 as emulsifier. The level of solvent and emulsifier were kept constant.

Experimental Protocol

Toxicity bioassay

For testing the insecticidal bio-efficacy, fifty gram of healthy chickpea seeds were taken in a plastic container (300 ml) were used as food mixed with different concentrations (0.5,1.0 and 2.0 %) of each extract (v/w) and then air dried for 30 minutes. Five pairs of newly emerged one day old adult beetles were released in each plastic container and the mouth was closed with its lid. Each treatment was replicated thrice including control. All treated containers were kept at ambient room temperature (27-30ºC) in the laboratory for mortality. After 6, 12 and 24 hours, dead and alive beetles were counted and removed from each container. The efficacy of plant materials as protectant of chickpea seeds against *C. chinensis* was assessed considering mortality percentage. Thus data was collected on the number of adults of pulse beetle, *C. chinensis* were dead on treated food and mortality over control was recorded. The data were arranged in tabulated form and graph formats. The mortality (%) was corrected by Abbotts’s formula (Abbott, W.S.) [1].

Table 1: Mean mortality percentage of *C. chinensis* at plant extracts irrespective periods

Treatments	6 hrs.	12hrs.	24hrs.	Mean mortality %
C. odorata	80.16	81.14	87.95	83.08
C. cinerariaefolium	70.41	72.21	80.16	74.26
C. intybus	67.20	67.50	71.11	68.60
I. racemosa	60.54	67.83	71.44	66.59
M. duriaeri	64.36	68.59	70.77	67.90
R. tingitana	59.92	64.57	68.00	64.16
R. acaule	53.86	56.08	61.28	57.07
S. undulate	55.77	67.97	72.78	65.50
S. paniculata	53.66	58.90	66.61	59.76
T. minuta	64.26	68.90	73.35	68.83
Control	0.00	18.44	18.44	12.26

(T_1, T_2, T_3 = Treatments and T.B.V.$_1$, T.B.V.$_2$, T.B.V.$_5$=Transformed Back Values)
C.D. for treatment x period means=0.078
C.D. for treatment means (plant extra = 0.037
C.D. for treatment means (control) = 0.162

The data depicted in table 1 and figure 1 showed that indicated that maximum early emerging adults mortality percentage of beetles was observed in *C. odorata* (83.08) followed by *C. cinerariaefolium (*74.26) > *T. minuta (*68.83) > *C. intybus* (68.70) > *M. duriaeri (*67.90) > *I. racemosa* (66.59) > *S. undulate (*65.50) > *R. tingitana (*64.16) > *S. paniculata (*59.76), whereas *R. acaule (*57.07) the least.

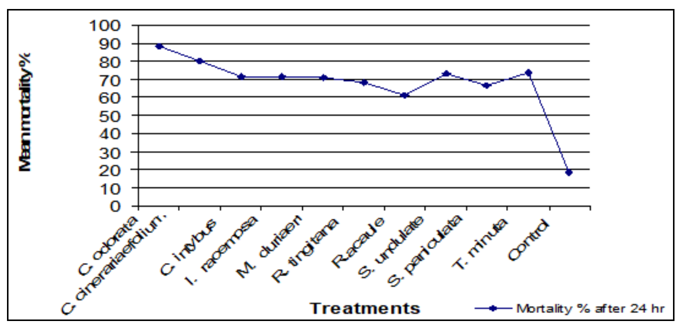

Fig 1: Mortality % of C. chinensis with a Steraceous Extract

2. Oviposition Deterrent and adult Emergence Bioassay

Fifty gram of healthy chickpea seeds were taken in a plastic container (300 ml) and mixed with 2.0% of each tested extract (v/w) and then air dried for 30 minutes. Five pairs of newly emerged one day old adult beetles were released in each plastic container and the mouth was closed with its lid. Each treatment was replicated thrice including control. All treated containers were kept at ambient room temperature (27-30ºC) in the laboratory for oviposition. After 7 days, dead and alive beetles were removed from each container and seeds along with eggs were kept in the laboratory for further development of the insect. The efficacy of plant materials as protectant of chickpea seeds against *C. chinensis* was assessed considering oviposition, adult’s emergence, seed infestation and seed weight loss done.

For the experimentation and observation of oviposition of the pulse beetle, *C. chinensis,* one hundred (100) seeds were collected randomly from each plastic container of each treatment and examined under 10x magnifying glass and the number of seeds along with eggs (egg bearing seeds) and the number of eggs deposited were counted. After each observation, the grains were returned to the respective containers for the further development of the beetle. The adults were counted and removed daily from each plastic container and the data were recorded It is obvious from Table-1 and figure-1 that maximum number of eggs laid per hundred grains *are as: M duri.aei* (257.00) > *I. racemosa* (69.00) > *S. undulate* (51.33) > *R. acaule* (48.33) > *S. paniculata* (48.33) > *C. intybus* (40.67) > *R. tingitana* (40.33) > *C. cinerariaefolium* (34.00) > *T. minuta* (26.67) > *C. odorata* (23.00), respectively.

Table 2: Bioefficacy of plant extracts oviposition and adult emergence done by pulse beetle, *C. chinensis* on chickpea seeds

Plant Extracts	No. of eggs/ 100 seeds	TBV	No. of eggs bearing seeds /100 seeds	TBV	No. of adults Emergence	TBV
C. odorata	23.00	1.35	22.00	1.33	36.67	1.56
C. cinerariaefolium	34.00	1.52	31.33	1.48	66.33	1.82
C. intybus	40.67	1.60	35.67	1.54	88.00	1.93
I. racemosa	69.00	1.83	55.67	1.74	143.00	2.15
M. duriaei	257.00	1.75	43.67	1.63	134.33	2.13
R. tingitana	40.33	1.60	38.33	1.57	93.33	1.96
R. acaule	48.33	1.68	40.33	1.60	72.00	1.85
S. undulate	51.33	1.37	42.00	1.62	107.00	2.02
S. paniculata	48.33	1.68	40.33	1.60	72.00	1.85
T. minuta	26.67	1.33	19.67	1.28	49.00	1.68
Control	94.33	1.97	77.67	1.89	190.00	2.28

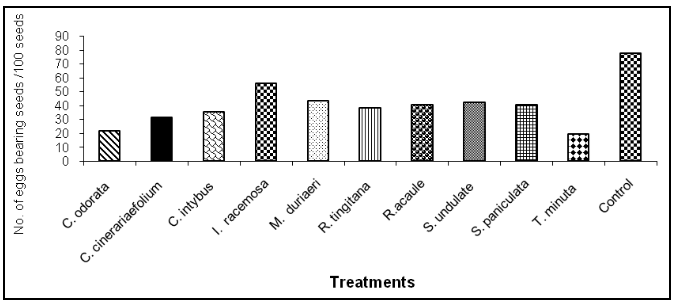

Fig 2: Bioefficacy of plant extracts showing no. of C. chinensls eggs bearing seeds/ 100 seeds

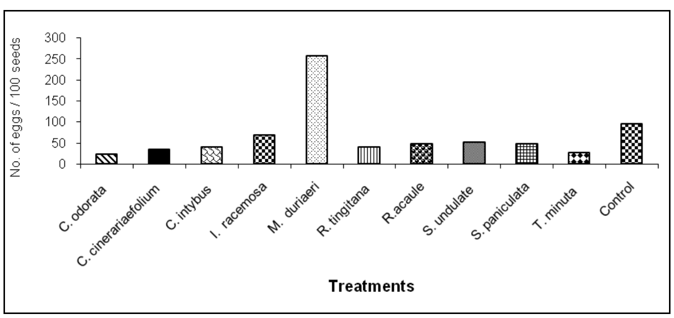

Fig 3: Bioefficacy of plant extracts showing no of C. chinensls eggs laid/ 100 seeds

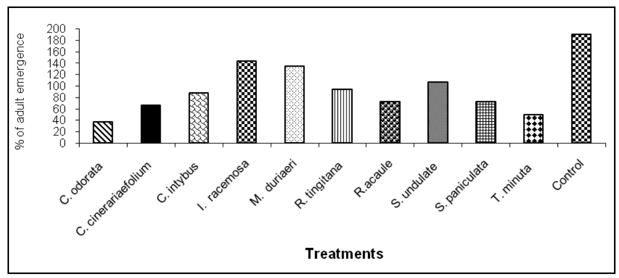

Fig 4: Bioefficacy of asteraceous extracts adult emergence done by pulse beetle, c. Chinensis on chickpea seeds.

The data depicted in table 1 and figure 2 indicated that maximum number of eggs bearing seeds per hundred seeds was observed in *I. racemosa (*55.67) followed by *M. duriaei (*43.67) > *S. undulate (*42.00) > *R. acaule (*40.33) > *S. paniculata (*40.33) > *R. tingitana (*38.33) > *C. intybus (*35.67) > *C. cinerariaefolium (*31.33) > *C. odorata (*22.00), whereas *T. minuta (*19.67) the least.
Similarly, table 1 and figure 3 indicated that maximum adults emergence was found in *Inula racemosa (*143.00) followed by *Mantisalca duriaei (*134.33) > *Scorzonera undulate (*107.00) > *Reichardia tingitana (*93.33) > *Cichorium intybus (*88.00) > *Rhaponticum acaule (*72.00) > *Spilanthes paniculata (*72.00) > *Chrysanthemum cinerariaefolium* 66.33) > *Tagetes minuta (*49.00) and *Chromolaena odorata (*36.67) the least effective.

Table 3: Bioefficacy of asteraceous extracts on seed infestation and seed weight loss done by pulse beetle, *C. chinensis* on chickpea seeds

Plant Extracts	% Infestation	TBV	%Weight Loss	TBV
C. odorata	13.26	21.30	0.58	0.75
C.cinerariaefolium	23.39	28.11	1.25	1.11
C. intybus	29.28	32.64	1.87	1.36
I. racemosa	45.85	42.57	2.90	1.69
M. duriaei	42.68	40.76	2.98	1.72
R. tingitana	29.03	32.49	1.98	1.40
R. acaule	26.03	29.96	1.54	1.24
S. undulate	35.07	36.29	2.12	2.12
S. paniculata	25.03	29.96	1.54	1.24
T. minuta	15.76	23.27	1.23	1.10
Control	63.57	52.90	4.31	2.07

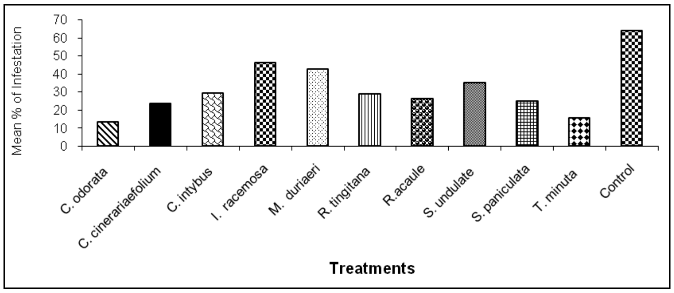

Fig 5: Potential of asteraceous extracts on seed infestation done by pulse beetle, C. chinensis on chickpea seeds

Effect of botanical extracts on seed infestation and weight loss of *C. chinensis*:

For the experimentation and observation of oviposition of the pulse beetle, *C. chinensis* % seed infestation and weight loss were determined at the completion of adult emergence. The sample of each replicate were examined carefully and damaged and healthy seeds were separated, cleaned, counted and weighed. Percent seed infestation and weight loss were computed by using the following formulae. Percent infestation = N_b / T_n X 100 Where, N_b = Number of damaged seeds, T_n = Total number of seeds

Percent weight loss = UN_d-DN_u / U (N_d-N_u) x 100, Where, U = Weight of healthy seed, D = Weight of damaged seed, N_u = Number of healthy seeds, T_d = Total Number of damaged seeds;

It is seen from the summary table 2 and figure 4 that maximum per centage of infestation was recorded in *Inula racemosa* (45.85) followed by *Mantisalca duriaei (*42.68) > *Scorzonera undulate (*35.07) > *Cichorium intybus (*29.28) > *Reichardia tingitana (*29.03) > *Rhaponticum acaule (*26.03) > *Spilanthes paniculata (*25.03) > *Chrysanthemum cinerariaefolium (*23.39) > *Tagetes minuta (*15.76) > *Chromolaena odorata (*13.26), respectively. The data depicted in table 2 figure 5 indicated that maximum per cent weight loss was observed in *Mantisalca duriaei* (2.98) > *Inula racemosa* (2.90) > *Scorzonera undulate (*2.12) > *Reichardia tingitana (*1.98) > *Cichorium intybus (*1.87) > *Rhaponticum acaule (*1.54) > *Spilanthes paniculata (*1.54) > *Chrysanthemum cinerariaefolium (*1.25) > *Tagetes minuta (*1.23) > *Chromolaena odorata (*0.58), respectively.

Seed germination test

The viability of treated and control seeds were tested 3 months after the asteraceous plant extract application. For this assay, chickpea seeds were separately treated with the *Chromolaena odorata* and *Tagetes minuta* extracts at the rate of 5 ml extract per kg seeds (1% v/w). The control seeds were treated with the solvent, but no extracts was applied to the untreated seeds. The treated and control seeds were air-dried for 2–3 hours. Then 25 seeds from each treated, control or untreated group were placed separately in glass jars, under laboratory conditions but without insects, for 3 months. Each treatment was replicated three times. The germination of seed was evaluated for each treatment. The seeds was placed on moist filter paper in Petri dishes. The dishes were kept in an incubator at 25°C and 12:12 L:D conditions. The dishes were observed for the germination of seeds for the next two months. Seed qualities were judged by tasting and smelling the treated seeds. Data were analyzed. For mortality tests, original data were corrected by Abbott's (1925) formula, transformed into arcsin & percentage values and then data were analyzed by probit analysis (Finney 1971).

Finally, it can be concluded on the basis of number of seed treated and per cent germination of seed 2 % extracts of *C. odarata* and *A. calamus* showed 92 % seed germination while, *T. minuta* extracts gave 82 per cent and in control, it was recorded 84 per cent only.

Table 3: Effect of different asteraceous plant extracts on chickpea seed viability

Plant Extracts	No. of Seed Treated	Germination %
C. odorata	25	92
T. minuta	25	82
Control	25	84

Discussion

In the support of above findings the works of various entomologist on oviposition deterrency on eggs deposition, their emergence and seed weight loss and treated seed germination was also studied [15, 17, 24]. Srivasthava and Mann [28] have observed this type of reduction in adult emergence of *C. chinensis* from grains treated with extract of *Peganum harma*. Remarkable decrease in the adult emergence of *C. chinensis* with increase in concentration of Pongam oil was also reported by Prakash and Rao [21] and Negi *et al.* [18]. Haridasan and Gokuldas [14] reported the effects of *Vitex negundo* leaf extracts on adult emergence of the stored product pest, *Tribolium castaneum*. Their studies revealed that there was a significant reduction in adult emergence with increased exposure period and concentration of the extract. These earlier findings are in conformation with the results of the present study. The data shown in the Table 4 reveals the effect of leaf extracts on adult emergence of pulse beetle. Considerable reduction in adult emergence was found in different concentrations. Jayakumar *et al.* [15] reported that plant extract has obvious effect on post embryonic survival of the insects and reduction in adult emergence. Annie Bright [3] and Raja *et al.* [30] reported that when the eggs were laid on treated seeds, the toxic substance present in the extract may enter into the egg through chorion and suppress their embryonic development. The results thus are in agreement with the earlier studies thus suggesting that adult emergence was greatly reduced in treated seeds than control seeds.

In earlier days, various plant products s have been used for the control of *Callosobruchus species*, mainly neem oil [29, 12, 26]. Study of Bhatnagar et al. [6] revealed that neem oil has significantly higher repellent, oviposition deterrent and ovicidal effects against the pulse beetles tested. Powdered leaves and extracts of *Vitex negundo* [22],, *Acorus calamus* [7], cardamom and clove powder [30], aqueous extracts of *Calotropis gigantia*, *Phyllanthus amarus*, *Ocimum tenuiflorum* and *Catharanthuses roseus* [27] leaf extract of *Clitoria ternatea* [4] were reported to have significant oviposition deterrent, and other biological activity against stored grain pest. The present study was carried out to determine the oviposition inhibition and repellent effects of a local aromatic and medicinal plant, *Clerodendrum infortunatum* on the pulse beetle *Callosobruchus chinensis*.

Conclusion

Conclusively, the present investigation revealed that there appears prospects in selected botanicals *Chromolaena odorata*, *Chrysanthemum cinerariaefolium, Tagetes minutia* registered promising mortality (83.08 to 68.63%) to the early emerged *C. chinensis* adults, when compared to other extracts. The oviposition deterrent, growth potential and viability of treated seed done by selected extractives. The result reveals that minimum number of eggs per hundred seeds and egg bearing seeds, number of adult emergence, per cent seed infestation and weight loss treated extracts *C. odorata* was recorded most effective followed by *Mantisalca duriaei, Inula racemosa* and *Scorzonera undulate*.. Female beetles were deterred from ovipositing in chickpea seeds treated with selected extracts applied at the rate of 2% (w/w). At rates of 2*% C. odorata* extract had similar effects on F_1 progeny, seed damage, weight loss and inhibition rates followed by *T. minuta* and control, respectively. This study therefore opens a new line of investigation for the management of stored grain pests using indigenous asteraceous plant materials in a very safe way, without operational and residual hazards that are usually part of the use of synthetic insecticides.

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