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Efficacy of extracts of plant *Prosopis* on egg laying performance of pulse beetle *Callosobruchuschinensis* Linn.

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Abstract

A very important aspect of food production is its proper conservation during and after harvest, so that crop losses during storage are reduced. Insects cause tremendous losses in storage and looking into the health and environmental hazards caused by synthetic chemical insecticides, use of botanicals have gained significance. Therefore, during the present study two plants viz. *Prosopisjuliflora* and *P. cineraria* belonging to family Leguminosae (now Fabaceae) were selected to screen their efficacy against the pulse beetle *Callosobruchuschinensis* (Coleoptera: Bruchidae) by documenting the egg laying performance of the beetle. Different formulations using some parts (bark, fruit, leaf) and their combinations (*P. juliflora* leaf + *P. cineraria* leaf and *P. juliflora*bark + *P. cineraria* fruit) were employed in the form of aqueous extract, ether extract and aqueous suspension using different dose concentrations namely 1%, 2.5%, 5% and 10%.Among the various treated sets, minimum egg laying of 6 eggs/pair was observed in sets treated with 10% aqueous suspension of bark of *P. cineraria*, while maximum (40.33eggs/pair) was found in sets treated with 1% ether extract of leaf of *P. cineraria*.

Introduction

Production of food grain has been the endeavour of human race since the ushering in of civilization. The increasing population pressure since then has resulted in ever-increasing need for agricultural produces. A very important aspect of food production is its proper conservation during and after harvest, so that crop losses during storage are reduced. The protection of stored grain from insect pest is of considerable importance owing to chances of severe infestation and damage in a short period (Swamiappan et al. 1976). The worldwide losses in storage due to insects and rodents have been estimated by FAO to be about 20%, the figures ranging from 10% in Europe and North America and 30% in Africa and Asia (Hill 1992).

Heavy reliance on modern pesticides and their increased use has its apparent benefits. Use of pesticides seemed to have resulted in immediate high returns and it seemed to fit well into high-tech and intensive agriculture. With time, the ill effects associated with heavy and indiscriminate use of pesticides started becoming visible. The adverse side effects, development of resistance in some pests and environmental and health hazards, have been of such magnitude and lasting that there has been a universal appreciation of the problem (Reddy, 1993).

The use of synthetic pesticides thus had to be restricted for their environmental toxicity, erosion of beneficial natural enemies and pest resurgence. Use of plant bioproducts became an alternative, protecting nature from pesticidal pollution (Prakash et al. 1989, Tiwari et al. 1990). The efforts have been applauded by all, and the efficacy of botanicals have been found against stored grain pests (Rao et al. 1990, Prakash et al. 1990). Thus in recent years, an impetus has been on developing and evaluating botanical insecticides in view of their relative safety to the environment (Schumutterer, 1990).

The present study has focused attention on the pulse beetle *Callosobruchuschinensis*Linn. (Coleoptera: Bruchidae), which has widely been acclaimed to be one of the major pest causing significant damage to stored pulses resulting in heavy losses to public exchequer annually. It has been found to cause weight loss, decreased germination potential and reduction in commercial value of the seeds (Booker, 1967; Caswell, 1981). According to Borikar&Pawar(1996)who studied the life cycle of *C. chinensis* the pest has a short life span but a very high degree of reproductive capacity. Keeping the aforesaid facts in mind a study was designed to assess the losses by *C. chinensis*and screening of certain botanical formulations to manage the beetle population.

Botanical insecticides are broad spectrum in pest control and many are safe to apply, unique in action, and can be easily processed and used. A number of plants have been identified in several developing countries for their pesticidal activities. Pyrethrum ranks first in popularity and effectivity as a natural plant based insecticide. Nicotine obtained from *Nicotianatabacum* and *N. rustica*have been used in Europe, Rotenone obtained from *Derris* spp. in Singapore, *Ryania* spp. used in West Indies and Mexico, *Quassiaamara* in Europe *Sobadilla*powder in South and North America (Solanki &Shanker, 2001). Following these, *Azadirachtaindica*in the only tree that has gained worldwide recognition and in India alone it has been evaluated against 105 insects (Singh &Kataria, 1986).

Plants contain a large number of secondary metabolites and those categorized under terpenoids, alkaloids, glycosides, phenols, tannins etc. play a major role in plant defense and cause behavioural and physiological effects on insects. Over the past 50 years, more than 2000 plant species belonging to different families and genera have been reported to contain toxic principles. (Solanki &Shanker, 2001). A large number of plant extracts have been screened for their activities against insects and have been found to possess insecticidal, repellent or anti-feedent properties. (Grainage& Ahmed, 1988; Arnason et al., 1989; Jacobson, 1989).

Leguminosae is a wide and chemically rich family (Pascual, 1978). The major alkaloids present were discovered to be rotenoids, which were one of the first insecticides discovered (Ahmed et al.1989). The family probably contains the largest number of plants, poisonous to fishes and many of the genera viz. *Butea, Millettia, Mundulea, Pongamia, Sophora*and *Tephrosia*have been recorded as poisonous to insects (Chopra et al., 1965). Uddin & Khanna (1978) have identified rotenones in *Crotolaria*through tissue culture studies. Besides this compound deguelin, tephrosin, cytesine are some other toxic substances which have been reported from the members of this family (Chopra et al. 1965).

Silva et al. (2007) reported alkaloids from *P. juliflora*to be cytotoxic. Plant growth inhibitory alkaloids were extracted from *P. juliflora* leaves by Nakano et al. (2004 b). Certain biologically active alkaloids from the aerial parts of five Argentinian *Prosopis* species were studied by Tapia et al. (2000) and the main active constituent was identified as catechin. The alkaloids obtained from *P. juliflora*have also been tested against plants and have been found to inhibit growth by Nakano et al. (2004). *Prosopis* has been found to contain 5-hydroxytryptamine, apigenin, isorhamnetin-3-diglucoside, 1-arabinose, quercetin, tannin and tryptamine. Tapia et al.(2000) reported that aerial parts of *P. alpataco, P.argentina, P. chilensis*and *P. pugionata* contain tryptamine and phenethylamine derivatives. Muhammad &Amusa (2005) reported medicinal properties in *P. africana*and suggested the bark and root to help improve immunity. Besides, the plant *P. juliflora* has been found by Oliveira et al. (2002) and Franco et al. (2002) to contain proteinase inhibitors that could impede the digestion process of the pest insects. Sivakumar et al. (2005) purified this component from *P. juliflora*seeds and found a remarkable in-vitro activity against *T. castaneum*and *C. maculatus*. Therefore, during the present study two plants viz. *Prosopisjuliflora* and *P. cineraria* belonging to family Leguminosae (now Fabaceae) were selected to screen their efficacy against the pulse beetle *Callosobruchuschinensis* (Coleoptera: Bruchidae) by documenting the egg laying performance of the beetle. Different formulations using some parts (bark, fruit, leaf) and their combinations (*P. juliflora* leaf + *P. cineraria* leaf and *P. juliflora*bark + *P. cineraria* fruit) were employed in the form of aqueous extract, ether extract and aqueous suspensionusing different dose concentrations namely 1%, 2.5%, 5% and 10%.

Materials and method

This study was carried out in the Laboratory of Entomology, Post Graduate Department of Zoology, Govt. Dungar College, Bikaner, Rajasthan. The test insect selected for the study was *Callosobruchuschinensis* Linn. A pure line culture was raised from its single pair. The seeds of cowpea *Vignaradiata*, were cleaned and disinfested by exposing them to 60^0c for 4 h. The insects were reared on these grains kept in glass jars covered with muslin cloth. The jars were kept in BOD incubator maintained at 28 ± 2^0c temperature and 70% relative humidity.

The plant material used in the study was collected from Bikaner city and its vicinity (situated between $27^011'$ & $20^003'$ North latitude and $71^054'$ & $74^012'$ East longitude). The plant parts used were bark, leaf and fruit. The plant parts were picked from the tree. After washing

they were shade dried for 10 – 15 days and were ground separately in electric grinder and kept in air tight plastic containers for further use.

Different parts namely bark, fruit and leaf of the two plants were used separately and in combination (*P. julifloral*eaf + *P. cineraria* leaf and *P. julifloral*bark + *P. cineraria* fruit). Only two combinations were selected for the present study based on the preliminary findings. The powdered plant powders were used in theform of liquid extract and powder suspension. The liquid extract of the plant parts was made in two media, inorganic (water) and organic (petroleum ether) as the solvent of active ingredient was obscure. For aqueous extract, 1g of powdered plant material was kept in a thimble and placed in a flask containing 50 ml of distilled water and boiled till the volume reduced to 10 ml. Thus, 10 percent concentration was obtained. further dilutions were made by adding required amount of distilled water for getting lower concentrations viz. 5, 2.5 and 1 percent. For preparation of ether extract, 1g of dried and powdered plant material was taken in a thimble, placed in a soxhlet extraction unit with petroleum ether. The extract so obtained was made to fixed volume of 10 ml having concentration of 10 percent. This was used as stock solution. Further dilutions were made to have 5, 2.5 and 1 percent concentration from the stock solution. The ether extracts were prepared fresh at the time of application to avoid evaporation loss and concomitant alterations in the concentrations. The powdered plant parts were weighed to get required concentration of 10, 5, 2.5 and 1 percent and suspension was prepared by adding distilled water.

10 g of host grains were taken and treated with 1 ml of specific extracts. Five pairs of test insects were released into each experimental set of different doses viz. 10, 5, 2.5 and 1 percent. For the study each experimental set was taken in ten replicas.

The egg laying or fecundity was calculated by counting the total number of eggs laid per pair of adult insects after three days of introduction of the adult into the treated sets and presented as No/pair. ANOVA was applied using SPSS software(2004).

Result

The egg laying (No./pair) by *C. chinensis*under different treatments has been presented in Figs. 1-2. The result of ANOVA with respect to egg laying has been presented in Tables 1-7.

The oviposition by the test insect observed during the present study was 42.33 eggs/pair. In control sets formulated with distilled water it was 41.22 while it was 40.33 with those formulated with ether. Among the various treated sets, minimum egg laying of 6 eggs/pair was observed in sets treated with 10% aqueous suspension of bark of *P. cineraria*, while maximum (40.33eggs/pair) was found in sets treated with 1% ether extract of leaf of *P. cineraria*.

Effect of formulations of plant *P. juliflora*

Minimum egg laying by *C. chinensis*(6 eggs/pair) was found in sets treated with 10% aqueous extract of bark, while 10% formulations of bark, fruit, leaf, 5% aqueous extract of bark and leaf and 5% aqueous suspension of fruit were found to moderately reduce egg laying to about 6 to 20 eggs/pair although these were significantly different from normal (41.07 eggs/pair).

Effect of formulations of plant *P. cineraria*

Minimum egg laying of 4.66 eggs/pair was found in sets treated with 10% aqueous suspension of bark. 2.5, 5 and 10% formulations of bark and fruit, 10% formulations of leaf, 5% aqueous extract of leaf, 1% aqueous extract and suspension of bark and 1% ether extract of fruit were observed to have a moderate deterrant effect on egg laying by *C. chinensis*reducing the egg laying to about 21 eggs/pair.

Effect of formulations of combination of plant parts

(a) *P. juliflora*and *P. cineraria* leaf

The sets treated with mixed plant part formulation of extract had the minimum egg laying (9.89 eggs/pair). Egg laying was observed to moderately decline by treating the sets with 5 and 10% formulations.

(b) *P. juliflora*bark and *P. cineraria* fruit

Minimum egg laying (8.56 eggs/pair) was found in sets treated with 10% aqueous suspension. 5 and 10% formulations also effectively reduced the egg laying by the bruchid to upto 21 eggs/pair as compared to normal where 41.07 egg laying/pair was observed.

For making overall comparisons between the two plants (*P. juliflora and P. cineraria*) and their plant parts (bark, fruit, leaf), the combinations (*P. juliflora* leaf + *P. cineraria* leaf, *P. juliflora*bark + *P. cineraria* fruit), various formulations (aquous extracts, ether extract, aqueous suspension) and different concentrations (1%, 2.5%, 5%, 10%). The treatments of plant *P. cineraria* were observed to be significantly effective ovipositional deterrents as compared to those of plant *P. juliflora*. Mean egg laying/pair was 16.8 in sets treated with *P. cineraria*, while it was 23.7 eggs/pair in *P. juliflora* treated sets.

The efficacy of different plant parts was found to vary in reducing egg/laying by *C. chinensis*. Although the sets treated with bark and fruit differed non-significantly from one another, they showed significant difference with leaf formulations.The treatments with aquaeous extract resulted in 18.8 eggs/pair and aqueous suspension in 19.8 eggs/pair which were significantly better as compared to ether extract treatments which resulted in 21.9 eggs/pair. Significant difference in the number of eggs laid/pair was also observed in experimental sets treated with extracts of different concentrations viz. 1, 2.5, 5 and 10%. Maximum ovipositional deterrence was observed in sets treated with 10% formulations (11.4 eggs/pair) followed by 5% (16.9 eggs/pair), 2.5% (23.3 eggs/pair) and 1% (29.2 eggs/pair).

The various treatments also showed significant differences among themselves, the most effective treatment in reducing egg laying by *C. chinensis*were formulations of *P. cineraria* bark and fruit (13.3 and 13.7 eggs/pair respectively).

Discussion

During present investigations, the findings with respect to egg laying revealed that the treatment of both the plants significantly reduced the number of eggs laid by *C. chinensis*, as compared to normal and control sets. The treatments of plant *P. cineraria* were found to be more effective as compared to the treatments of *P. julifloraı*n reducing oviposition by the bruchid.

The botanical insecticides have been in vogue since ancient times and these do possess some properties which hinder the pest insect to lay eggs. The results obtained during the present study are in conformation with the works of Kamakshi et al. (2000) who reported significant reduction in the number of eggs laid by *C. maculatus* when treated with *Menthaarvensis, Sesbaniaglandiflora* and *Ocimum sanctum* on compared to control. Delobel&Molonga (1987) also observed no or very few eggs being laid by the pest *C. serratus* when treated with *Nicotianatabacum* along with five other plants. A significant decrease in egg laying was also observed by Gupta (2004) using treatments of plants *Solanumsurattense, Solanummigrum*and *Withaniasominifera*. Mathur et al. (1985) found neem to impair oviposition by *C. chinensis.* A reduction in oviposition by *C. chinensis* was also recorded by Ghei (2001), who treated the pest with formulation of plants *Trigonella, faenumgraecum, Tephrosiapurpurea*and *Crotolariaburhia*.

Tinzaara et al. (2006) tested the potential of certain botanicals on *Cosmopolites sordidus* and found that oviposition was significantly low on corms treated with *M. azedarach, Tagetes*spp. and *R. communis.* Four pepper cultivars were used for the control of bruchids on stored cowpea seeds by Echezona (2006), viz. Sombo, Nsukka yellow, Tatashi and Tanjarawa. He also used 2% dust of primiphos methyl. There was no ovicidal effect of the protectants used earlier than 6 days after infestation, after that all the protectants significantly reduced number of eggs by the bruchid*C. maculatus*.

Oils of plant origin have been used by many of the workers against *Callosobruchus*spp. which have been found to reduce egg laying. These include the works of Naik&Dumbre (1984), who observed vegetable oils to reduce oviposition by *C. maculatus* and neem oil extracts to be most effective in hampering oviposition; Shukla et al. (1988) who found the oils of coconut, sesame, rape, soyabean, groundnut, mustard, palm, maize and Dalda to be effective in reducing the number of eggs laid by *C. maculatus* on cowpea seeds; Singhal& Singh (1990), who observed significantly reduced oviposition by *C. chinensis*when chickpea grains were treated with oils of groundnut, coconut, mustard, sesame, soyabean and rapeseed, while Babu et al. (1989) observed that the treatments of Karanj oil and castor oil effectively brought down the number of eggs laid by the bruchid; Uvah&Ishaya (1992) observed significant reduction in oviposition by *C. maculatus*when treated with groundnut and palm oils; Neem oil has been observed to significantly bring down egg laying by Yadav (1985) and Das (1986).

By applying ANOVA, it was observed that bark and fruit of the two plants were significantly more effective as compared to leaf in bringing down the egg laying by the pest insect. Earlier when leaves of *Vitexnegundo* were admixed with grains of black gram, reduction

in oviposition by *C. chinensis* was observed by Prakash & Rao (1989). Similar results were also observed by Miah et al. (1993) on chickpea. Dwivedi&Kumari (2000) observed reduced oviposition by *C. chinensis* when the grains were treated with *Ipomeapalmata*leaf extracts. Juneja& Patel (1994) observed a complete prevention of egg laying by *C. analis*until 60 days when the grains were treated with seed powder of custard apple, black pepper, leaves of mint, and peel of orange. A minimum egg laying by *C. maculatus* was recorded by Enchendu et al. (1988) when cowpea seeds were treated with dry ginger root powder and pulverised dried neem fruit. Bowry et al. (1984) reported the powdered neem cake to be more effective in reducing the number of eggs laid by *S. oryzae*infesting maize seeds.

Elhag (2000) studied the ovipositional deterrence of nine plant materials on *C. maculatus*on chickpea. Seed treatment with 0.1% crude extract of material resulted in a significant reduction in ovipositional preference of the bruchid. The highest repellency was found in *Rhazyastricta* leaves (82%), *A. indica* seeds (76.8%), *H. bacciferum*aerial parts (59.2%) & citrus peels (58.6%). F_1 females laid slightly fewer eggs in response to *R. stricta,* neem seeds, *E. caryophyllata,* cloves, *H. bacciferum,* citrus peels and *P. nigrum.*

During the present study, the aqueous extract was most effective formulation followed by aqueous suspension. Both of these formulations were significantly better than ether extract suggesting that the solvents do play a role in extracting the chemically active substance of the plant. The present findings get support by the works of Dwivedi&Maheswari (1997), who reported that acetone extract of *Croton,* petroleum ether extract of *Verbesinaenceliodes*and *Occidentalis*exhibited ovipositional deterrent activity against *C. chinensis*in stored cowpea; Teotia&Tewari (1977), who used ether and petroleum ether extracts of dharek drupes and sweet flag rhizomes against *S. cerealella*and found the petroleum ether extract to be more toxic than ether extracts; Dover (1985), who observed alcohol extracts of hyssop, rosemary, sage, thyme, white clover and the essential oils of sage and thyme to reduce oviposition by *Plutellaxylostolla,* 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& Garg (2000) who reported that acetone leaf extracts of *Tagetes, Ipomea* and *Acacia* exhibited nearly 50% reduction in oviposition by *C. cephalonica*; Mann (1997) who observed that in *R. dominica,* all the formulations of leaf of *Peganum* and ether extracts of leaf, stem and fruit of *Tribulus*and aqueous suspension of *Aerva*plant were effective in reducing egg-laying remarkably and in *C. chinensis* leaf formulation of *Fagonia*were highly effective in bringing down egg laying and ether and aqueous extracts of stem of *Tribulus*and leaf and root of *Peganum*also showed similar effects; Dwivedi& Kumar (1998), who reported petroleum ether extract and acetone extract of *Argemonemaxicana*leaves to possess maximum ovicidal properties against *T. granarium.*

Boeke et al. (2004) used aqueous extracts of plants, 13 volatile oils, 2 non-volatile oils and 8 slurries. Application of volatile oils led in most cases to a reduced number of eggs on treated beans. Repellent effects were found for *Clausenaanisata, C. citratus, C. nardus,* a mixture of *C. citratus*and *C. flexuosus, H. spicigera, Tagetesminuta*and for two samples of *O.*

*basilicum*while non-volatile oils were not repellent but had a toxic effect on beetles, but the Slurries obtained from *Carica papaya*, *Dracaena arborea*and *T. vogelii*were repellent, whereas the slurry from *A. indica*leaves was attractive.

The extract concentration also had a considerable effect on the number of eggs laid by the pest insect which was found to decrease significantly with the increase in concentration of the formulation during the present study. The 10% aqueous suspension of bark of *P. cineraria* treatment brought down the number of eggs laid to 4.66 (No./pair), while treatment of 10% aqueous extract of bark of *P. julifloral*so exhibited a similar effect reducing the egg laying to 6 (No./pair). It was observed that 10% concentration caused the maximum ovipositional deterrence, followed by 5%, 2.5% and 1%, all of which were significantly low as compared to the normal value. These results are in agreement with the works of Olaifa&Erhun (1988), who observed that although low concentration of the powder of *P. guineense*significantly reduced oviposition by *C. maculatus*, a complete suppression of oviposition was observed at a higher concentration of 42%. They further reported that volatile oil of *P. guineense*at 0.02% and 0.005% significantly reduced oviposition and complete suppression of oviposition was observed at 0.02%. Ghei (2001) reported that 10% aqueous suspension of roots and leaves of plant *Tephrosia* were found to reduce the average number of eggs laid per pair to 6.66.

Oil of *Cymbopogonmartinii* at 0.1% concentration and *Menthaarvensis* at 0.2% were observed to be most effective in preventing oviposition of *C. chinensis* by Srivastava et al. (1988). The treatement with neem, castor and karanj at 1.0% showed significant repellent action for egg laying by adult bruchidupto 100 days was reported by Kachare et al. (1994). Savitri&Subbarao (1976) observed that the powdered neem kernel mixed directly with paddy at 1% and 2% was effective in reducing the oviposition by *R. dominica*and *S. cerealella*respectively. Prasad et al. (1998) observed that the extract of *L. camara*in all the used concentrations checked the egg laying by *S. oryzae*. Dwivedi& Kumar (1998) reported that increase in extract concentration of *Argemonemexicana*increased its ovicidal properties against *T.granarium.* Tomar& Singh (2001) observed strong ovipositional deterrence by melon fruit fly, when 5% neem oil and 5% extract of rambans were used, while, mahua, mustard, sunflower, castor and olive oils, each at 5% concentration were found to result in less than 50% reduction in oviposition. Pitlehra&Borad (2001) investigated various indigenous plant materials against *L. trifolii* on castor, out of which neem seed kernel extract, ardusi leaf extract and kaner leaf extract were found effective, whereas, *Bougainvillea* and naffatia leaf extracts (3%) were found less effective in reducing the oviposition.

Mbaiguinam et al. (2006) found six seed oil extracted by methylene chloride from *A. indica*, *R. communis*, *T. nerifolia*, *B. eagyptiaca*, *M. oleifra*and *K. senegalensis*to significantly reduce the oviposition by *C. maculatus*, the most effective being *T. nerifolia*. Tebkew&Mekasha (2002) evaluated thirteen botanicals for their efficacy in controlling *C. chinensis* and found that *Mellettiaferruginea*deterred egg laying when mixed with grain at 5% w/w. The powder and ethanol extract of *T. diversifolia*leaves were tested for their efficacy at five concentrations

against *C. maculatus*by Adedire&Akinneye (2004) who found that mean number of eggs laid was reduced to 4.7 at 2% extract concentration, while control value was 20.7. In the powder treatments the egg laying reduced from 41.3 in the untreated to 17.3 at 2% concentration.

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Table 1. Report: Egg laying (No/pair) by *Callosobruchuschinensis*

Plant type		Egg laying (No/pair)
P. juliflora	Mean	23.7223
	N	108
	S.D.	8.58798
P. cineraria	Mean	16.7922
	N	108
	S.D.	8.89685
Total	Mean	20.2573
	N	216
	S.D.	9.38938

Table 2. ANOVA for egg laying (No./pair)

Source	Type III Sum of Squares	df	Mean Square	F	Significance
Plant	2593.414	1	2593.414	97.981	0.000
Plant part	971.568	2	485.784	18.353	0.000
Extract	701.008	2	350.504	13.242	0.000
Concentration	9209.507	3	3069.836	115.980	0.000
Error	5479.005	207	26.469		

a. R Squared = 0.711 (Adjusted R Squared = 0.700)

Table 3. Comparison of mean egg laying (No./pair) under different formulation ofsome parts of *P. julifloraand P. cineraria*

Plant Part	N	Subnet	
		1	2
Bark	72	18.6633	
Fruit	72	18.8539	
Leaf	72		23.2546
Significance		0.824	1.000

Means for groups in homogeneous subsets are displayed.

Based on Type III Sum of Squares

The error term is Mean Square (Error) = 26.469

a. Uses Harmonic Mean Sample Size = 72.000
b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type 1 error levels are not guaranteed.
c. Alpha = 0.05

Table 4. ANOVA for egg laying (No./pair) under formulation employing combination of some parts of plant *P. julifloraand P. cineraria*

Source	Type III Sum of Squares	df	Mean Square	F	Significance
Treatment	5050.195	7	721.456	39.212	0.000
Extract	515.850	2	257.925	14.018	0.000
Concentration	12907.777	3	4302.592	233.848	0.000
Error	5059.747	275	18.399		

a. R Squared = 0.785 (Adjusted R Squared = 0.776)

Table 5. Comparison of mean egg laying (No./pair) under different treatments

Treatments	N	Subset			
		1	2	3	4
P. cineraria bark	36	13.3361			
P. cineraria fruit	36	13.7722			
P. juliflora bark + *P. cineraria* fruit	36		18.4439		
P. juliflora leaf + *P. cineraria* leaf	36			21.5367	
P. juliflora leaf	36			23.2408	23.2408
P. cineraria leaf	36			28.2683	23.2683
P. juliflora fruit	36				23.9356
P. juliflora bark	36			`	23.9906
Significance		0.667	1.000		0.507

Means for groups in homogeneous subsets are displayed.

Based on Type III Sum of Squares

The error term is Mean Square (Error) = 18.399

a. Uses Harmonic Mean Sample Size = 36.000
b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type 1 error levels are not guaranteed.
c. Alpha = 0.05

Table 6. Comparison of mean egg laying (No./pair) under different extracts

Extracts	N	Subset	
		1	2
Aqueous	96	18.7996	
Aqueous suspension	96	19.7744	
Ether	96		21.9976
Significance		0.117	1.000

Means for groups in homogeneous subsets are displayed.

Based on Type III Sum of Squares

The error term is Mean Square (Error) = 18.399

a. Uses Harmonic Mean Sample Size = 96.000
b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type 1 error levels are not guaranteed.
c. Alpha = 0.05

Table 7. Comparison of mean Egg laying (No./pair) under different concentrations

Concentration	*N*	Subset			
		1	**2**	**3**	**4**
10%	72	11.4006			
5%	72		16.8785		
2.5%	72			23.2572	
1%	72				29.2258
Significance		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on Type III Sum of Squares

The error term is Mean Square (Error) = 18.399

a. Uses Harmonic Mean Sample Size = 72.000
b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type 1 error levels are not guaranteed.
c. Alpha = 0.05

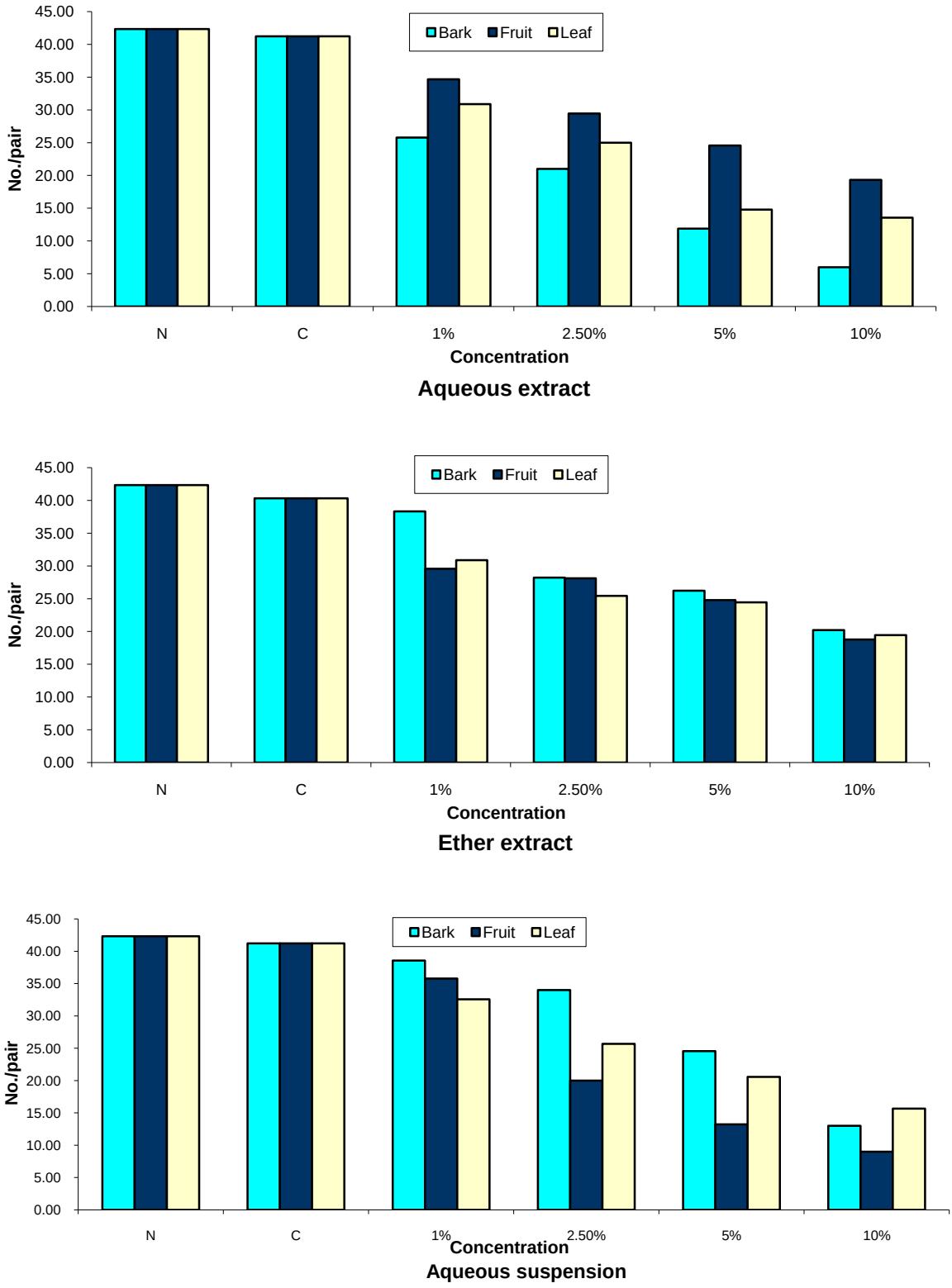

Fig. 1. Effect of different formulations of some parts of *P. julifloraon* the egg laying (No./pair) by *C. chinensis*

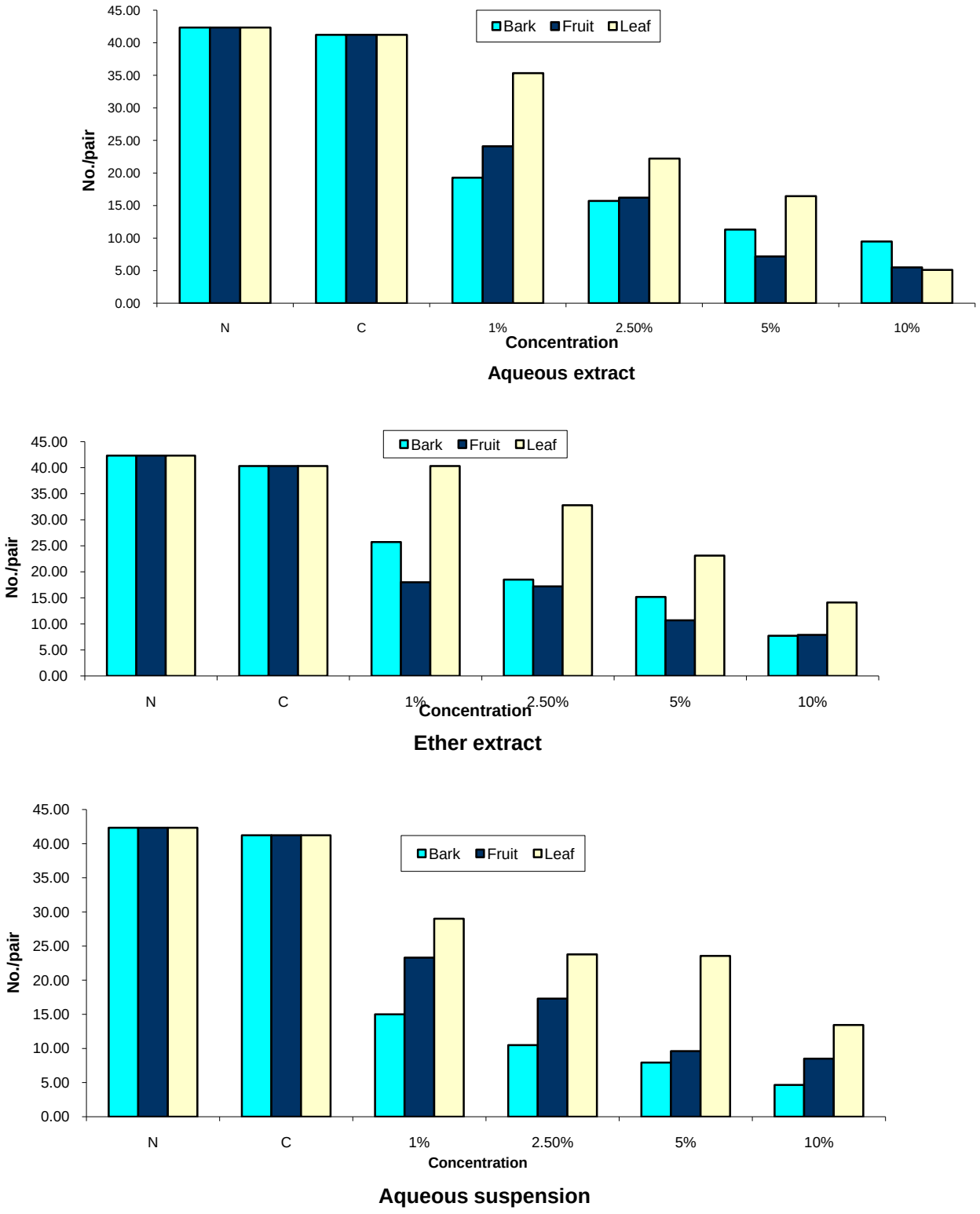

Fig. 2. Effect of different formulations of some parts of *P. cineraria* on the egg laying (No./pair) by *C. chinensis*