Studies on Biology and Host Specificity of *Callosobruchus Analis* (Coleoptera: Bruchidae) and their Management, using Indigenous Plant Extracts

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Abstract: ***Pulse beetle, Callosobruchus analis (Fab.) is one the most common destructive pest of stored pulses in India. The present study revealed that use of phytochemicals extracted from native aromatic plants (Lantana samara, Eupatorium odoratum, Azidirachta indica and Clerodendrum infortunatum) is perceived as a promising alternative to protect legumes. The biology and the host preference study of C. analis on five pulse grains (Bengal gram, Green gram, Green pea, Cowpea, Black gram) revealed that the larval developmental period had an influence on the hosts. Bengal gram, cowpea and green pea, thus showed the highest ovipositional preference. The growth index values of cowpea and green grams revealed that they are the most susceptible legume seeds and thus the most suitable hosts for C.analis. The insecticidal principles isolated from L.camara, Eupatorium odoratum, Azadirachta indica and Clerodendrum infortunatum were established. From the present study, it is recommended that farmers have to be advised not to store cowpea and green gram seeds in the same place and/or at the same time to avoid cross infestation because of their high susceptibility to C.analis and the 5% ethyl acetate and hexane extract of eupatorium and lantana treatment can be used effectively against C.analis.***

Keywords: pest, stored pulses, plant extracts, treatment, larva

1. Introduction

Pulses are one of the important segments of Indian Agriculture after cereals and oilseeds. They are also the cheapest food which provides proteins to most of the rural people. Cereal grains as well as pulses are usually attacked in stores by different insect pests.

Beetles belonging to the family Bruchidae (*CalIosobruchus* sp.) are the most important insect pests of stored legumes. Infestation by bruchids causes several damages like loss of weight, nutritional value and germination potential, and therefore the commercial value of the commodity may be reduced (Southgate 1978;Singh et *al.*, 1980: Dick and Credland, 1986a). The biology of pulse beetle, *CalIosobruchus* sp. on various stored pulses was earlier investigated by different workers (Singh and Kumari, 2000; Mandal *et al*., 2003)

The use of chemical pesticides had made a great contribution to the fight against pests and diseases. But the overuse of synthetic insecticides led to numerous problems unforeseen at the time of their introduction: acute and chronic poisoning of applicators, farmworkers, and even consumers; destruction of fish, birds and other wild life; disruption of natural biological control and pollination; extensive ground water contamination, potentially threatening human and environmental health and the evolution of resistance to pesticides in pest populations.

Extracts from different plants have been confirmed useful against a wide range of insect pests (Sarwar et al., 2012). The application of bio pesticides should be encouraged because of greater environmental protection and food safety concerns.. Such botanicals used have well-known volatile and act as natural fumigants that kill adult pests and their progeny (Sarwar et al., 2012). Considering the economic significance of gram, the severity of damage caused and the problems associated with the use of synthetic insecticides, need was felt to evaluate natural methods for the control of *C.analis* in storage. The present study investigated the efficacy of botanical insecticides against *C.analis* for a sustainable pest control strategy.

2. Materials and Methods

The bruchid infested cowpeas were collected from the local grain market. The insect cultures were maintained in cowpea (*Vignaunguiculata*) at a constant temperature of 30 ± 2°C and 70±5% rh. The biology of pulse beetle *C.analis*was carried out under laboratory conditions on cowpea during March to June, 2014. The morphological characters, fecundity, mating time duration of development was noted.

Healthy seeds of different pulses *viz.*, cowpea, green gram, black gram, Bengal gram, and green peas were collected from a supermarket. The insects were reared. The ovipositional preference, mean developmental period and per cent adult survival in different pulses were observed and recorded. Ten replications were maintained for the experiment.

The growth index (G. I) of bruchids reared on different pulses was determined using the formula: G.I = log S/T, where, S is the percentage of emerged adults and T = mean developmental time.

The per cent weight loss was calculated by using the following formula (Adams and Schulten, 1978).

$$\text{Percent weight loss} = \frac{U\,(Nd) - D\,(Nu)}{U\,(Nu+Nd)} \times 100$$

Number of damaged seeds (Nd), Number of undamaged seeds (Nu), Weight of damaged seeds (D), weight of undamaged seeds (U)

The percent germination was computed according to Ogendo *et al*. (2004) as follows:

$$\text{Viability index (\%)} = \frac{NG \times 100}{TG}$$

Where, NG = number of seeds germinated and TG = total number of seeds tested in each Petri dish.

Bio-assays

The plant species used in the experiments *Lantana camara*, *Azadirachta indica*, *Eupatorium odoratum* and C*lerodendrum infortunatum* collected from the field were shade dried, powdered and sieved to obtain the finest particles. The powder (100g) was then extracted with 500ml solvents (Hexane, ethyl acetate and water) with various polarities. The solvent was removed by filtration after 48 hours. Extracts were allowed to evaporate to get residue. It was dried and weighed and re-dissolved in known volume of respective solvents. Adults of *C.analis* were exposed with various increasing concentrations of each plant extracts separately. For this purpose, separate filter paper strips (3.5 cm) were coated with different concentrations (5 and 10%) of plant extracts were placed in the plastic bottles of 100ml capacity. The coated filter paper strips were air-dried before application. Only solvent treated filter papers were strips used to set control. Ten adult insects were released in the plastic bottles (100ml). For each extract, two different concentrations were used and for each concentration three replicates were set. Mortality in *C.analis* was recorded after 24 hr. The weevils were considered dead when there was no response after verifying from the abdomen by touching with a pin.

3. Results

Copulating males were attached firmly to females by their genitalia. Females usually start to kick males about 3-7 min after mating. Mating lasts for 6-7 minute. In *C.analis* second mating observed 10 min after the first mating.

The oviposition period in *C.analis* was 6.33± 0.57days. The fecundity of *C.analis* was 67.33±2.88. In *C.analis* the egg laying started 20 min after first mating. Usually one to three eggs was laid over an individual seed. The number of eggs per seed depends upon the availability of resources and size of the seed.

The mean incubation period was 4.66±0.57 days for *C.analis*. Most of the eggs are hatched at the fifth day after oviposition. The C-shaped larvae look like short cream-coloured maggots hatched out from the egg. The first instar larva enters into the seed cotyledon through seed coat. The second instar larvae increase its size and weight. They voraciously feed cotyledons and took more time for completing its second instar.

The third instar larva was similar to the second instar except for size and shape. The third and fourth larval instar periods were short. The fourth instar period was shortest. The fourth instar larvae moulted into pupae. However, prior to pupation larvae excavate a pupal chamber just below the seed surface, visible as a small transparent window in the seed coat. The female emerged first and the male emerged out only one or two days after the female emergence from the seed. The percentage of emergence was noted and was 55.31±4.64 %. The sex ratio of *C.analis* was found to be 1:1.8.

The larval development was completed within 13.00 ±1.00 days. The pupal period for the insects was observed as 6.33±0.57 days. The total time for the development was noted as 25.00±1.00 days. The female life span (7.33±0.57 days) and male life span (5.66±0.57 days) were significantly differed and the females live 2 or 3 days more than male.

Ovipositional preference of *C.analis* on different pulses was shown in Fig.1. Among the selected pulses, the green pea recorded significantly the lowest of 6.0 ±1.00 eggs /100 g seeds. Whereas significantly higher number of eggs (27.33 ± 1.52 eggs /100 g of seeds) were observed in green pea.

The developmental period ranged from 24.67 ± 2.08 to 34.33 ±1.15 days in different pulses (Fig.1). Among the selected pulses, the Green gram recorded significantly the lowest developmental period (24.67 ± 2.08 days) which was on par with Cowpea. In black gram a maximum of 34.33 ±1.15 days developmental period was observed. The result revealed that the green gram is the most preferred host for the insects. The incubation period and the pupal period were not significantly differing among the pulses (Fig.1). The highest larval period was taken by the insects developed in the black gram (21.67±1.15 days) and lowest recorded in the green gram (14.67±0.57days).

In *C.analis*, the mean adult survival among different pulses ranged from 4.49±1.28 to 62.76±2.42 per cent. Black gram recorded significantly lowest adult emergence of 4.49±1.28 per cent. The highest adult emergence recorded on green gram (62.76±2.42%) followed by cowpea (53.24±1.68%).

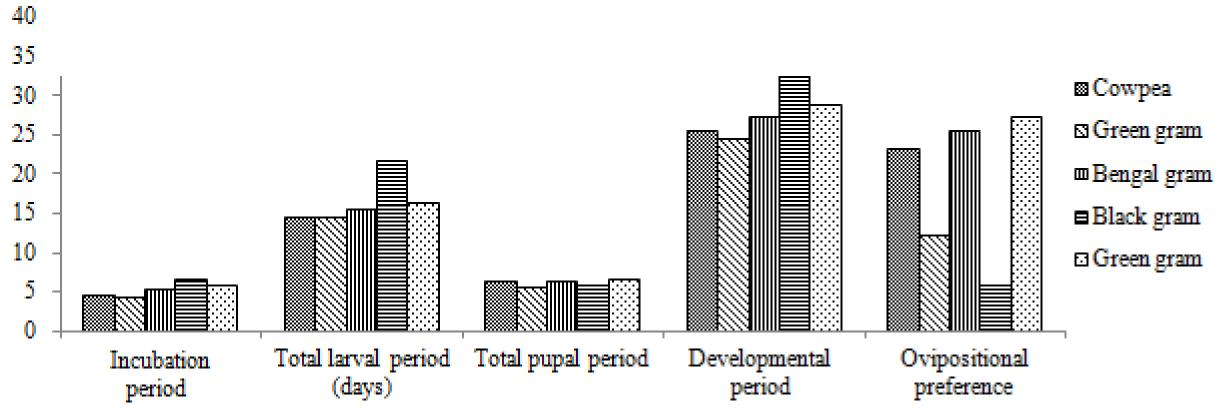

Figure 1: Host preference of *C.analis* under laboratory conditions

The significant difference was observed in the growth index of the insects developed in various pulses. The highest growth index was recorded in the insects developed from the green gram (0.40±0.03) and was followed by cowpea 0.31±0.01. Significantly lowest growth index was recorded in the insect developed from black gram (-0.89±0.14), bengal gram (-0.04±0.01) and green peas (-0.13±0.03) (Table 1).

Table 1: Weight loss, Adult emergence, and Growth index of *C.analis*

Host	Weight loss of seeds (%)	Percentage of emergence	Growth index
Bengal gram	18.34±1.48[c]	24.74±1.62[b]	-0.04±0.01[d]
Green gram	28.56±0.68[d]	62.76±2.42[d]	0.40±0.03[e]
Green peas	9.83±0.77[b]	21.26±1.54[b]	-0.13±0.03[c]
Cowpea	25.78±0.56[d]	53.24±1.68[c]	0.31±0.01[b]
Black gram	4.25±1.48[a]	4.49±1.28[a]	-0.89±0.14[a]

The Duncan's Multiple Range Test (P<0.05) was used and the significant differences in each column are indicated by different letters. There were 6 replicates for each variable (mean ± SE).

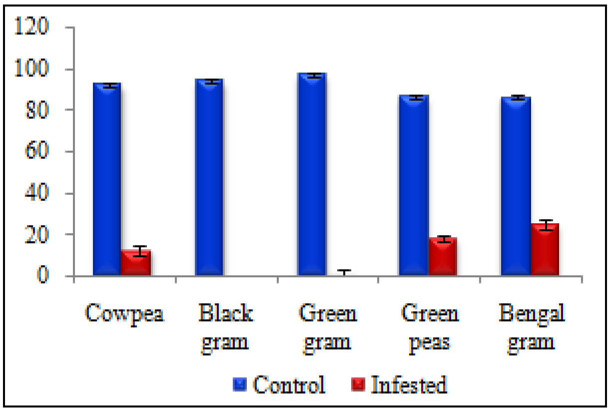

Figure 2: Germination of pulse seeds due to the infestation by C. analis

As a result of the feeding activities of the weevils, weight loss was recorded end of the first generation. Among the pulses maximum seed damage of 28.56±0.68 % was found in green gram followed by cowpea 25.78±0.56 and minimum seed damage was observed in black gram (Table 1). The present study revealed that maximum damage caused by the feeding of grubs of the pest was incurred in green gram and cowpea seeds. Further it was also observed during the study that the number of grains with more than one egg was very small.

The percentage seed viability of different pulses infested by different *C. analis* is recorded (fig 2). The *Callosobruchus* infestation significantly affects the seed viability. The data obtained showed that infestation of pulse seeds with different *Callosobruchus* species reduced seed germination significantly, the infested black gram not germinated. The infested Bengal gram, green peas and soybeans germinated some extent.

Bio-assay of phytochemicals

Among the hexane extract, the lantana gave the highest mortality (20.0±10.0%) after 24 hrs exposure. when the concentration was doubled the mortality increased to 20%. And when the 5% *clerodendron* extract treated no mortality observed after 24 h. But when the concentration increased, the percentage of mortality also increased. The ethyl acetate extract gives the highest mortality among all the treatments. The ethyl acetate extract of lantana gave highest percentage of mortality (56.7±5.77) among treatment. The lowest mortality observed in the neem extract. In all treatments the percentage of mortality of control and in the aqueous extracts are 0.0±0.0 (Table 2).

Table 2: Bioactivity of hexane and ethyl acetate extracts on *C.analis*

Plant	Concentration	Percentage of mortality	
		Hexane	Ethyl acetate
Eupatorium odoratum	5%	6.67±5.77	36.67±5.77
	10%	13.33±5.77	43.33±5.77
Lantana camara,	5%	13.3±5.77	23.33±5.77
	10%	20.0±10.0	56.7±5.77
Azadirachtaindica	5%	3.33±5.77	20.0±10.0
	10%	13.3±5.77	36.67±5.77
Clerodendruminfortunatum	5%	0.0±0.0	0.0±0.0
	10%	3.33±5.77	6.67±5.77

4. Discussion

Although several species of Bruchids are known to damage grains of legumes, the *C.analis* show greater specificity to attack stored pulses (Krishnamurthy and Rao, 1950). The mean incubation period obtained was 4.66±0.57 days in the present study. These results are also in agreement with Butani et al. 2001;Varma and Anandhi (2010). The eggs laid by *C.analis* was 85.76 per cent viable. This result corporated with Raghvani (1986) and Singh and Kumari (2000).Varma and Anandhi (2010) reported fecundity in *C. chinensis* 85.6 of eggs which are different from the present study (67.33). Butani, *et al*. (2001) recorded a *C.chinensis* single female laid as many as 97 eggs during its life span.

Host preference study of *Callosobruchus spp* revealed that strong oviposition preference exhibited for Bengal gram seeds followed by green peas this may be due to large grain size. Apple baum *et al.* (1970) reported that *Callosobruchus spp*. are known to prefer to feed and develop on cowpea as their main hosts. Maximum adults were seen emerging from stored green gram seeds. The results of the present study were more or less similar to the findings of Singh (1997), Singh and Kumari (2000). This probably eliminated the physical barriers and indicates a high toxicity and/or a low nutritional value as the cause of death

From this study, it can be concluded that seeds of cowpea and green gram are the most susceptible legume seeds and thus the most suitable hosts for *C. analis*. These hosts had highly oviposited and percent adult emergence, the shortest developmental period, highest growth index and largest weight loss. Conversely, black gram and green beans seeds were found to be less susceptible. From host study, it may recommended that farmers have to be advised not to store cowpea and green gram seeds in the same place and/or at the same time to avoid cross infestation because of their high susceptibility to *C.analis*.

Pulse seeds suffered the highest weight loss among highly infested seeds. Green peas, Bengal gram, and black gram had the lowest weight losses. The result of the present experiment is comparable with those reported by Dias and Yadav (1988), Ofuya and Bambigbola (1991), Dwivedi and Sharma (1993). Weight loss in cowpea seeds was as a result of the larvae eating up the endosperm (Baidoo *et al.*, 2010). The cowpea and green gram seeds being the most susceptible compared to other pulse grains used in the present study. They made use of the dry matter from the seeds thereby reducing the weight of pulses.
The infestation of grains influences the viability of the seed. The infested green pea and black gram sees did not germinate. This is due to the consumption of endosperm by larvae or damaging the plant embryo. The percentage of infestation decreases the viability increases.

The present laboratory results clearly demonstrated that various botanical extract containing certain volatiles/ alkaloids adversely affected the survival of the weevil *C.analis.* Earlier, many plant derived materials were observed possessing repellant and insecticidal activities against the insects of the stored food products (Sukumar, 1993; Desmarchelier, 1993; Coelho *et al*., 2010). During the present investigations, hexane and ethyl acetate plant extract of *Eupatorium* and C*lerodendron* are proved more efficient for the control of *C. analis.* The sustainable use of these botanical pesticides will boost the food security in those environments where investment in synthetic pest control is uneconomical. However, the promotion of botanicals as an alternative to synthetics must acknowledge that natural product toxicity could be high. Future studies must still confirm whether a botanical treatment can be safely used as a food additive before promotion to farmers.

5. Conclusion

Beetles belonging to the family Bruchidae are the major pests of pulses, both in the field on dry seeds and in the warehouses. Among this group *Callosobruchus* spp. has been identified as the devastating pest on cowpea, lentil, green gram and black gram. Usage of synthetic pesticides to control these pests is highly discouraged because of their adverse effect on human beings and environment. Although a large number of plants in various forms have been screened against major pests of stored grain, thousands are still untouched. The present study confirmed that among the four plants screened, bioactive principles from eupatorium and lantana can effectively use against *C.analis*.

References

[1] Adams JM and Schulten GBM (1978): Losses caused by insects, mites and microorganisms. (Harris KL and Lindblad CJ eds.) In: Post Harvest Grain Losses Assessment Methods. American Association of Cereal Chemists, St. Paul.
[2] Applebaum SW, Tadmor U and Podoler H (1970).The effect of starch and of heteropolysacharide and fecundity of *Callosobruchus chinensis*. EntomologiaExperimentalisetApplicata, 13: 61–70.
[3] Baidoo PK, Mochiah MB and Owusu-Akyaw M (2010). The effect of time of harvest on the damage caused by the cowpea weevil *Callosobruchus maculatus*(Fab.) (Coleoptera: Bruchidae). Journal of Stored Product Postharvest Research, 1(3): 24 - 28.
[4] ButaniPG,Motka MN, and Kapadia MN (2001).Storage pests and their management.Bulletin published by Department of Agricultural Entomology, College of Agriculture, Gujarat Agrciultural Univesity, Junagarh. pp. 25-27.
[5] Coelho MB, Macedo MLR, Marangoni S, Silva DS, Cesarino I and Mazzafera P (2010) Journal of Agriculture and Food Chemistry.58 3050.
[6] Desmarchelier JH(1993) Grain Protectants: Trends and Developments, Stored Product Protection, CAB International, Walling Ford, UK. 722.
[7] Dias CAR and Yadav TD (1988). Incidence of pulse beetles indifferent legume seeds. Indian Journal of Entomology, 50:457–461.
[8] Dwivedi SC and SharmaMK (1993). Extent of damage caused and host preference of *Callosobruchus chinensis* Linn. Bioved4(2):249-254)
[9] Krishnamurthy B and Rao DS (1950).Some important insect pests of stored grains and their control.Mysore Entomological Series Bulletin, 14.
[10] Mandal S (2003). In: Proc. National Sympo. on “Frontier Areas of Entomological Research” organized by Division of Entomology : 205.
[11] Ofuya TI and Bambigbola KA (1991). Damage potential, growth and development of the seed beetles *Callosobruchus maculatus*(Coleoptera: Bruchidae) on some tropical legumes. Tropical Agriculture, 68 (1):33- 36.

[12] Ogendo JO, Deng AL, Belmain RS and Musandu AAO (2004). Effect of insecticidal plant materials *Lantana camara*L. and *Tephrosia vogelli* Hook on the quality parameters of stored maize. The Journal of Food Technology in Africa, 9: 29–36.
[13] Pokharkar PK and Mehta DM **(**2011**).**Biology of pulse beetle, *Callosobruchus chinensis* in stored chickpea. Progressive Agriculture**,**11(1): 34- 36.
[14] Raghvani B R (1986). Varietal resistance and susceptibility of chickpea (*Cicerarietinum*L.) to pulse beetle, *Callosobruchus chinenisis*(L.).*M.Sc. (Agri.)* thesis submitted to the Gujarat Agricultural University, Sardar Kurshinagar. pp. 136.
[15] Sarwar M, Ahmad N, Bux M and Tofique M (2012). Potential of plant materials for the management of cowpea bruchid *callosobruchus analis*(coleoptera: bruchidae) in gram *cicerarietinum* during storage. The Nucleus 49:1 61-64
[16] Singh SC, Kumari R (2000). A study of the biology of *Callosobruchus chinensis*(Linn.) infesting stored pulses (grain legumes) in India. Indian Journal ofEntomology, 62(4):319-322.
[17] Singh Y, Saxena HP and Singh KM (1980). Exploration of resistance to pulse beetle III. Growth, development of *C. maculates* F. Indian Journal of Entomology, 42: 622- 626.
[18] Southgate BJ (1978).The importance of Bruchidae as pests of grain legumes, their distribution and control.In: Pests of Grain Legumes: Ecology and Controled. Singh et al. (Eds.). Academic Press. London. : 219-229.
[19] Sukumar K (1993) Role of allelochemicals in the control of phytophagous insects. In: T.N. Ananthakrishnan and A. Raman, (Editors), Chemical Ecology of Phytophagous Insects, Oxford and IBH, New Delhi. 82.
[20] Varma S and AnandhiP(2010).Biology of pulse beetle (*Callosobruchus chinensis*L.,Coleoptera:Bruchidae) and their management through botanicals on stored mung grains in Allahabad region. Legume research,33 (1):38.