Bond for their technical assistance in maintaining insect cultures and in executing fumigation bioassays.

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Response of *Callosobruchus chinensis* L. to plant extracts and to the parasitoid *Anisopteromalus calandrae*

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Abstract

Present investigation was carried out to elucidate the extracts of botanicals i.e., *Cichorium intybus, Glycyrrhiza glabra, Trachyspermum ammi* and *Terminalia chebula*, for their possible toxic effect against *C. chinensis* population. The results revealed that mortality was highest (94.649%) in case of *T. ammi* treatment, followed by *T. chebula* with mortality value 56.929%. Mortality was 52.363% where application of *C. intybus* was carried out. Minimum mortality (34.500%) was observed in *G. glabra* treated grains. A natural ecto-parasitoid, *Anisopteromalus calandrae* was used to manage *C. chinensis* population. *A. calandrae* male and female adults (5, 10 and 15 pairs) were released to analyze the parasitism efficiency. *A. calandrae* was reared in the laboratory on *C. chinensis* larvae. Honey was offered as a suitable food to parasitoid. The parasitism data was recorded after the adult emergence of brunchid beetles. The experiment conducted under Completely Randomized Design and results statistically evaluated using statistical software at 5% level of significance. *A. calandrae* parasitized both larval and pupal stages of *C. chinensis* and preferred 4th instar larvae of *C. chinensis*. Large amount of *A. calandrae* may efficiently control the *C. chinensis* population. As compared to control (1558.7 host adult), the minimum host emergence (699.00 host adult) was observed with high population density of *A. calandrae*. It was also obvious from the results, that mortality was increased with the increase in concentration so, a direct dose-mortality response was observed.

Key words: Callosobruchus chinensis, Plant Extracts, Anisopteromalus calandrae, Mortality,

1. Introduction

Mungbean is highly infested by particularly three species included *Callososbruchus chinensis* (L.), *C. maculatus* (F.) and *C. analis* (F.); caused significant losses during storage (Angus, 2010). *C. chinensis* is a well known insect pest of stored mung bean, chickpea and other pulses. Use of synthetic pesticides is the main method to control the insect pest due to their high cost, environmental pollution and development of resistance in insects, alternative approaches have been developed to manage insect pest problems. To control the insects in this sense, essential oils are the best alternative (Perez *et al*., 2010).

Trachyspermum ammi is traditionally widespread used medicinal plant to treat various illnesses. The essential oil of this plant has antimicrobial activity (Kaur and Arora, 2009). Due to the insecticidal activities of *T. ammi*, its essential oil has been used against *C. chinensis* (Chaubey, 2011). *Glycyrrhiza glabra* has antifungal and antimicrobial efficiency. It has small cellular toxicity, anti-tumor and anti-virus (Wang *et al*., 2003). *Terminalia chebula* has antibacterial and anti-pathogenic potential (Malekzadeh *et al.,* 2001). The roots of *Saussurea lappa* have distinct antimicrobial and anti-inflammatory potential and used as a traditional drug for the treatment of several ailments (Pandey *et al*., 2006). *Cichorium intybus* is a popular folk medicinal plant used in curing the urinary tract inflammation, gallstones and liver disorders. It helps in maintaining healthy gastrointestinal tract and metabolism (Roberfroid and Slavin, 2000).

Anisopteromalus calandrae has a wide host range including *Sitophilus granarius* (Ghani, and Sweetman, 1955), *Sitophilus oryzae* (Lucas and Riudavets, 2002), *Lasioderma serricorne* (Ahmed and Khatun, 1988) and *Rhyzopertha dominica* (Menon *et al.,* 2002). *Anisopteromalus calandrae* gave effective control for *C. maculatus* in Cameroon. It was used as an adult parasitoid and gave efficient results (Ngamo *et al.,* 2007). *Anisopteromalus calandre* ecological and biological investigation were made under laboratory condition and showed that it preferred 4th larval instars over pupa and then 2nd instars for parasitism (Kazemi *et al*., 2004).

In the light of above discussion the present study was carried out with the objective to develop environmentally friendly IPM, to check the biological activity of some plants including *Trachyspermum ammi, Glycyrrbiza glabra, Terminalia chebula* and *Cichorium intybus* and to evaluate the efficiency of an ectoparasitoid *A. calandre* to manage *C. chinensis* populations.

2. Materials and Methods

Collection and Rearing of Insects

Callosbruchus chinensis population was collected from grain market in Faisalabad. Insect population was reared on mung bean in sterilized jars which kept in the incubator at temperature 30±2ºC, 70±5% relative humidity and 12:12 L:D photoperiod to get the homogeneous population. Thirty insects were released in each jar which contains 500 g of mung bean. The jars were covered with muslin cloth so that to avoid insects escape. After five days adults were separated from the mung bean and the grains containing eggs were kept again in the incubator to get another generation. The grains containing adults were also kept in the jar to get homogenous population.

Preparation of Plant Extracts

Plant materials including *Trachyspermum ammi* (Ajowin), *Terminalia chebula* (Hararr), *Glycyrrhiza glabra* (Mulathi) and *Cichorium intybus* (Kasni) were purchased from a medicinal plant shop, Faisalabad. The material was cleaned to avoid contamination. The materials were grinded to get powder. The extraction of plant extracts was accomplished using rotary shaker by dipping 50 grams of powder in 250 ml acetone. The extracts which were obtained were placed in clean bottles and stored in refrigerator.

Mortality Bioassay

The experiment was carried out in 60 small jars. Different concentrations of plant extracts were applied on the inner side of jar and allowed to get dry. Twenty adults of test insects were released in each jar and then the jars were covered with muslin cloth. Mortality of the adults was recorded three times after equal intervals of 24 hours.

Parasitism Bioassay

Anisopteromalus calandrae was reared on the adults and pupae of *C. chinensis*. The trial contained 36 jars with 20 g of mungbean grain. Thirty adult females of *C. chinensis* were released in each jar for egg laying. After one week the adults were removed and the eggs were placed in jars with grains till emergence. With the start of emergence, the parasitoids were introduced on cowpeas infested by *C. chinensis*. This allowed the synchronisation of the life cycles of the parasitoid and its host. The jars were placed in an incubator and the adult emergence of *C. chinensis* was checked after 27-42 days to record the parasitism data.

Statistical analysis

After the completion of the experiment the recorded data was analyzed using statistical software and the corrected mortality was measured using Abbott's formula. The data was analyzed using Completely Randomized Design and suitable statistic software.

3. Results

Effect of plant extracts against adult mortality of *Callosobruchus chinensis*

Results showed that impact of plants and duration of insects to plant extracts has a highly significant effect on mortality of *C. chinensis*. Interaction of plants and time and interaction of plants and concentrations also have significant impact on mortality. But concentrations, interaction of time and concentrations and interaction of plants, time and concentrations have no significant impact on mortality of test insect. Figure 1 shows the mean comparison of percent mortality of *C. chinensis* of various plant extracts. The results showed that maximum mortality (93.65%) was recorded of *T. ammi* extract and it was statistically different to *C. intybus, T. chebula* and *G. glabra* with percent mortality of 64.67, 63.67 and 44.42% was observed respectively. The results regarding mean comparison of percent mortality of *C. chinensis* at various time exposures revealed that maximum mortality (80.20 %) was recorded after 72 hours and it was statistically different to 48 and 24 hours with percent mortality of 69. 63 and 49.98% was observed respectively (Figure 2). Mean comparison of percent mortality of *C. chinensis* at various time exposures of plant extracts is given in Figure 3. The results showed that maximum mortality (69.33%) was observed at 5% concentration. It was statistically similar to 15 and 10% concentrations, where mortality was 65.46 and 65.01%, respectively.

The results regarding mean comparison of percent mortality of adults of *C. chinensis* of plant extracts and various time periods showed that the effect of interactions of plant extracts and various time exposures was significant (Figure 4). Similarly, the results in Figure 5 show that the effect of interactions of plant extracts and various concentrations was significant.

Maximum mortality (97.62%) was observed with *T. ammi* after 72 hours while minimum mortality (26.16%) was observed with *G. glabra* after 24 hours of exposure. In the interactions of plant extracts and concentrations, maximum mortality (98.21%) was observed with *T. ammi* at higher (15%) concentrations and minimum mortality (39.85%) was observed with *G. glabra* at 10% concentrations. With the interaction of exposure time and concentrations the maximum mortality

(83.92%) was recorded after 72 hours at 5% concentration. In plant, exposure time and concentrations interaction the maximum mortality was observed with *T. ammi* after 72 hours at 15% concentrations and minimum mortality (23.19%) with *G. glabra* after 24 hours at 15% concentration.

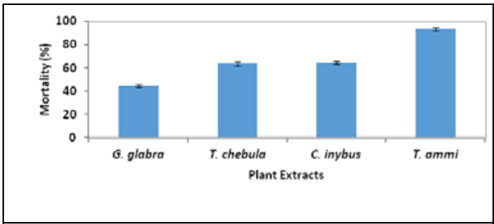

Fig. 1 Comparative effect of four plant extracts against mortality of adults of *C. chinensis*

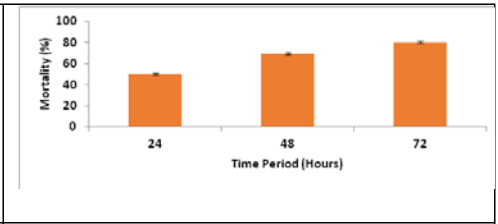

Fig. 2 Comparative effect of plant extracts on the mortality of *C. chinensis* at different time exposure

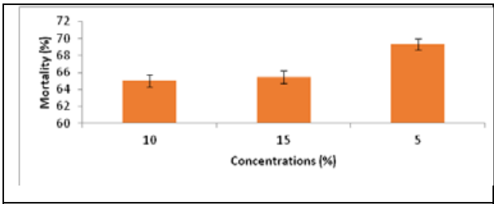

Fig. 3 Comparative effect of plant extracts on the mortality of *C. chinensis* at different concentrations

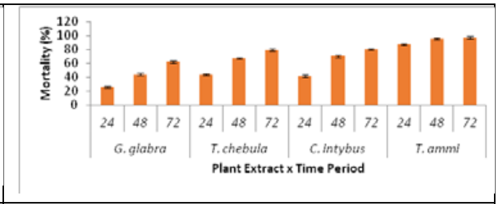

Fig. 4 Impact of interaction of plant extracts and time period on adult mortality of *C. chinensis*

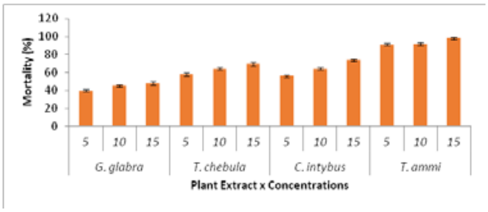

Fig. 5 Impact of interaction of plant extracts and contraptions on adult mortality of *C. chinensis*

Effect of Anisopteromalus calandrae on parasitism (%) of Callosobruchus chinensis

With respect to biological control, the response of *A. calandrae* was also observed. *A. calandrae* was released in three different treatments with 5, 10 and 15 pairs of the parasitoids on *C. chinensis*, and observations were made after 15 and 45 days.

After 15 days of Host Emergence

Results showed that impact of treatments of host insects to parasitoid has a significant effect on adult emergence of *C. chinensis*. Results in Table 1 show the mean comparison of adult emergence of *C. chinensis* in the presence of parasitoid. The finding revealed that the effect of parasitoid on adult emergence differed significantly. Minimum percent adult emergence (699.00) was observed with the release of fifteen pairs of parasitoid and it was statistically different from the other treatments. Maximum of percent adult emergence (1558.7) was observed in control. The trend of adult emergence in respect of parasitoid was mentioned in order to Fifteen pairs < Ten pairs < five pairs < Control.

After 45 days of Host Emergence

Results regarding after 45 days of host emergence revealed that the effect of parasitoid on adult emergence differed significantly among treatments (Table 1). Minimum percent adult emergence (17536.00) was observed with the release of fifteen pairs of parasitoid and it was statistically different from others. Maximum of adult emergence (36754) was observed in control. The trend of adult emergence in respect of parasitoid was mentioned in order to Fifteen pairs < Ten pairs < five pairs < Control.

Tab. 1 Comparative effect of different treatments on parasitism (%) of adults of *C. chinensis* after 15 and 45 days of host emergence

Treatment	Parasitism (%) After 15 days	Parasitism (%) After 45 days
Control	1558.7 a	36754 a
5 pairs	1132.0 b	31893 b
10pairs	961.7 c	24165 c
15 pairs	699.0 d	17536 d

Discussion

Overall results revealed that maximum percent mortality 93.65% of adults of *C. chinensis* was recorded with *T. ammi* and minimum 44.42% was observed with *G. glabra*. These results are in line with the findings of Pereira *et al*. (2008) who reported that the oils of *Piper aduncum*, *Lippia grcillis* and *Cymbopogon martinii* gave 100% mortality against *Callosobruchus maculatus*. Moreover, similar results of several plant extracts have been observed by Shimizu and Hori (2009) against *Callosobruchus maculatus*, while other studies show good efficacy of certain plant extracts for the control of *Callosobruchus* spp. (Roberfroid and Slavin, 2000; Wang *et al.*, 2003; Pandey *et al*., 2007).

Results regarding parasitism effect showed that after 15 days of host emergence, minimum adult emergence (699.00) was observed with fifteen pairs of parasitoid and maximum adult emergence (1558.7) was observed in control. At the same time, after 45 days of host emergence, minimum adult emergence (17536) was observed with fifteen pairs of parasitoid and maximum adult emergence (36754) was observed in control. However, from these results it was concluded that maximum parasitism was achieved at highest number of pairs of *A. calandrae* and after highest time interval (45 days) while at lower number of pairs and time interval test insect percent mortality was not sufficient. Our findings are also related to Utida (1943) who conducted as series of experiments with the same host-parasitoid complex, and found that the species can coexist for 50 generations. He described that *A. calandrae* showed functional response of type III. Our results are also in accordance with the findings of Ngamo *et al.* (2007) who reported a significant reduction in progeny emergence of *C. chinensis* due to the presence of *A. calandrae.* Previous studies have also provided similar findings of the effect of *A. calandrae* against *Rhyzopertha dominica*, *Sitophilus oryzae*, *Lasioderma serricorne* and *Tribolium confusum* (Mahal *et al.*, 2005, Ghrimire and Phillips, 2007, Belda and Riudavets, 2010).

From these results it is concluded that the use of plant extracts and bio-control agents could be a better alternative to our conventional synthetic insecticides and could be an integral part of stored grain IPM programs.

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Detection of hidden insect *Sitophilus oryzae* in wheat by low-field nuclear magnetic resonance

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Abstract

Insects, either adults or larvae, living inside grains are difficlut to detect but can cause enormous loss of grain. Therefore, we explored the use of low-field nuclear magnetic resonance (LF-NMR) techniques to detect *Sitophilus oryzae* hidden inside wheat. Significant difference in transverse relaxation times (T_2/ms) and the T_2 components proportion (P_2/%) was observed between wheat and *S. oryzae* at its four different growth stages (small larvae, large larve stage, pupal stage and adult stage). The transverse relaxation signals on the infested