Abstract

Plant materials have been used for pest control for centuries but recently, preservation of cereals products in storage has relied upon chemical insecticides to control stored grain pests but having drawbacks of toxicity to non-target organisms, human health hazards, development of pest resistance and environmental pollution. The present study was conducted to control the serious cereal’s (wheat) stored grains pest Tribolium castaneum Herbst. Calotropis proceras Aiton (Ak) and Datura alba (Dhatura) have been reported by many researchers as natural insecticides against stored grain pests. Five concentrations of leaf extracts of C. sprocera and D. alba (20%, 40%, 60%, 80% and 100%) were compared to see their efficiency to control Tribolium castaneum. Different parameters like repellency, growth inhibition, mortality rate, infestation/spoilage of grains, insect population and losses in grain weight were tested after application of treatments. Three months storage of wheat grains showed maximum repellency, mortality at higher concentrations of C. sprocera and D. alba, but there was less infestation/spoilage, loss in grain weight and insect population at higher concentrations (80%, 100%).

Keywords: Tribolium castaneum; Triticum Aestivum; Stored wheat; Bio-pesticide; Calotropis Proceras; Datura alba

Introduction

Loses due to insect infestation are the most serious threat in grain storage, particularly in developing countries, where poor sanitation and inappropriate storage facilities encourage insect attack [1,2]. It was estimated that more than 20,000 species of field and storage pests destroy approximately 1/3rd of the world’s food production, valued more than US$ 100 billion annually, among which the highest losses (43% of potential production) occur in developing Asian and African countries [3]. In Pakistan it has been estimated that 5 to 7% loss of food grains occurs due to poor storage conditions [4]. Tribolium castaneum is found in stored grains of different cereals. Control of this insect relies mainly on the use of synthetic insecticides and fumigants that lead to problems such as disturbances of environment, increasing cost of application, pest resurgence, resistance to pesticides and lethal effect on non-target organisms in addition to direct toxicity to users [5]. Tissues of plants from several families contain chemical compositions that are considered as defensive substances against their enemies. They include oils, alkaloids, organic acids and other compounds [6]. The insecticidal and acaricidal properties of number of plants have been discovered long ago, and some of the plants can compete with synthetic means of control [7]. To minimize the use of pesticides and to avoid environmental pollution, natural antifeedant, deterrent and repellent substances have been searched for pest control during recent times [8,9] reported the successful results of crude extracts of C. procera and D. alba against the termites in the sugarcane crop. Calotropis Proceras and Datura alba have been reported by many researchers as natural insecticide against stored grain pests [10,11]. There is a possibility to develop a method of stored product protection without or with reduced use of synthetic chemicals [12]. Present study was undertaken to check the efficacy of Calotropis Proceras and Datura alba extracts against the Tribolium castaneum in stored wheat. The leaves of these medicinal plants, growing under regional environmental conditions were used to investigate their potential in controlling insect infestation. Alam et al., [13] reported significant insecticidal activity of root bark of Calotropis gigantea and its chloroform and petroleum ether solutions against larvae and adults of Tribolium castaneum.

Materials and Methods

The study was conducted in Entomological Laboratory of College of Agriculture, Dera Ghazi Khan, Pakistan during the year 2010. Adult Tribolium castaneum were collected from food storage Godowns of Punjab Food Department and from local grain markets of D. G. Khan District of province Punjab, Pakistan.

Rearing of Insects

Insects were reared in already damaged grains of local wheat variety (Triticum Aestivum L.) under controlled conditions of 27 ± 1°C temperature and 55 ± 5% RH in the growth chamber. Each three liter jar was filled with 800 gm of wheat flour and damaged wheat grains and about 150 beetles were added to each jar. The jars were covered with muslin cloth, tied with rubber bands to avoid the escape of beetles and to prevent any unwanted entry. The insects were placed in the chamber for two months for rearing. Adults of first generation were shifted to next jars for collection of eggs and these eggs were further transferred to damaged grains in new jars. Adult insects were shifted to the experimental and control jars for data recording.

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Preparation of Botanical Extracts

Mature plant leaves of C. procera and D. alba were collected from surrounding villages of D.G. Khan District, dried for 3–4 days under shade and then was chopped into 2 cm pieces with electric fodder cutter (local made). Chopped leaves were soaked in distilled water in ratio of 1:10 for 24 hours [14]. The extract was filtered with the help of fine sieve of 10 and 60 meshes gradually and boiled at 100°C to evaporate water to decrease volume up to 20 times. The grains were treated with concentrations (20%, 40%, 60%, 80% and 100%) of botanical extract solution. Insects were released in jars @ 16 insects/100 g of wheat grains [15]. Jars were covered with muslin cloth and rubber band.

Preparation of different concentrations

From a stock solution with 100% concentration, different concentrations (20%, 40%, 60%, 80% and 100%) of D. alba and C. sprocera were prepared. Total volume of water required for pasting the grains was 100 ml.

1. 20 ml botanical extract + 80 ml distilled water
2. 40 ml botanical extract + 60 ml distilled water
3. 60 ml botanical extract + 40 ml distilled water
4. 80 ml botanical extract + 20 ml distilled water

Each concentration was replicated thrice in experimental jars. For repellency studies, these concentrations were applied to a filter paper in the petri dishes. Data regarding bio-insecticides applied to grains was obtained after three months storage in experimental jars while for repellency studies, these concentrations were applied to a filter paper strip.

Infestation/spoilage (%)

Infestation of stored wheat grains by T. castaneum was measured by calculating the percentage grains weight loss. At the end of trials completion after three months, C. procera and D. alba treated jars with different concentrations 20%, 40%, 60%, 80% and 100% of leaf extracts containing wheat samples, were weighed and compared with control samples.

Loss in grain weight (gm)

Loss in grain weight caused by T. castaneum was measured by calculating the difference between grain weight before releasing the insects and after trial completion in C. sprocera and D. alba treated jars containing wheat samples, at different concentrations 20%, 40%, 60%, 80% and 100% of leaf extracts containing wheat samples was weighed and compared with control samples.

Mortality Percentage (%)

The mortality of T. castaneum was measured by using following formula.

\[
\text{% age mortality} = \frac{\text{No of insects alive intest } \times 100}{\text{No of insects alive in control}}
\]

Mortality of Tribolium castaneum was measured after releasing the specific no. of insects in the treated jars of wheat grains of C. procera and D. alba at different concentrations 20%, 40%, 60%, 80% and 100% of leaf extracts, and also no. of insects in the control wheat grain jars.

Insect population count

Insect were counted by releasing a specific number of insects in treated wheat grains along with control. The effect of C. procera and D. alba was observed at their different concentration level of 20%, 40%, 60%, 80% and 100% of leaf extracts.

Repellency studies

Repellency studies were carried out by using paper strip method. Filter paper (Whatmann No. 1) was cut into strips of 8x10 cm. Half filter paper strips was treated with 1 ml of extract and was allowed to air dry for 10 minutes. Each treated half strip was then attached lengthwise, edge-to-edge, to a control half strip with adhesive tape and was placed in a petri dish. Twenty adult insects were released in the middle of each filter paper circle in a petri dish. Insects settled on each half of the filter paper strip were counted continuously for 5 hours with the interval of 1 hour. Repellency against red flour beetle on the basis of time interval of one hour at different concentrations (20%, 40%, 60%, 80% and 100%) of C. procera and D. alba were examined.

Result and Discussion

The Data presented in table 1 shows that number of alive adult T. castaneum in control treatments were maximum (40) and at 20% concentration, no. of alive individuals were (36) and (37) of C. procera and D. alba leaf extracts respectively and at 40% concentration, no. of alive insects were (30.33) and (34) of C. procera and D. alba leaf extracts respectively. At 60% concentration, no. of alive insects were (23.33) and (27.67) of C. procera and D. alba leaf extracts respectively and at 80% concentration, no. of alive insects were (20) and (19.33) of C. procera and D. alba leaf extracts respectively. However at 100% concentration of C. sprocera and D. alba, numbers of alive insects were (26.94) and (29.67) respectively. It is clear from the given data that the no. of insects alive in C. procera treated jars was less than D. alba as compared to control.

Loss in grains weight

The data presented in table 2 shows that maximum loss in grain weight was observed at control (51 and 47.33) caused by T. castaneum. Minimum grain weight loss was observed at 60% concentration of C. procera and D. alba (5) and (7.67) respectively and at 40% concentration, loss in grain weight caused by T. castaneum was (23.67)

| Concentrations | C. sprocera | D. alba | Total |
|----------------|-------------|---------|-------|
| Control        | 40a         | 40a     | 40a   |
| 20 %           | 36b         | 37ab    | 36.5b |
| 40 %           | 30.33cd     | 34bc    | 32.17c|
| 60 %           | 23.33e      | 27.67d  | 23.5d |
| 80 %           | 20ef        | 19.33f  | 29.67e|
| 100%           | 12g         | 20ef    | 16f   |
| Total          | 26.94b      | 29.67a  | 29.67a|

Table 1: Means comparisons for the insects count of Tribolium castaneum at different concentration of C. sprocera and D. alba leaf extract

| Concentrations | C. sprocera | D. alba | Total |
|----------------|-------------|---------|-------|
| Control        | 51a         | 47.33a  | 49.17a|
| 20 %           | 30.67b      | 33.33b  | 32b   |
| 40 %           | 23.67bc     | 12cdef  | 21c   |
| 60 %           | 5f          | 7.67ef  | 17.83c|
| 80 %           | 18.33bdf    | 23.67bc | 16.67c|
| 100 %          | 11.33def    | 22bdc   | 6.33d |
| Total          | 23.33a      | 24.33a  | 24.33a|

Table 2: Means comparisons for Loss in grains weight at different concentration of C. sprocera and D. alba leaf extract caused by Tribolium castaneum
and (12) of C. procera and D. alba leaf extracts respectively. At 60% concentration, loss in grain weight caused by T. castaneum were (5) and (7.67) of C. procera and D. alba leaf extracts respectively and at 80% concentration, loss in grain weight caused by T. castaneum were (18.33) and (23.67) of C. procera and D. alba leaf extracts respectively. At 100% concentration, loss in grain weight was (11.33) and (22) in C. procera and D. alba treated jars respectively. Comparatively loss in grain weight caused by T. castaneum was less in C. procera treated jars than D. alba treated jars.

### Infestation/ Spoilage of grains

The data presented in table 3 shows that maximum infestation/spoilage of grains (20.49, 18.93) was observed in control treatments. Minimum infestation/spoilage (2, 2.8) was observed at 60% concentration of C. procera and D. alba respectively and at 40% concentration, infestation/spoilage of grains caused by T. castaneum were (9.5) and (3.5) of C. procera and D. alba extracts respectively. At 60% concentration, infestation/spoilage of grains caused by T. castaneum were (2) and (2.8) of C. procera and D. alba leaf extracts respectively and at 80% concentration, infestation/spoilage of grains caused by T. castaneum were (7.33) and (10.8) of C. procera and D. alba leaf extracts respectively. At 100% concentration infestation/spoilage was (4.53) and (8.8) in C. procera and D. alba treated jars respectively. Comparatively infestation/spoilage of grains was less in C. procera treated jars than D. alba treated jars.

### Mortality Percentage

The data presented in table 4 shows that maximum mortality of T. castaneum (70, 50) was observed at 100% concentration of C. procera and D. alba respectively. Minimum mortality of T. castaneum (0.0) was observed at control treatments.

Mortality of T. castaneum at 20% concentration was 10 and 7.5 of C. procera and D. alba leaf extract respectively, and at 40% concentration, mortality of T. castaneum were (24.17) and (15) of C. procera and D. alba leaf extracts respectively. At 60% concentration, mortality of T. castaneum were (41.67) and (30.83) of C. procera and D. alba leaf extracts respectively and at 80% concentration, mortality of T. castaneum was (70) and (50) in C. procera and D. alba treated jars respectively. Comparatively mortality of T. castaneum was less in C. procera treated jars than D. alba treated jars. At 100% concentration of C. procera treated jars T. castaneum adults were found alive underside of the muslin cloth but not in the treated grains, similar results were observed for D. alba treated jars.

### Repellency Studies

The data presented in tables 5 and 6 show that maximum repellency of T. castaneum (16.97) was observed at 40% concentration of C. sprocera and D. alba. Minimum repellency of T. castaneum (6.97) was observed at 80% concentration while at 20% concentration of C. procera and D. alba leaf extracts, repellency of T. castaneum was (10.07) respectively. At 60% concentration, repellency of T. castaneum were (41.67) and (30.83) of C. procera and D. alba leaf extracts respectively and at 80% concentration, repellency of T. castaneum were (50) and (51.67) of C. procera and D. alba leaf extracts respectively. At 100% concentration, repellency of T. castaneum was (14.67). Comparatively repellency of T. castaneum was less in C. procera (9.92) than D. alba treated (13.87) treatments.

The data packed in table 7 shows that maximum repellency of T. castaneum (15.93) was observed after 5 hour interval. Minimum repellency of T. castaneum (9.37) was observed after 1st hour interval. Whereas after 2nd hour interval, repellency showed by the T. castaneum was (10.13), while repellency in 3rd hour interval was (11.73) and 4th hour interval (12.3) was observed. The results also supported to clarify that with the passage of time after application of extracts, repellency increased and maximum repellency of T. castaneum (15.93) was observed after 5th hour.

| Concentrations | C. sprocera | D. alba | Total |
|----------------|-------------|---------|-------|
| Control        | 20.4a       | 18.93a  | 19.67a|
| 20 %           | 12.3b       | 13.33b  | 12.8b |
| 40 %           | 9.5bc       | 3.5ef   | 9.1c  |
| 60 %           | 2f          | 2.6ef   | 6.67c |
| 80 %           | 7.33cde     | 10.8bc  | 6.47c |
| 100%           | 4.53def     | 8.8bcd  | 2.4d  |
| Total          | 9.33a       | 9.69a   |       |

Table 3: Means comparisons for Infestation/Spoilage of grains at different concentration of C. sprocera and D. alba leaf extracts caused by Tribolium castaneum

| Concentrations | C. sprocera | D. alba | Total |
|----------------|-------------|---------|-------|
| Control        | 0           | 0       | 0     |
| 20 %           | 10f         | 7.5fg   | 8.75e |
| 40 %           | 24.17d      | 15ef    | 19.58d|
| 60 %           | 41.67c      | 30.83d  | 36.25c|
| 80 %           | 50bc        | 51.67b  | 50.83d|
| 100 %          | 70a         | 50bc    | 60a   |
| Total          | 32.63a      | 25.83b  |       |

Table 4: Means comparisons of Mortality Percentage of Tribolium castaneum at different concentration of C. sprocera and D. alba leaf extracts

![Image](image_url)

**Table 5:** Means comparisons for Repellency of Tribolium castaneum at different concentration of C. sprocera and D. alba leaf extract Table of Means

| Extracts | Means |
|----------|-------|
| D. alba  | 13.87a|
| C. sprocera | 9.92b|

**Table 6:** Repellency of Tribolium castaneum at different concentration of C. sprocera and D. alba leaf extract

| Time Interval | Means |
|---------------|-------|
| 1 hour        | 9.37d |
| 2 hour        | 10.13c|
| 3 hour        | 11.73b|
| 4 hour        | 12.3b |
| 5 hour        | 15.93a|

**Table 7:** Means comparisons for the Repellency of Tribolium castaneum at different concentration of C. sprocera and D. alba leaf extract with respect to time interval

Variance of Concentrations = 1.53

Variance of Time interval = 1.53

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