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Food consumption and utilization by *Tribolium confusum* du Val (Coleoptera: Tenebrionidae) larvae and their susceptibility to the acetone extract of *Nerium oleander* L. (Apocynaceae) leaves in relation to three types of flour

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ABSTRACT

The nutritional indices of *Tribolium confusum* larvae reared on wheat, barley and corn flour and their susceptibility to acetone extracts of *Nerium oleander* leaves were studied. In addition, the concentrations of total protein, carbohydrate and lipid in the flours and the larvae reared on them were also determined. Although the lowest consumption index and relative growth rate (RGR) were obtained in larvae reared on corn flour, these showed the highest weight gain. No significant difference was apparent between the three types of flour in terms of digestibility, or between the RGR of larvae reared on barley and corn flour. In contrast, the RGR of larvae reared on wheat flour was significantly higher than that for those reared on barley and corn flour. The highest food utilization, in terms of the efficiency of conversion of ingested and digested food into biomass, was reached in larvae reared on corn flour. Larvae reared on wheat and corn flour had the highest and lowest total protein contents, respectively, while larvae reared on corn and barley flour had the highest and lowest total lipid contents, respectively. On the other hand, no relationship was evident between larval and flour total carbohydrate content. The present study showed that larvae reared on corn flour were more tolerant to acetone extracts of *N. oleander* leaves than those reared on wheat or barley flour. The relationship between the total protein, carbohydrate and lipid contents in the flour and the larval nutritional indices, and also the susceptibility of larvae to the botanical extract, were discussed.

1. Introduction

The confused flour beetle, *Tribolium confusum* du Val, is one of the most common and destructive insect pests for flour and grain and other food products stored in silos, warehouses, grocery stores and houses. Moreover, *Tribolium* spp. secrete carcinogenic chemicals known as quinones when they occur in large numbers (Hodges et al., 1996). The suitability of locally available plant extracts as alternative control agents to synthetic chemicals is being widely researched and extracts of *Nerium oleander* L., a common plant in the Mediterranean region, have shown potential in this respect (El-Shazly et al., 2000; Hameed et al., 2012).

The quality of the breeding medium can influence fitness of the insects reared and the toxicity of applied insecticides (Cichy, 1971; Longstaff, 1995; Casadío and Zerba, 1996; Xue et al., 2010). This study therefore had the following objectives:

(I) Evaluating the suitability of wheat, barley and corn flour as rearing media for the development of *T. confusum* larvae by determining the nutritional indices of larvae relative to each diet.
(II) Exploring the relationship between the nutritional values (total protein, carbohydrate and lipid contents) of wheat, barley and corn flour and the nutritional indices of *T. confusum* larvae.
(III) Elucidating the relationship between the total protein, carbohydrate and lipid contents accumulated in *T. confusum* larvae, due to feeding on wheat, barley and corn flour, and their susceptibility to acetone extracts of *N. oleander* leaves.

2. Materials and methods

2.1. Insects

Adults of *T. confusum* were collected from a flour store at Misurata city, Libya, and identified following the taxonomic characters

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given by Freeman (1980). The flour used was previously sifted through a 250 μm opening sieve and autoclaved at 60 °C for 90 min. Twenty grams of wheat, barley and corn flour were separately put in three round plastic containers (150 mL). Each container was supplied with 50 adults of mixed age and sex and tightly closed with a muslin cloth held in position by a rubber band. The containers were held in dark incubators at 28 °C and 65% r.h. for 3 d after which the adults and eggs were removed by sieving. A 840 μm sieve retained the adults, allowing flour and eggs to pass through, while a 250 μm sieve retained eggs. Eggs were placed in 9 cm glass Petri dishes and held at 28 °C and 65% r.h. and examined daily for hatchability.

2.2. Nutritional indices

Wheat, barley and corn flour of a known weight were offered respectively to three batches of newly-moulted last instar larvae of *T. confusum* of a known weight until prior to pupation (late last larval instars). Each batch consisted of 30 larvae. The larvae were confined in plastic containers (15 cm in diameter) covered with perforated covers. Every day the surviving larvae, faeces excreted and the remaining food were weighed. The experiment was incubated, in darkness, under controlled conditions as previously mentioned. Four replicates were set up. The nutritional indices of the larvae were calculated according to the equations given by Waldbauer (1968) as follows:

- Consumption index (CI) = F/TA,
- Relative growth rate (RGR) = G/TA,
- Approximate digestibility (AD) = $(F - E/F) \times 100$,
- Efficiency of conversion of ingested food into biomass (ECI) = $(RGR/CI) \times 100 = (G/F) \times 100$,
- Efficiency of conversion of digested food into biomass (ECD) = $(G/F - E) \times 100$.

Where:

A = Mean weight of insect during the feeding period, E = Weight of faeces, F = Weight of food eaten, G = Weight gain at the end of the feeding period and T = Duration of the feeding period.

2.3. Preparation of stock extract and larvicidal bioassay

Nerium oleander leaves were collected from a park at Misurata city. The leaves were thoroughly washed with distilled water and shade dried for a week. Then they were finely ground in a small electric kitchen mill. The resulting powder was soaked in acetone, as a solvent, for 48 h in a tightly sealed conical flask (1 L) at a ratio of 1:4 (w/v). The solution was occasionally stirred, using a magnetic stirrer, and the crude extract was extracted using soxhlet apparatus. The extract was then allowed to evaporate in a rotary vacuum apparatus. The collected concentrated extract (stock extract) was lyophilized and stored in a refrigerator till use.

For the experiments aliquots of the stock extract of *N. oleander* leaves were dissolved in acetone solution (w/v). Three batches of newly-moulted last instar larvae of *T. confusum* were set up. Each batch comprised of 30 larvae. The batches were fed respectively on wheat, barley and corn flour. Late last larval instars in each batch were topically treated along the dorsum with 0.5 μL of extract acetone solution using a micropipette (Socorex 841). Five to six concentrations of the extract in acetone were applied: 0.5, 1.5, 4.0, 8.5 and 26.0 μg/mL for larvae reared on wheat flour; 1.0, 1.5, 4.5, 15.0 and 35.0 μg/mL for larvae reared on barley flour; 0.5, 1.5, 2.0, 10.0, 15.0 and 50.0 μg/mL for larvae reared on corn flour. Each concentration was replicated four times. Treated larvae were held in plastic containers, as above. A parallel control of larvae treated with acetone only was also run. The experiment was carried out under the controlled conditions mentioned above. The mortality count was made 24 h post-treatment.

2.4. Determination of total protein, carbohydrate and lipid content

Three batches of newly-moulted last instar larvae of *T. confusum* were prepared, as described above, and were fed on the three types of flour. The larvae were incubated under the same previously mentioned conditions. Then, the total protein, carbohydrate and lipid contents in late last larval instars and their rearing media were estimated as follows:

2.4.1. Total protein content

Protein extraction was made by 0.1 N NaOH and estimated by the Folin-phenol method (Lowry et al., 1951) for soluble proteins using albumin as a standard. Total protein content was measured spectrophotometrically, using a Schimadzu UV-160 spectrophotometer, at 750 nm.

2.4.2. Total carbohydrate content

Total carbohydrate content was estimated by the method of Singh and Sinha (1977) using anthrone reagent and glucose as a standard. The content was measured spectrophotometrically at 620 nm.

2.4.3. Total lipid content

Lipid was estimated by sulphophosphovanilun method (Barnes and Blackstock, 1973) using cholesterol as a standard. Samples were extracted with chloroform – methanol (2:1) (v/v). Total lipid content was measured spectrophotometrically at 540 nm.

All experiments were replicated four times. The content was expressed as μg/mg fresh larval body wt. or μg/mg flour.

2.5. Statistical analysis

For larvicidal bioassay of the botanical extract, insect mortalities were corrected by Abbott's formula (Abbott, 1925). Median lethal concentrations were calculated using probit analysis (Finney, 1971) and were expressed as mg/mL acetone solution.

The data obtained from determination of nutritional indices and those of total protein, carbohydrate and lipid contents were statistically analyzed using one-way analysis of variance (ANOVA) (SAS Institute, 2002). The equation of the standard error, *t*-statistic values and probabilities (P) were used. Statistical significance was set at $P < 0.05$ for all analyses.

3. Results

Table 1 shows that in spite of the lowest CI obtained in *T. confusum* larvae reared on corn flour; the highest weight gain was attained. Conversely, although the highest CI occurred in larvae reared on barley flour, the lowest weight was gained. The same pattern was found for food utilization efficiencies (ECI and ECD), as they were inversely proportional to CI. The highest values of ECI and ECD were achieved in larvae reared on corn flour and the lowest in larvae reared on barley flour. No significant ($P > 0.05$) differences were obtained for the means of AD among the three types of flour, or between the means of RGR of larvae reared on barley and corn flour. However, the mean RGR of larvae reared on wheat flour was significantly higher than that due to rearing on barley and corn flour and there was some consistency between the RGRs and weight gain of larvae fed on wheat and barley flour.