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Insecticidal efficacy of *Chrysanthemum cinerariaefolium* Seed Oil Against Cowpea Beetle and Its Impact on the Physicochemical and Technological Characteristics of Cowpea during Storage

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ABSTRACT

Essential oil from *Chrysanthemum cinerariaefolium* seeds was extracted using a soxhlet extractor for 48 hours. The oil extract's insecticidal activity against adult cowpea beetles (Callosobruchus maculatus) was investigated at concentrations of 0.01, 0.03, 0.05, 0.1, and 0.18%. Data showed that seed extract caused significant insect mortality. The mortality percentage reaches 100% after three days of exposure at a concentration of 0.18% of the extract. Data was carried out on the effect of oil on some biological aspects of *C. maculatus* (total number of eggs, egg hatchability, adult emergence, and reduction percentage). The LC_{95} (0.4%) of *Chrysanthemum* oil was assessed for physicochemical and technological characteristics during a storage period of 12 weeks. The treated cowpea seeds showed an unchanged chemical composition at zero time. During storage periods, the tested treatment reduces the rate of decreasing length, width, lightness, and redness. The 1000-weight seed and yellowness value were higher in treated seeds than in untreated seeds. A decrease in the cooking time and total soluble solids of treated cowpea seeds during storage. While the water uptake ratio, hydration and swelling capacity, the moisture, protein content and in vitro protein digestibility of treated cowpea seeds were higher than those of the untreated at the end of storage, the treated cowpea seed samples were acceptable in all tests of sensory acceptability. This study recommended using *Chrysanthemum* seed oil (0.4%) for management of the *C. maculatus* population while maintaining the nutritional and technological quality of cowpea seeds during storage and avoiding risks from chemical insecticides.

1. Introduction

Pulses (legumes) are very valuable biologically and nutritionally. They play a major role in improving malnutrition in developing countries. Pulses are a good source of protein and have been added to human meals due to their favorable and rich content of dietary proteins, fiber, vitamins, and some favorable phytochemicals (Yanni et al., 2024). Cowpea is considered an inexpensive source of protein for poor people, whose habits largely depend on starchy foods such as wheat, maize, sorghum, millet, and cassava, making it an excellent crop to contribute to the reduction of nutritional deficiency with a mean protein content of 25.4%, 1.8% fat and 63.3% carbohydrates, and a good supply of vitamins and phosphorus (Horn et al., 2022 and Hisseine et al., 2023). Egypt produced 7216 tons of cowpeas (FAOSTAT, 2021). While the risk and scope of that damage increase as long as the cowpea seeds are stored, the producers have limitations in technologies for reducing storage loss (Kadjo et al., 2013). The majority of common ways of storing seeds normally do not provide safeguards against insect pest

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infestations (Gitonga et al., 2013) and have been stated to lead to significant seed damage of approximately 25 percent of the harvested cowpea seeds (Moussa, 2006). While the chemicals (insecticides) used to control insects are so expensive, they are commonly exposed to abuse, leading to economic, environmental, and health concerns.

Callosobruchus maculatus, commonly known as the cowpea beetle, is considered to be one of the most important insect pests during cowpea storage (Deshpande et al., 2011). This species is capable of damaging up to 100% of the seeds of pulses (Gbaye et al., 2011) during 45 to 90 days of storage at an optimal temperature of 30±1 C and relative humidity (RH) conditions of 75±3%. The neonate larvae pass through the seeds, leading to major harm resulting in seeds losing weight, decreased germination ability, a decline in the nutrient content (Oke and Akintunde, 2013), and the cowpeas becoming inappropriate for consumption (Elhag, 2000). Adults of certain insect species attack pulse seeds and cause severe damage, reducing the quality and quantity of nutrients in stored pulses (Kedia et al., 2015). The chemicals in insecticides can kill the intended insect and non-intended organisms, such as useful organisms. Also, they accumulate in food and numerous human body systems and lead to cancer. Additionally, the insect will have the ability to resist some kinds of chemical pesticides. Hence, it is necessary to assess the components of natural sources as biopesticides due to their safety to both humans and the environment (Shawkat et al., 2011). Currently, synthetic pesticide fumigants provide an alternative method of protecting food, feed for animals, stored crops, and other agricultural commodities from insect damage (Ghorab and Khalil, 2016). However, continuous or excessive use of synthetic insecticides causes many problems, including decreasing ozone layer potential, air pollution, increasing the cost of use, residues of pesticides in the environment, and food products (Ccanccapa et al., 2016). Nowadays, immediately environmentally friendly solutions have become available, such as the use of biopesticides (Shimira et al., 2021). For the long-term storage of cowpeas, essential oils derived from plants and their constituent parts have been regarded as a valuable natural resource that offer safe substitutes for pesticides. They can be used to create novel fumigants, and their quick degradation and local availability may make them superior to standard fumigants (Ilboudo et al., 2015 and Chaudhari et al., 2021). *Chrysanthemum* is considered a good source of essential oils owing to its containing a group of essential compounds that have various effectiveness and advantages for health, as it can be utilized as food, medicine, antibacterial, anti-inflammatory, immune regulation, antioxidation, antitumor, and regulation of cardiovascular function, etc. (Ren et al., 2018 and Shao et al., 2020). *Chrysanthemum* can be used as a spice in food and can be added to several food products and medicines as a natural additive for preservation, comprising antioxidants (Youssef et al., 2020).

Chrysanthemum cinerariaefolium, a genus of *chrysanthemum* in the Asteraceae family, is one of the plants that has the ability to produce the insecticide pyrethrum (Li et al., 2014). (Yunos et al., 2024 and Haouas et al., 2013) explored the use of *Chrysanthemum* leaves oil in producing organic insect repellents, while (Haouas et al., 2013) demonstrated the insecticidal effect of essential oils on stored products (grains) against *T. confusum*a dults. There are many studies on the effect of using essential oils from different sources against many insects in stored cereals and pulses, but there are limited studies to evaluate the effect of using these essential oils on the nutritional and technological properties of stored cereals and pulses treated with essential oils. Therefore, it was necessary to evaluate the use of essential oil on the nutritional, technological and sensory quality of cowpea seeds during storage.

The present study aims to evaluate the efficacy of *Chrysanthemum cinerariaefolium* seed oil extract against cowpea beetles (*Callosobruchus maculatus*) and their effects on the physicochemical and technological characteristics of cowpea during storage periods of 12 weeks.

2. Materials and Methods

Materials

Insect cultures

The cowpea beetle species, *Callosobruchus maculatus*, was reared on the seeds of cowpeas in the Stored Grains Pest Research Department, Plant Protection Research Institute, Agriculture Research Center (ARC), Giza, Egypt. Adult male and female insects were placed in each jar to lay eggs and covered with muslin with a rubber band to prevent insect escape. The jars that contained insects were kept incubated at 28±2°C and 75±5% RH. for one week. Then the parent adults were sieved out and discarded. Cowpea seeds (Kareem 7 variety) were obtained from the Agricultural Research Center in Giza, Egypt. *Chrysanthemum* seeds from the nursery of the Ministry of Agriculture, Dokki, Giza, Egypt. Pepsin and pancreatin enzymes were obtained from Sigma-Aldrich Chemical Co., St. Louis, USA. All the chemicals used were of analytical grade.

Methods

Chrysanthemum oil extraction

Chrysanthemum oil was extracted from the seeds. 50 g of the powder and 0.5 liter of petroleum ether (40°C) were used to extract the oil using a Soxhlet extractor for 48 hours. This produced 50 milliliters of pure yellow oil, and a rotary evaporator was used to collect the ether. Until the oil was needed, it was stored at 4 °C in the dark, as described in (Sahile et al., 2012).

Evaluation of the insecticidal efficiency of *Chrysanthemum* oil on *C. maculatus* in cowpea seeds

Ten grams of disinfested cowpea seeds were kept in each glass jar and sprayed with tested percentage concentrations (0.0, 0.01, 0.03, 0.05, 0.1, and 0.18%) of the tested oil. To make sure the cowpea seeds were evenly coated with the tested oil, each jar was shaken for 2 minutes. The treated seeds were allowed to dry, and then twenty newly emerged adults of *C. maculatus were* released into each glass jar. The jars were covered with nylon mesh and held in place with rubber bands. The treated jars were kept at an ambient temperature of 28 ± 2 oC in the incubator. The insect mortality data was recorded after 1, 2, 3, 5, and 7 days post-exposure to different concentrations of tested oils. Three replicates of both treated and untreated grains were used.

Residual activity of the insecticidal efficiency of *Chrysanthemum* oil on *C. maculatus* adults after different storage periods

Tubes measuring 1x3 inches with 10g in each of the tested cowpeas treated with LC_{95} concentrations of *Chrysanthemum* oil were divided into several groups. Each group consists of three replicates. Following the cowpea and *chrysanthemum* oil mixture, the tubes were stored in a laboratory. Treated cowpea seeds were stored for two weeks. 25 adults of *C. maculatus* were placed in each of the three replicates, then covered with muslin cloth secured with elastic bands and kept under laboratory conditions.

Mortality counts were carried out at 2 days post-treatment for each 2 weeks. Three replicates of untreated seeds were used as controls. The infested-treated seeds with eggs were kept under laboratory conditions and observed regularly until adult emergence. The total number of emerged offspring was recorded after seven weeks, and the percent reduction in progeny was calculated according to the following equation (1):

$$\text{Reduction in progeny (\%)} = \frac{\text{No. of adults emerged in control.}}{\text{No. of adults emerged in treatment.}} \times 100 \quad (1)$$

Effect of *Chrysanthemum* oil on some biological aspects of *C. maculatus*

Number of eggs, hatchability and oviposition

To estimate the number of eggs laid by mated female adults of *C. maculatus* exposed to *Chrysanthemum* oil, glass tubes measuring 1 × 7.5 cm contained 5 gm of cleaned cowpea seeds treated with LC_{50} (0.04%) and LC_{95} (0.4%) of Chrysanthemum oil (for insects) were placed in glass tubes measuring 1×7.5 cm in order to estimate the number the number of eggs by mated female adults of *C. maculatus* exposed to the oil. Each treatment had three replicates. Two pairs of newly emerged adults (18 hours old) were put in each tube, coated with a plastic covering, and kept in the incubator until their deaths. After ten days, the insects were removed, and the number of hatched and non-hatched eggs was counted and recorded, and hatchability was determined in addition to the reduction in hatchability. Non-hatched eggs are transparent and glossy, but hatched eggs are distinguished by the presence of food waste, which turns the egg milky white as newborn larvae burrow into the seed or black color, which was classified as the larva's head capsule. (Giga and Smith, 1987). Thus, the total numbers of white (or black) and translucent eggs on the seeds indicate bruchid oviposition, and the numbers of white eggs indicate the number of larvae entering the seed (Dharmasena et al., 2001). The hatchability of eggs was indicated when they turned white, showing that eggs had hatched and larvae had penetrated the seeds (Ahmad et al., 2001). Percentage hatchability and the reduction in hatchability percentages were recorded with the following equations: 2 and 3.

$$\text{Hatchability (\%)} = \frac{\text{No. of hatched eggs}}{\text{Total no. of eggs}} \times 100 \quad (2)$$

$$\text{Reduction in hatchability (\%)} = \frac{\text{No. hatched eggs}_{\text{untreated}} - \text{No. hatched eggs}_{\text{treated}}}{\text{No. hatched eggs}_{\text{untreated}}} \times 100 \quad (3)$$

The collected cowpea seeds bearing deposited eggs were placed in clean glass tubes and kept in an incubator under constant conditions till progeny emergence.

Percentage adult emergence

The infested seeds treated with eggs were incubated and observed regularly until adult emergence. Emerging adults were counted and discarded for a period of 10 days (equation 4). The adult emergence percentage was calculated from the number of hatched eggs and F_1 adult progeny, and the reduction in F_1 progeny was also calculated from equation (5).

$$\text{Adult emergence (\%)} = \frac{\text{Total no. of emerged adults}}{\text{Total no. of hatched eggs}} \times 100 \quad (4)$$

$$\text{Reduction in adult (\%)} = \frac{\text{No. emerged eggs}_{\text{untreated}} - \text{No. emergedeggs}_{\text{treatment}}}{\text{No. emerged eggs}_{\text{untreated}}} \times 100 \quad (5)$$

The lethal concentration LC_{95} (0.4%) of chrysanthemum seed oil was calculated using the LDP line program., which is the best concentration to protect the cowpea seeds from cowpea beetle (*Callosobruchus maculatus*) infection during storage. The cowpea seeds were treated with this percentage (0.4%) of *chrysanthemum* seed oil and evaluated for nutritional, sensory, and technological characteristics compared with untreated (control) seeds during storage.

Proximate Analysis of Cowpea Seeds

Moisture, protein, fat, crude fiber, and ash contents of the cowpea seeds before and after treatment with *Chrysanthemum* seed oil were determined according to (AOAC 2019). Nitrogen content was determined by the Kjeldahl method using a factor of conversion of 6.25. The total carbohydrate content was calculated by subtracting the contents of protein, fat, ash, and crude fiber from 100 g of samples. The proximate composition values were averages from three replicates. Zinc, iron, magnesium, calcium and potassium contents were determined in samples according to the method outlined in the AOAC (2019) using the Agilent Technologies Microwave Plasma Atomic Emission Spectrometers (Model 4210 MPAES, USA). Phosphorus was determined by the colorimetric method of (Trough and Mayer 1929).

Physical Properties of Cowpea Seeds

Control and treated cowpea seeds with 0.4% *Chrysanthemum* seed oil were examined for physical properties.

Length and width Seeds: The width and length (mm) of randomly chosen seeds were measured using a Vernier caliper reading of 0.01 mm. Three replicates with an average of ten determinations each are reported (Milani et al., 2007).

1000-seed weight (g): The total weight of 1000-seeds of cowpea seeds was measured according to (AOAC 2019).

Color parameters: The external cowpea seed color was measured using a hand-held chromameter (model CR-400, Konica Minolta, Japan). Color parameters were expressed as the values of lightness (L^*), redness (a*), and yellowness (b^*). All measurements used three replicates to get an average.

Technological Evaluation

Cooking time

A standard hot plate laboratory was used to maintain uniform and stable temperatures during cooking time determination. 200 ml of distilled water was boiled in 500-mL spout-less beakers fitted with condensers to reduce water loss during cooking. 20 g of seeds from the control and tested samples were separately put into them. Boiling was continued, and samples (4–5 seeds) were withdrawn using a spatula at 5-min intervals, approximately 30 min, and then every 2 min, and checked for softness by pressing between finger and thumb as explained by (Singh et al., 1984). The time from the addition of seeds until the achievement of the desired softness was recorded as the cooking time.

Total Soluble Solids

5 g of cowpea seed samples were cooked for 40 minutes at 100°C (where it increased during storage) in 100 ml of distilled water. Following cooking, measurements of the total soluble solids (TSS) and water uptake ratio were taken (Wani et al., 2013). Total soluble solids (TSS) were detected by drying the cooking water containing soluble materials in an oven at 100°C for 16–18 hours. Following that, the residue was weighed and calculated as a percentage of the initial weight of the seeds before being cooked to calculate the total soluble solids percentage as shown in equation (6).

$$\text{Total soluble solids (TSS)\%} = \frac{\text{Weight of residue (g)}}{\text{Initial weight of seeds (g)}} \times 100 \quad (6)$$

Water Uptake Ratio (g/g): Previously cooked cowpea seeds were drained and weighed. The water uptake ratio was calculated using the following equation 7:

$$\text{Water uptake of cooked seeds (g/g)} = \frac{\text{(Weight of cooked seeds − Initial weight of seeds)}}{\text{Initial weight of seeds}} \quad (7)$$

Hydration and Swelling Capacities: The hydration (g/seed) and swelling (ml/seed) Capacities were determined according to (Adebowale et al., 2005) equations 8, 9

$$\text{Hydration capacity (g/ seed)} = \frac{\text{Weight after soaking} - \text{Weight before soaking}}{\text{Number of seed}} \quad (8)$$

$$\text{Swelling capacity (ml/ seed)} = \frac{\text{Volume after soaking} - \text{Volume before soaking}}{\text{Number of seed}} \quad (9)$$

***In vitro* Protein Digestibility (%):** *In vitro* protein digestibility was determined as the method of (Akeson and Stahmann 1964). 1 g sample was mixed with 15 ml of pepsin (1.5 mg pepsin, dissolved in 0.1 M HCl) and then incubated at 37°C for 3hrs. The suspension was neutralized with NaOH (0.2 M), then added 7.50 ml pancreatin (4 mg in 0.2 M phosphate buffer, pH 8.0) and then the mixture was shaken and incubated at 37°C for 24 hrs. The two samples were treated with 10% trichloroaceticacid and centrifuged for 20 min at room temperature at 5000 xg. The Protein in supernatant was determined using the Kjeldahl method (AOAC, 2019). The percentage of protein digestibility was calculated according to the following equation (10)

$$\text{IVPD (\%)} = \frac{(\text{N in supernatant} - \text{N in Blank})}{\text{N in sample}} \times 100 \quad (10)$$

Where, IVPD = *In vitro* protein digestibility, N= Nitrogen

Sensory Evaluation

The cooked cowpea samples were coded and given to ten member panelists from Food Technology Research Institute staff for evaluation using the method described by (Larmond 1977). Panelists were asked to score the cooked cowpea samples on a 9-point hedonic scale (9 = like extremely and 1 = dislike extremely) for the tested quality attributes (color, taste, texture, flavor, and overall acceptability).

Statistical Analysis

The computed percentage of mortality was plotted versus the corresponding concentrations using the LDP line software program to obtain the toxicity regression lines. The lethal concentrations LC_{50} and LC_{95} were statistically analyzed, and the variance ratios were calculated. The method of ANOVA involves using a SAS computer program. Means were detected and compared by Duncan multiple range tests at the 0.05 probability level (Duncan, 1955). The collected data of untreated and treated (with 0.4% *Chrysanthemum* seed oil) cowpea samples were statistically analyzed in triplicate, with the exception of the sensory evaluation (n = 10). The analytical data was presented as mean values and standard deviation. The obtained data were subjected to an independent t-test and analysis of variance (because it compares the means of two independent groups (control and treated cowpea with 0.4% *Chrysanthemum* seed oil) in order to determine whether there is statistical evidence or the two samples are different from one another) at $p<0.05$ by using SPSS version 21 (Elliott and Woodward 2007).

3. Results and Discussion

Evaluation of the *Chrysanthemum* oil treatment on adult *C. maculatus*

Data in Table 1. showed percentages of mortality of *C. maculatus* adults' post-exposure for various periods (days) on cowpea seeds treated with different percentages of *Chrysanthemum* oil.

Findings stated that the adult's mortality increased with increasing concentrations of oil. *Chrysanthemum* oil at a percentage of 0.01, 0.03, 0.05, 0.1, and 0.18% on cowpea seeds gave 26, 32, 54, 64, and 97% mortality, respectively, for *C. maculatus* adults after two days of exposure. The patients treated with 0.1% were capable of giving 100% mortality after five days of exposure; the percentage of 0.18% gave 100% mortality after three days post-exposure. This activity can be attributed to the composition of the tested essential oil.

Actually, a chemical analysis of essential oils obtained from *Chrysanthemum* species suggested the effective content of pyrethrin (Haouas et al., 2012). By the mechanism of pyrthrin, they enter the nervous system of the insect by penetrating its cutile when they come into contact with its body. This causes the nerve cells to stop functioning and the insect nervous system to close, which ultimately causes the insect to die (Venn et al., 2021).

C. cinerariaefolium contains pyrethrin, a potent natural insecticide that acts on insects' nerve systems systems to provide a "knockdown effect" (Kikuta et al., 2012).

Residual activity of *Chrysanthemum* oil

Table 2. indicated that the percentage mortality of *C. maculatus* adults exposed to *Chrysanthemum* oil started at 100% at the initial treatment and reached 70% at the 6 weeks of storage period. After that, the percentages of mortalities decreased gradually from 70 to 5%, as shown in Table 2.. After 5 days of infestation with *C. maculatus* adults, the percentage mortality caused by *Chrysanthemum* oil was 100% until 6 weeks of storage of *Chrysanthemum* oil, then this percentage decreased from 80 to 0% at the end of the tested storage period for *Chrysanthemum* oil (12 weeks). Some oils not only maintain the viability and seed protein content, but they may also prevent other deleterious factors from appearing during storage (Lee et al., 2002). He also proved that in tested oils, their high lipophilieity permits them to rapidly penetrate into insects and interfere with their physiological functions. Due to its high volatile content, oil has fumigant and gaseous action and might be of importance for stored product insects (Ahn et al., 1998). (Li et al., 2014) proved the relationship between the ability of *Chrysanthemum* species to produce insecticides (pyrethrum) and toxicity in insects.

Table 1. Mortality (%) of *C. maculatus* in cowpea seeds treated with *Chrysanthemum* oil at different concentrations.

Chrysanthemum oil (%)	Corrected percentage mortality indicated (days)				
	1	2	3	5	7
0.01	24.33	26.0	40.5	53.33	64.5
0.03	27.33	32.0	58.33	74.0	90.0
0.05	48.00	54.0	72.0	93.0	100
0.10	52.00	64.0	91	100	---
0.18	81.33	97.0	100	---	---

Table 2. Percentage mortality of *C. maculatus* in cowpea seeds treated with *Chrysanthemum* oil LC_{95} (0.4%) after different periods:

Storage time (weeks)	% Corrected mortality of exposed insects after		
	2 days	3 days	5 days
Initial	100	—	—
2	100	—	—
4	100	—	—
6	70	96	100
8	40	65	80
10	20	25	32
12	5	0	0

LC=lethal concentration.

Table 3. showed the effect of *Chrysanthemum* oil in LC50, which recorded a few egg-laying (21.3) eggs. The application of *Chrysanthemum* oil prevented the laying of eggs by *C. maculatus* at the LC95 (0.4%). The treatment that resulted in the absence of egg-laying did not show any adults, i.e., 100.

Table 3. The effect of *Chrysanthemum* oil on mean no. of reproduction potential of *C. maculatus* adults and progeny emergence of *C. maculatus*

Chrysanthemum oil (%)	Mean no. of eggs / female					Progeny emergence				
	NO. of hatched eggs	No. of un-hatched eggs	Total no. of eggs	Hatchability %	Reduction %	No. of Female	No. of Male	No. of emerged adults	% of emergence	Reduction %
LC_{50} (0.04%)	19[b]	2.3	21.3[b]	89.2	79.1	7 [b]	5 [b\]	12 [b]	63.2	85.2
LC_{95} (0.4%)	0[a]	0	0[a]	0	100	0 [a]	0 [a]	0 [a]	0	100

LC: Lethal Concentration. Means with the same letter are not significantly different in the same column.

Physicochemical and Cooking Quality of Cowpea Seeds

Reduction of pulse quality during storage is one of the major problems and often becomes unacceptable due to significant quality loss (Karathanos et al., 2006). The insecticidal efficacy of *Chrysanthemum cinerariaefolium* seed oil against *C. maculatus* was studied. The relationship between the treatment of cowpea seeds with tested oil and nutritional, cooking quality, and sensory changes in the seeds should be known for assessing the acceptability of treated seeds.

Proximate chemical composition of cow-pea seeds

The chemical profile of the treated cowpea seed sample with 0.4% *Chrysanthemum* seed oil and untreated seeds is presented in Table 4. According to our findings, there were no changes in chemical composition between untreated and treated cowpea seeds after treatment with the tested oil. Moisture and protein content were equal in values for untreated and treated cowpea samples (11.10 and 25.83%). The moisture content of dry legume seeds ranges between 8% and 12%, as reported by (Ahenkora et al., 1998). The protein content of cowpea seeds ranged from 20.1% to 30% (Coelho et al., 2010). From the same results in Table 4. the mineral content showed non-significant differences ($p > 0.05$) between treated cowpea seeds with *Chrysanthemum* seed oil and untreated cowpea seeds for zinc, iron, magnesium, calcium, potassium, and phosphorus, these results agree with (Famata et al., 2013 and Inobeme et al., 2014) with the content of mineral elements in cowpea, which are rich in potassium, magnesium, zinc, and iron. Minerals are important co-factors found in the structure of certain enzymes and are indispensable in numerous biochemical pathways (Soetan et al., 2010). Cowpeas are good sources of minerals such as potassium, phosphorus, sulfur, calcium, iron, magnesium, and zinc (Affrifah et al., 2021).

Minerals are inorganic nutrients that are ordinarily needed in small quantities, ranging from less than 1 to 2500 mg per day, depending on the mineral (Soetan et al. 2010). In the overall findings from the proximate and mineral composition conducted in this Table 4., the untreated and treated cowpea seeds contain non-significant ($p>0.05$) quantities of nutrients, which indicates that there is no effect of treating cowpea seeds with *Chrysanthemum* seed oil.

Physical characteristics and color param-eters of cowpea seeds

The seed's apparent quality is the criteria for consumers, as shown in Figure 1. It is appreciated through the seed size, defined by the length, width (seed dimensions), and the seed's exterior color. Additionally, determining seed weight is important as it is the most discriminating variable, representing 93% of the variance in in physical characteristics (Henshaw, 2008). Table 5. represents the length, width, 1000-seed weight, and color parameters of untreated and treated cowpea seeds during storage (12 weeks). From the results, it could be observed that there was a slight reduction in length, width, and 1000-seed weight during storage periods for the two tested samples. At the end of the storage period under investigation, the length, width, and 1000-seed weight of the cowpea treated with *Chrysanthemum* seed oil were higher (9.27, 6.42 mm, and 196.12 g) than those of the untreated (9.23, 6.37 mm, and 194.95g), respectively. Although the length, width, and 1000-weight seed of the cowpea seeds decreased during storage, the seeds treated with tested oil were able to approximately maintain their seed dimensions and seed weight. with tested oil were able to approximately maintain their seed dimensions and seed weight. These results are in agreement with (Hassan 2012), who recorded that the weight loss of cowpea infested with *Callosobruchus maculatus F.* was significantly (*P\>0.05*) increased from 46.33% after one month to 67.33% after storage for four months. This loss in weight may be due to two reasons. The first is that the loss of cowpea weight is owed to the decrease in moisture content of the untreated sample during storage periods, as shown in Table 7.

(Davies and Zibokere 2011) confirmed that there is a correlation between decreasing the moisture content of cowpea seeds and decreasing the dimensions (length, width, and thickness) and weight of the seeds. The second reason for the loss in cowpea weight would be attributed to the feeding behavior of insects inside the seeds (Sharma et al., 2010). Results agree with (Mahmoud et al., 2010), who estimated the percentage of weight loss for broad bean seeds treated with jojoba oil to prevent *Callosobruchus maculatus* and found a weight loss of 8.07% in the treated broad bean with jojoba oil at a concentration of 875 ppm, compared to the control sample's 16.04% weight loss at the end of storage. The color characteristics of the two cowpea samples are displayed in the same Table 5. The color of the seed coat of pulses such as cowpea influences consumer acceptability. The cowpea seeds treated with *Chrysanthemum* oil recorded non -significant (p > 0.05) changes in lightness (*L**) and redness (*a**) during all storage periods. Regarding yellowness (*b**) values, there was a significant increase (*p<0.05*) during the storage periods as a result of treating the cowpea seeds with tested oil compared to the control sample. This increase in yellowness of treated cowpeas is due to the treatment with *Chrysanthemum* oil, which tends to yellowness as mentioned by (Shawkat et al., 2011).

Table 4. Proximate chemical composition of untreated and treated cowpea samples

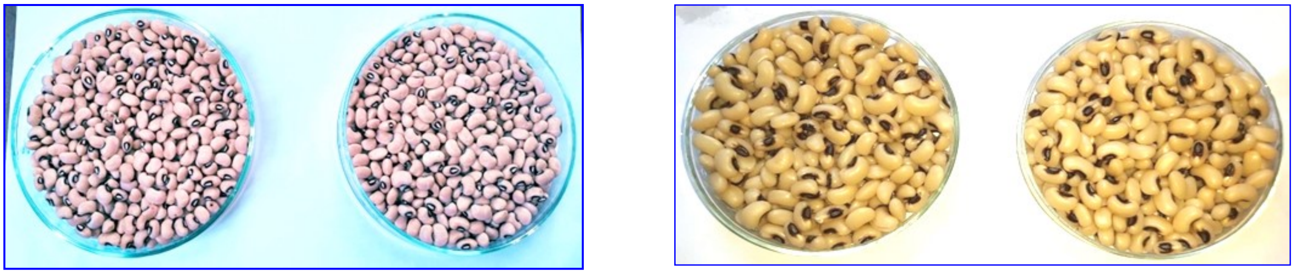
	Untreated	Treated	t value	Sig. (2-tailed)
Chemical composition (g/100g)				
Moisture	11.10±0.01	11.10±0.01	0.707	0.519
Protein	25.83±.0.01	25.83±0.00	1.000	.0374
Ash	3.25±0.01	3.24±0.01	0.707	0.519
Fat	1.21±0.01	1.22±0.01	-2.121-	0.101
Crude fiber	4.41±0.00	4.40±0.01	1.000	0.374
Total carbohydrates	65.30±0.02	65.31±0.02	-0.267-	0.802
Minerals (mg/100g)				
zinc	4.61±0.02	4.61±0.01	-0.277-	0.808
Iron	9.52±0.01	9.51±0.01	0.447	0.698
Magnesium	85.55±0.07	85.45±0.07	1.414	0.293
Calcium	86.45±0.07	86.50±0.00	-1.000-	0.423
Potassium	780.45±0.07	779.85±0.78	1.086	0.391
Phosphorus	490.00±1.41	491.00±1.4	-0.707-	0.553

Untreated= Control cowpea seeds. Treated= cowpea seeds treated with 0.4% *Chrysanthemum* seed oil. Chemical composition contents except moisture were calculated based on dry weight. Data in each row are analyzed to independent t-test and analysis of variance at (p<0.05). Values are means of three replicates±SD.

Table 5. Physical characteristics and color values of untreated and treated cowpea samples during storage

Parameters	Zero time		4 weeks		8 weeks		12 weeks	
	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated
Length (mm)	9.35±0.07	9.35±0.07	9.34±0.01	9.35±0.07	9.27±0.00	9.30±0.01	9.23±0.01	9.27±0.02
t value	0.000		-0.299-		-2.236-		-2.530-	
Sig. (2-tailed)	1.000		0.793		0.155		0.127	
Width (mm)	6.45±0.07	6.50±0.00	6.45±0.07	6.50±0.00	6.42±0.02	6.45±0.04	6.37±0.02	6.42±0.01
t value	-1.000-		-1.000-		-1.043-		-3.051-	
Sig. (2-tailed)	0.423		0.423		0.406		0.093	
1000 seeds weight(g)	198.80±0.00	198.85±0.07	197.99±0.01	198.10±0.14	195.96±0.02	196.22±0.03	194.95±0.03	196.12±0.01
t value	-1.000-		-1.095-		-10.200-		-46.600-	
Sig. (2-tailed)	0.423		0.388		0.009		0.000	
L*	69.02±0.07	68.80±0.12	68.25±0.04	68.21±0.01	67.98±0.02	68.02±0.02	67.87±0.03	67.96±0.07
t value	2.282		1.300		-1.342-		-1.671-	
Sig. (2-tailed)	0.150		0.323		0.312		0.237	
a*	4.73±0.08	4.82±0.01	4.79±0.02	4.84±0.04	4.81±0.00	4.86±0.05	4.82±0.00	4.86±0.04
t value	-1.480-		-1.342-		-1.116-		-1.151-	
Sig. (2-tailed)	0.277		0.312		0..380		0.369	
b*	19.68±.07	20.96±.03	18.98±0.04	19.57±0.06	19.09±0.00	19.29±0.01	19.10±0.04	19.30±0.01
t value	-21.187-		-10.817-		-17.441-		-7.614-	
Sig. (2-tailed)	0.002		0.008		0.003		0.017	

Untreated= Control cowpea seeds. Treated= cowpea seeds treated with 0.4% *Chrysanthemum* seed oil. Data in each row are analyzed to independent t-test and analysis of variance at p (<0.05). L* = Lightness, a* = redness and b* = yellowness. Values are means of three replicates±SD.



Untreated dry cowpea Treated dry cowpea Untreated cooked cowpea Treated cooked cowpea

Figure 1. Cowpea seeds images of untreated and treated with 0.4% *Chrysanthemum* seed oil

Cooking Quality

Cooking time, hydration capacity, and swelling capacity have been the most discriminating among the cowpea-studied treatments (Gull et al., 2017). The data in Table 6. revealed that cooking time slightly increased during the storage of cowpea seeds (treated and untreated samples). It could be observed that the optimal cooking time of cowpea was considerably reduced when treated with *Chrysanthemum* seed oil, which will save energy and time for processing. The increase in cooking time for untreated cowpea seeds may be due to the decrease in moisture content of the sample compared to the treated sample; this is explained by (Sofi, et al., 2022) reducing the moisture content in pulses reduces the separation of the outer layer during cooking, and thus increases the cooking time. Also, (Bressani et al., 2003) concluded that cooking time was shorter when pulse moisture content was high. Cooking time ranged from 57 to 84 minutes, as reported by (Appiah et al., 2011). Cooking times for seeds are influenced by chemical component proportions, such as those of protein and carbohydrates (Henshaw et al. 2003). (Arruda et al., 2012) reported a relationship between cooking time and storage periods by increasing storage period the cooking time increased. Total soluble solids of cooked control seeds were found to be higher than cooked treated cowpea seeds. So, the results showed that the treated cowpea seeds had a decrease in TSS during the storage period compared to the control. Regarding water uptake ratio, hydration capacity, and swelling capacity decreased for all samples during the storage periods, but the treated sample with *Chrysanthemum* seed oil was higher than the untreated sample. (Traore et al., 2022) reported hydration capacity ranging from 0.16 to 0.22 g/seed and swelling capacity from 0.17 to 0.27 ml/seed in cowpea seeds. Hydration capacity plays an important role in the food preparation process; swelling capacity provides an indication of an increase in volume upon absorption of water. The high swelling and water absorption capacity indicate that this could be useful in food systems because this increase is greater after cooking. Hydration capacity is correlated with swelling capacity and seed weight, indicating that seeds with a higher seed weight have higher water absorption, which leads to higher swelling (Traore et al., 2022).

Moisture, protein, and *in vitro* protein digestibility of treated and untreated cowpea

Table 7. represents the moisture, protein, and *in vitro* protein digestibility for untreated and treated samples during storage periods of 12 weeks. Results revealed that there were significant differences in moisture content during the storage period. Untreated cowpea seeds recorded a significant decrease from 11.10 to 10.13% after a storage period of 12 weeks, while treated cowpea seeds recorded a decrease in moisture content during storage from 11.10 to 10.94%. By comparing the two samples after the end of the storage period, it was found that there was a noticeable and significant decrease in the moisture content of the untreated sample compared to the treated sample. This indicates the

ability of the treated sample to safeguard seeds from moisture loss during storage. (Nyakuni et al., 2008) clarified the decrease in the moisture content indicated that moisture had been lost during storage, the last percent of moisture after storage plays a role for assessing the changes in the other characteristics of the Pulses. Regarding the protein content of the two samples, there was a decrease in protein during all the storage periods, while the untreated sample recorded the highest decrease (24.98 %) compared to the treated sample (25.82) %. The findings align with those of Abd El-Raheem et al. (2022), which reported that stored grains (rice, corn, and wheat) had decreases in total protein due to insect infestation during storage than grains treated with certain essential oils (garlic, clove, peppermint, orange, onion, and camphor).

This reduction in protein content of untreated cowpea sample during storage may be due to insect infestation. The protein mainly is an endosperm feeder insect reported by (Affrifah et al., 2021). Also, (Jood et al., 1993) reported that infestations of stored wheat, maize, and sorghum grains by *T. granarium* and *R. dominica* caused a significant reduction in protein content during storage. Concerning the data on *in vitro* protein digestibility (%), generally, the digestibility of protein decreased during storage. At the end of the storage period, the *in vitro* protein digestibility of treated cowpea seeds was higher than that of the untreated (65.79 to 69.99%). The higher digestibility of protein in treated cowpea seeds may be due to the higher protein content than untreated seeds during storage, as mentioned above in the same table. In a similar investigation, (Asiedu et al., 2021) confirmed that the storage of cowpea seeds can reduce their viability and nutritional quality if the seeds are stored without any treatment. The improvement in protein digestibility may be due to the destruction or inactivation of various anti-nutritional factors such as phytic acid and tannins (Khattab and Arntfield, 2009). Higher protein digestibility is dependent on the hydrolysis of peptide bonds that are characteristic of proteins. The digestibility of plant proteins is lower (<80%) than that of animal proteins (≥90%) (Annor et al., 2017).

Sensory Acceptability Scores

Sensory acceptance scores of cooked cowpea seeds with respect to color, taste, texture, flavor, and overall acceptability are represented in Table 8. An independent t-test was performed to compare the effect of *Chrysanthemum* seed oil treatment during storage periods of 12 weeks of seeds with that of untreated cowpea. Data showed that *Chrysanthemum* seed oil treatment did not significantly ($p > 0.05$) affect the color, taste, and texture during storage periods up to 12 weeks. While there was a significant ($p<0.05$) increase in the flavor and overall acceptability of cowpea treated with *Chrysanthemum* seed oil compared to the control after 12 weeks of storage, Generally, the overall acceptability of treated cowpea seeds was higher than that of untreated seeds.

During the storage period of 12 weeks, there were non-significant ($p>0.05$) changes in the sensory characteristics of treated and control cowpea seeds. This indicates that although the cowpea seeds were treated with *Chrysanthemum* seed oil, the sensory characteristics of the cowpea seeds were acceptable. Essential oils effectively increased the shelf lives of cereals and crops without affecting their quality (Bhavaniramya et al., 2019).

Over the years, herbs and essential oils have been one of the main components among the extracts used in the culinary tradition as preservatives for flavor and aroma (Embuscado, 2015).

These results are similar to those of (Cetin and Güdek, 2020) who found that using essential oil from the leaves of rosemary used in the control of Callosobruchus maculatus of the chickpea did not affect the sensory characteristics.

Table 6. Cooking quality of untreated and treated cowpea seeds during storage.

Parameters	Zero time		4 weeks		8 weeks		12 weeks	
	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated
Cooking time (min)	57.25±0.35	57.0±0.00	58.50±0.71	57.00±0.00	59.50±0.70	59.00±0.00	60.00±0.70	59.00±0.00
t value	1.000		3.000		1.000		2.000	
Sig. (2-tailed)	0.423		0.095		0.423		0.184	
T.S.S.* (%)	7.55±0.01	7.52±0.01	7.62±0.04	7.54±0.05	7.82±0.03	7.76±0.06	8.16±.01	7.75±.01
t value	2.236		1.844		1.342		28.991	
Sig. (2-tailed)	0.155		0.207		0.312		0.001	
Water uptake ratio (g/g)	1.25±0.01	1.26±0.01	1.25±0.01	1.24±0.01	1.03±0.01	1.05±0.00	0.98±0.01	1.00±0.01
t value	-1.1342-		1.414		-1.342-		-1.414-	
Sig. (2-tailed)	0.312		0.293		0.312		0.293	
Hydration capacity (g/seed)	0.22±0.01	0.23±0.01	0.22±0.01	0.23±0.01	0.21±0.01	0.23±0.00	0.20±0.00	0.22±0.01
t value	-0.447-		-1.342-		-5.000-		-2.886-	
Sig. (2-tailed)	0.698		0.312		0.038		0.102	
Swelling capacity (ml/seed)	0.22±0.00	0.23±0.00	0.20±0.00	0.21±0.00	0.20±0.00	0.20±0.00	0.19±0.00	0.20±0.00
t value	-1.000-		-1.414-		-2.121-		-4.025-	
Sig. (2-tailed)	0.423		0.293		0.168		0.057	

Untreated= Control cowpea seeds. Treated= cowpea seeds treated with 0.4% *Chrysanthemum* seed oil. *TSS= Total soluble solids after cooking.
Data in each row are analyzed to independent t-test and analysis of variance at ($p<0.05$). Values are means of three replicates±SD.

Table 7. Moisture, protein and *in vitro* protein digestibility of untreated and treated cowpea seeds during storage

Parameters	Zero time		4 weeks		8 weeks		12 weeks	
	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated
Moisture (%)	11.10±0.01	11.10±0.01	11.02±0.01	11.04±0.01	10.56±0.10	10.99±0.10	10.13±0.10	10.94 ±0.10
t value	0.707		-2.214-		-52.664-		-99.204-	
Sig. (2-tailed)	1.000		0.091		0.000		0.000	
Protein (%)	25.83±.0.01	25.83±0.00	25.82±0.005	25.83±0.00	25.48±0.01	25.82±0.01	24.98±0.01	25.82±0.01
t value	0.519		-1.000-		-46.510-		-112.698-	
Sig. (2-tailed)	0.374		0.374		0.000		0.000	
IVPD (%)	72.56±0.57	72.55±0.58	71.02±0.01	71.01±0.01	70.22±0.03	70.35±0.03	65.79±0.01	69.99±0.03
t value	0.007		0.171		-6.327-		-282.192-	
Sig. (2-tailed)	0.995		0.873		0.003		0.000	

Untreated= Control cowpea seeds. Treated= cowpea seeds treated with 0.4% *Chrysanthemum* seed oil. IVPD: *in vitro* protein digestibility. Data in each row are analyzed to independent t-test and analysis of variance at ($p<0.05$). Values are means of three replicates±SD.

Table 8. Sensory acceptability of untreated and treated cooked cowpea seeds during storage.

Parameters	Zero time		4 weeks		8 weeks		12 weeks	
	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	treated
Color	8.62±0.44	8.56±0.50	8.56±0.18	8.50±0.28	8.31±0.53	8.38±0.44	8.19±0.59	8.25±0.38
t value	0.266		0.552		-0.256-		-0.251-	
Sig. (2-tailed)	0.794		0.590		0.802		0.805	
Taste	8.69±0.37	8.75±0.27	8.56±0.32	8.75±0.27	8.19±0.26	8.44±0.18	8.12±0.44	8.37±0.44
t value	-0.386-		-1.271-		-2.256-		-1.128-	
Sig. (2-tailed)	0.705		0.224		0.041		0.278	
Texture	8.75±0.38	8.75±0.27	8.68±0.26	8.62±0.23	8.18±0.26	8.31±0.26	7.88±0.64	8.13±0.35
t value	0.000		0.509		-0.966-		-0.966-	
Sig. (2-tailed)	1.000		0.619		0.350		0.350	
Flavour	8.62±0.35	8.81±0.26	8.37±0.35	8.43±0.18	8.25±0.27	8.43±0.18	7.75±0.27	8.18±0.37
t value	-1.210-		-0.447-		-1.655-		-2.701-	
Sig. (2-tailed)	0.246		0.662		0.120		0.017	
Overall acceptability	8.81±0.26	8.75±0.27	8.63±0.23	8.60±0.19	8.25±0.46	8.38±0.23	7.94±0.18	8.38±0.23
t value	0.475		0.235		-0.683-		-4.249-	
Sig. (2-tailed)	0.642		0.818		0.506		0.001	

Untreated= Control cowpea seeds. Treated= cowpea seeds treated with 0.4% *Chrysanthemum* seed oil. Data in each row are analyzed to independent t-test and analysis of variance at ($p<0.05$). Values are means of n=10 replicates±SD.

4. Conclusion

The cowpea beetle, *Callosobruchus maculatus,* is a common pest that damages stored pulses in Egypt and has a negative impact on food security. Finding new natural, low-priced, and locally available sources of biopesticides that are both effective and maintain the nutritional, sensory, and technological characteristics of pulses is therefore critically needed. The application of *Chrysanthemum* seed oil prevented the laying of eggs by *C. maculatus* at the LC_{95} (0.4%). The tested essential oil not only prevents the cowpea seeds from infestation by insects during storage but also maintains the physicochemical and technological characteristics of the cowpea seeds. For this, *Chrysanthemum cinerariaefolium* seed oil can play an important role in controlling *Callosobruchus maculatus* and reducing the need to use harmful chemical insecticides without effect on the nutritional value, technological characteristics, and sensory acceptability of the cowpea seeds during storage. Finally, it can be recommended to use *Chrysanthemum* seed oil (0.4%) as part of an integrated control program for the cowpea beetle *Callosobruchus maculatus* during cowpea storage for producers and consumers.

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