International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 08-11-2025, Accepted: 07-12-2025, Published: 22-12-2025
Volume 10, Issue 12, 2025, Page No. 83-97

Impact of bruchid beetles infestation and mite activity on the nutritional quality of pulses and the role of ozone treatment in enhancing nutritional quality

Rasha A Zinhoum[1], Azza A Omran[2], Eman F Ebian[1], Enas M K Kassem[3*]
[1] Department of Stored Products Pests, Plant Protection Research Institute, Agricultural Research Center (ARC), Giza, Egypt
[2] Department of Crops Technology Research, Food Technology Research Institute, Agricultural Research Center (ARC), Giza, Egypt
[3] Department of Cotton and Field Crops Mite, Plant Protection Research Institute, Agricultural Research Center (ARC), Giza, Egypt

Abstract

Pulse beetles pose a major risk to legume pulse storage. Because of their rapid reproduction, beetle populations can expand quickly. Infestation starts either in the field on mature pods and continues during storage or begins after the crops are stored. Within a few months of storage, the studies show that 100% of the stored pulses may be damaged if proper management is not adopted. The sensitivity of locally cultivated pulses (faba bean, chickpea, common bean, and cowpea) to two bruchid beetles [*Acanthoscelides obtectus* (Say) & *Callosobruchus maculatus* (Fabricius)] and two mites [*Acarus siro* (Linnaeus) & *Caloglyphus berlesei* (Michael)] infestations and their impact on stored nutrients value was investigated. The effects of ozone gas concentrations (100, 200, 300, 400, and 500 ppm) for six different exposure periods between (1-6 h) on controlling all stages of *A. obtectus* and mites infesting common bean seeds. The physicochemical quality properties (physical parameters, chemical composition, phenolics, antioxidant activity, sensory evaluation) of ozonated bean seeds were studied. Cowpea showed the most significant weight loss from *C. maculatus* infestation, whereas common bean was most susceptible to *A. obtectus*. In contrast, faba bean and chickpeas experienced the lowest weight loss caused by both beetle species. Infested pulses increased moisture but decreased protein, crude fibres, fibre components, ash, fats, and total phenolics content. Ozonated common beans showed decreased weight of 100 seeds, starch granule sizes, moisture, protein, and phenolic contents, but increased lightness values, fats, and protein digestibility. Ozone enhanced the water absorption percentage and reduced the cooking time of beans, and they were acceptable for their sensory attributes. Exposure to ozone gas may assist in reducing the usage of pesticides, and preventing infestation. Besides, it enhanced quality parameters, improving cooking characteristics and protein digestibility while maintaining sensory acceptability.

Keywords: Pulse seeds, Pulse pests, Ozone gas, quality parameters, Sensory evaluation

Introduction

Legumes are rich in protein, fiber, and minerals. Consuming legumes in the diet provides numerous physiological benefits and helps reduced metabolic disorders such as diabetes, colon cancer, and coronary heart disease (Angeles *et al.*, 2021) [4]. The legumes also have potent antibacterial and antioxidant properties (Amarowicz 2020) . Edible pulses (dried legumes such as beans, lentils, and peas) are utilized in various forms: soaked, cooked, germinated, ground, puffed, and roasted. Soaking is the most common method, followed by cooking (Zhou *et al.*, 2024) [64]. Poor storage of pulse seeds may be associated with a declining quality of seeds, either used for planting or for human consumption (Chidananda *et al.* 2014) [11]. Post-harvest losses in production and storage, primarily due to infestations by storage insect pests of legumes, are expected to impact marketing demand. Pulse producers, merchandisers, and processors lose hundreds of millions annually due to damage caused by insects, mites, fungi, and sprouting (Espinal *et al.* 2004; Chidananda *et al.* 2014) [11, 16].

Legume beetles spend their entire life cycle in pulse seeds. The adult lays eggs on the seed, and the larvae bore into it, consuming its nutrients and emerging as mature adults to continue the infestation (Kumar 2017) [34]. This infestation causes decreased germination potential, weight loss, market decay, and nutritional value of the pulses (Jat *et al.* 2013) [30]. The bean weevil, *Acanthoscelides obtectus* (Say) (Coleoptera: Chrysomelidae: Bruchinae), is one of the most devastating insect pests of pulse beetles that infest pulses both in the field and during storage (Hervet *et al.* 2023) [27]. *A. obtectus* is estimated to cause 10 % weight loss in one generation, and 100% weight losses can be recorded in a 3-4 months storage period (Gad and Abied 2019) [20]. The larvae of the insects start feeding on the embryo and continue to eat up the whole seed, thus making it hollow while the seed coat is intact (Ahmed *et al.* 2019) [5].

The cowpea weevil [*Callosobruchus maculatus* (Coleoptera: Bruchidae)] is the major insect pest of pulse crops, resulting in significant losses during storage (Ekoja and Ogah 2020) [15]. The larvae causes the most damage to pulses though feeding from the cowpea seed cotyledon causing seed perforations, loss of seed weight and poor seed germination. One of the most damaging activities is the complete hollowing out of seeds caused by larval feeding on the cotyledon, which compromises the seed's structural integrity and nutritional reserves (Deshpande *et al.*, 2011) [13]. Infestation intensity can be as high as 100% of the seeds within the first couple of months due to the fast growth of this population of insects (Silva *et al.* 2018) [52]. Both beetles also increase the temperature and moisture content of seeds, which affects the physiological quality of those seeds (Mofunanya and Namgbe 2016) [41].

On the other hand, mites significantly contribute to losses in stored products, affecting food quality and quantity. Mites can pierce hard seeds, reduce germination, modify moisture levels, and disperse mold, posing a threat to various products (Taha 1985) [59]. *Acarus siro* (Linnaeus) (Sarcoptiformes: Acaridae) and *Caloglyphus berlesei* (Michael) (Sarcoptiformes: Acaridae) are the most common found mites in stored pulses (Malik *et al.* 2018) [37]. The behavior of mites can lead to deterioration of foodstuff in various ways. For example, a 52 % reduction in seed germination viability after three months of infestation, direct weight loss, and qualitative degradation through contamination by hazardous (e.g., fungi, dead mites, excrement, eggs, and food fragments) (Stejskal *et al.* 2014) [57].

Various management practices have been implemented within the storage premises to control insect pests. The control practices against stored-product pests developed to date have mainly relied on fumigant phosphine and synthetic pesticides (Gad *et al.* 2023; Riaz *et al.* 2024) [22, 50]. Recent research has reported on the overuse of chemicals, leading to toxicity in humans and non-target organisms, pesticide residues, the development of resistance, and environmental pollution (Kaur *et al.* 2019) [31]. In response, there has been a concept of searching for environmentally friendly pest control methods (Silva-Filho *et al.* 2014) [53]. Ozone is a safe, eco-friendly, and non-thermal alternative to thermal processing. The benefits of using ozone in grain storage over conventional insecticides are well recognized. It has several applications in the seed industry, including pesticide residual and mycotoxin eradication, protein and starch modifications (FDA 2001; Kaur *et al.* 2022) [32]. It inhibits mould growth and destroys the spore forms of some bacteria (Acar 2024). During grain storage, ozone gas prevents several insect species from infesting the grain (Pandiselvam *et al.* 2019; Abreu *et al.* 2022) [2, 47]. There have not been cases of resistance of insect pests to ozone, and its sensitivity is based on cultivar, genus, species, and dose (Sousa *et al.* 2016) [55]. Due to its anti-microbial activity without any residues in food products, there is assurance that physiochemical, nutritional and sensory aspects of pulses are preserved (Pawar *et al.* 2021) [48]. This has been extended to pulse seeds as it affects functional groups, thermal behavior, pasting characteristics, cooking time, and morphological aspects, making them more digestible and effective in healthy food production (Nickhil *et al.* 2022) [43].

Therefore, the present study aims to investigate the susceptibility of the most important locally cultivated pulses to infestations by two bruchid beetles (A. obtectus and C. maculatus) and two mites (A. siro and C. berlesei), and their impact on nutrient elements. Furthermore, the effect of ozone gas treatment on all life stages of A. obtectus beetles and two mites was studied, beyond this, its implications for the physiochemical, quality, and sensory attributes of ozonated bean seeds.

Material and Methods

Experiments were conducted in the Stored Products and Grains Pest Department, Plant Protection Research Institute, Agricultural Research Center, and in Crops Technology Research Department, Food Technology Research Institute, Agricultural Research Center in Giza, Egypt.

Test insects, pulses and reagents

Callosobruchus maculatus and *Acanthoscelides obtectus* were obtained from a laboratory culture maintained for several generations on dry cowpea and common bean seeds, respectively, in glass jars (0.50 L) at the Stored Products and Grains Pests Department, Plant Protection Research Institute (PPRI), ARC. Mites (*A. siro* and *C. berlesei*) were obtained from the Cotton and Field Crops Acarology Department, Plant Protection Research Institute (PPRI), ARC. Pulse seeds were obtained from Field Crops Research Institute [for faba bean (*Vicia faba* L., Nubaria 1 variety), and chickpea (*Cicer arietinum* L., Giza 531, and Giza 3 varieties)], and Horticulture Research Institute, ARC [for common bean, (*Phaseolus vulgaris* L., Nebraska variety), and cowpea seeds (*Vigna unguiculata* L., Dokki 126 variety)]. Pancreatin, pepsin enzymes, and bovine serum albumin were purchased from Sigma-Aldrich Chemical Company, USA, and all other chemicals used were graded as analytical.

Weight loss and susceptibility index of beetles and mites in different pulse seeds

Frist experiment

Different pulse seeds were examined to assess their susceptibility to infestation by *C. maculatus* and *A. obtectus* under ambient conditions of 25±2°C and 60±10% RH. The pulse seeds were exposed to -5°C for 4 weeks to disinfect them and maintain their moisture content before the experiments. Five pairs of 24-hour-old beetles were randomly selected in this test and placed in glass jars containing 50 g of each pulse seed (faba bean, chickpea, common bean, and cowpea). Four replicates for each treatment were used. Then, the jars were placed in an incubator at 5±2°C and 60±10% RH after being covered in muslin and fastened with elastic bands. After a 3-day infestation, the insects were removed, and the infested pulse seeds were kept in under the same conditions until the new adults emerged. As soon as beginning adult emergence, counting starting until no further adults emerged from different jars. The date of the first adult emergence, counting started until no adult emerged from different jars. The mean developmental period (MDP) was estimated from egg laying to adult emergence (AE) from foodstuffs. In addition, after adult emergence ceased, the sample was reweighed to record the damage as wet weight loss. The weight loss (WL) (%) was calculated according to the equation of Khare and Johari (1984) [33]. The developmental period of the immature stages served as the criterion for calculating susceptibility indices, as described by Dobie (1974) [14] as follows:

$$\text{Weight Loss (\%)}=[(\text{Initial weight} - \text{Final weight})/\ \text{Initial weight}] \times 100$$

$$\text{Susceptibility index (SI)}= [(\log F) / D] \times 100$$

Where: F= total number of adult emergencies, D= Mean of the development period (day).

Second experiment

After the beetle experiment end, the infested pulse seeds were cleaned and stored in a refrigerator at 5°C for 48 hours to stop their activity before being affected by the mites, to assess the damage caused by the mites alone, without the influence of the beetles. Subsequently, each pulse seed was divided into three replicates, each containing 5 grams of seeds. These were then infested with 10 adult mites from each of the two tested species (A. siro and C. berlesei) separately and incubated at 27±2°C and 70±5% relative humidity. After one month, the seeds were examined, and the number of mobile mite stages was recorded.

Ozone gas application

Ozone gas was produced using an ozone generator (OZO Max Ltd., Shefford, Quebec, Canada) from purified extra dry oxygen feed gas at the Food Toxicology and Contaminants Laboratory, NRC. The ozone generator produces ozone steadily at 30g O_3/h (ozone output). The feed gas flow rate was 60 cubic feet per hour (CFH) at 15 pounds per square inch (28.30 L/min). Ozone concentration was regulated by a feed-gas-flow meter with a plug-in sensor that can adjust ozone concentration (0–10 wt%). For ozonation samples, five concentration levels of ozone gas ranging between 0.214 and 1.070 g/m^3 with 100-500 ppm levels were utilized using feed gas flow adjustment and a high-voltage voltmeter.

Efficacy of ozone application on different pests

Experiment technique

Five concentration levels of ozone gas (100, 200, 300, 400, and 500 ppm) were tested against all stages of A. obtectus and adults of both mites. The different exposure periods of each treatment were 0.50, 1, 2, 3, 4, 5, and 6 hours. Freshly deposited eggs (0–24 hours) were gently moved to glass tubes (1×5 cm) using a fine brush (100 eggs/tube), wrapped in muslin, and fastened firmly with rubber bands. Following the methods of Ahmed et al. (2019) [5], an investigation into the life history of A. obtectus, kidney beans were experimentally infested with A. obtectus eggs, and the seeds were collected after 25 and 33 days, respectively, to obtain 4th-instar larvae and 3-day-old pupae within the seeds. For each replicate, about 10 g of kidney bean seeds infested with fourth-instar larvae or three-day-old pupae were placed in fine porous bags, sealed with a rubber band. Newly emerged adults of A. obtectus (0-24 h old) were carefully transferred to glass tubes (1×5 cm) by sieving (20 adults/tube) and covered with muslin, secured tightly with rubber bands. A. siro and C. berlesei were placed in a small plastic tube (5ml) using a fine brush (30 mites/tube), and the tube was covered with a bored plastic lid for aeration. Then, all treatments were introduced into the Dreshel flask. Untreated bags and tubes were used as controls at each stage, and four replicates were used for each treatment. Then, all immature stages of A. obtectus were transferred to a new glass jar and incubated until adults emerged, and the percentage reduction at each adult emergence was calculated. Adults of A. obtectus and mites were observed after 24 hours to count the numbers alive and dead and calculate the percentage mortality. The percentage reduction formula in adult emergence was calculated using Henderson and Tilton (1955) [26].

$$\text{Reduction } \% = [(Nu-Nt)/Nu] \times 100$$

where Nu = number of adults emerged from untreated seeds and Nt = number of adults emerged from treated seeds.

Germination test

The germination tests were conducted for the control (uninfested) and infested pulse samples with C. maculatus and A. obtectus to examine the impact of pest infestation on seed viability. Additionally, experiments were performed to assess the effect of ozone gas treatment at 500 ppm for different exposure periods on bean seed germinability, using the Fritz (1965) [18] method to determine germination percentage.

Germination percentage% = (Total number of germinated seeds / Total number of planted seeds) × 100

Physicochemical analysis and technological evaluation

The bruchid infestation results of different dried pulse seeds (control and highly infested with) with both pulse beetles were examined using proximate analysis, fiber components, and total phenolics only. Common beans were chosen as a model, exposed to different ozone gas treatments (500 ppm for, and 6 hours), and evaluated for physicochemical, sensory, and technological parameters. Seeds were ground using a high-speed grinder (MDY-2000, China) to obtain whole-meal powder, packed, and stored until further analysis.

Physical analysis of ozonated common bean

Scanning Electron Microscopy (SEM)

A Scanning Electron Microscope (Model JSM-IT200, JEOL Ltd., USA) was used to obtain SEM images of control and ozonated common bean powder (for 3 and 6 h of ozone exposure). Samples were mounted on an aluminium stub with carbon paste, coated with gold to a thickness of 400 Å in a Sputter-Coating Unit, and examined; the micrographs were taken at a magnification of 1000×.

Fourier transform infrared spectroscopy (FTIR) analysis

The FTIR analysis of control and ozonated common bean samples was conducted using the FTIR spectrophotometer (Bruker, Opus-Invenios, Germany) to identify the primary functional groups. The powdered common bean samples were placed in the sample chamber, and the spectra of the samples were scanned in the range of 400 to 4000 cm^{-1} at a resolution of 4 cm^{-1} using the Lab Solutions IR software.

Physical properties of common beans

The physical properties of control and ozonated common bean seed samples (weight of 100 seeds, density, water absorption percentage after soaking, seed components, and colour parameters) were measured according to AACC (2002). The weight of 100 common bean seeds was measured by counting and weighing. Seed volume was estimated using the water displacement method, and seed density was calculated using the weight (g) and volume (cm^3) results. Ten seeds of each bean sample were weighed and soaked in 100 mL of water for 12 hours at room temperature for the seed part percentages. The seeds were weighed to calculate the water absorption percentage after soaking by dividing the mass gain by the seeds' mass before soaking. Then, the seeds were manually dehulled to obtain seed coats and cotyledons, dried at 60°C to a constant weight for 12 hours and weighed to calculate the percentages of the seed coat and the cotyledons. The color parameters of the exterior coat seeds were expressed as lightness, redness, and yellowness, and assessed using a handheld Chromameter (model CR-400, Konica Minolta, Japan), All measurements were averaged over three.

Proximate analysis

The moisture, protein, crude fibers, ash, and fat contents of the control, infested pulses, and ozonated common beans were determined (AOAC 2019). The carbohydrate content was calculated by subtracting the contents of ash, protein, crude fiber, and fat from 100 g of the sample. Fiber components (lignin, cellulose, and hemicellulose) of control and infested pulses were measured using AOAC (2019) methods.

Determination of total phenolics

The total phenolics were determined for control, infested pulses, and ozonated common bean seeds by mixing 2.50 g of sample with 25 ml of 80% methanol, shaking for 2 hours, and filtering the mixture (Singleton and Rossi 1965) [54]. Then, 0.50 ml of extract was added to 0.50 ml of Folin-Ciocalteu phenol reagent, 1.00 ml of sodium carbonate solution (7.50%), and 8 ml of water. Samples were kept in the dark for 30 min at room temperature, and the absorbance was measured at 725nm using a Jenway spectrophotometer (Model 6715-UV/Vis, Cole-Parmer Ltd, Staffordshire, UK), and the results were expressed as mg/100g gallic acid.

In vitro protein digestibility of common bean

One gram of sample ((untreated and ozonated samples) was mixed with 15.0 ml of 1.50 mg pepsin (dissolved in 0.1 M HCl) and incubated for three hours at 37°C, according to Akeson and Stahmann (1964) [6]. Then NaOH (0.20 M) and 7.50 ml of pancreatin (4 mg in 0.20 M phosphate buffer, pH 8.0) were added, the mixture was shaken and incubated at 37°C for 24 hours. Then, samples were centrifuged for 20 min at 5000 xg, and the percentage of protein digestibility was calculated using the following equation:

In vitro protein digestibility (%) = [(N in supernatant - N in Blank) / N in sample] × 100
N= Nitrogen.

Cooking quality of common bean seeds

10 g of common beans (untreated and ozonated samples) were boiled in a beaker with 200 mL of water until soft or until the white core in the cotyledons disappeared (50±5 min). The water absorption (imbibed water) and total soluble solids, or water-soluble materials (TSS), were measured after cooking (AACC, 2002). The cooked seeds were drained and weighed. TSS was determined by drying the cooking water at 100 °C in an oven until a constant weight was reached, and then weighing the residue. The water absorption and TSS were calculated using the following equations:

Water absorption (%) = [(Seeds weight after cooking - Seeds weight before cooking) /
Seeds weight before cooking] × 100

Total soluble solids (TSS) % = weight of residue (g) / Initial weight of seeds (g) × 100

Sensory evaluation

The cooked common bean seeds were coded and submitted to a 10-member panel from the Food Technology Research Institute for evaluation (Larmond 1977) [35]. Panellists were asked to rate the cooked bean samples on a 9-point hedonic scale (9 = like extremely and 1 = dislike extremely) for color, odor, texture, taste, and overall acceptability. The panellists received information about the study and the ozonated beans to be evaluated before granting their consent.

Statistical analysis

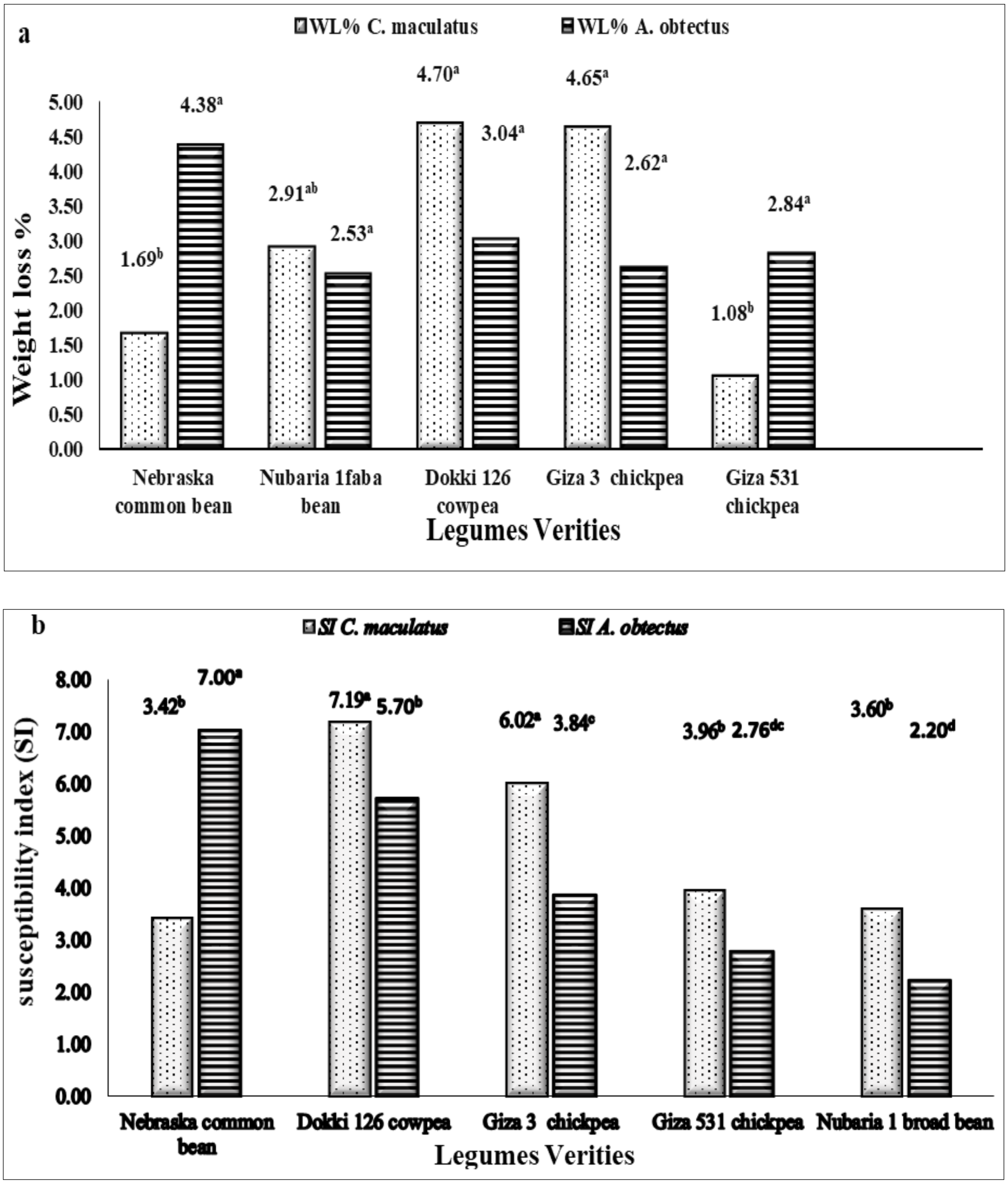
To identify lethal concentration values (LC50 and LC90), mortality percentages after treatment at both the adult and egg stages, or reductions in adult emergence from both larval and pupal stages, were used. The data were subjected to probit analysis (Finney 1971) [17] and analyzed using the Ldp Line program, as described by Noack and Reichmuth (1978) [45]. Data for weight loss, susceptibility index, and developmental period of insects and mites across different pulse types and the data for physicochemical, quality characteristics, and sensory evaluation were analyzed in triplicate [except for the pest expermints (n=4) and sensory evaluation (n=10)] using Costat statistical software and subjected to one-way analysis of variance (ANOVA) (at p<0.05), followed by Duncan's new multiple range tests to evaluate the differences between the groups' mean values. The collected data were statistically analyzed for mean and standard deviation (Steel *et al*., 1997) [56].

Results and Discussion

This research examined the susceptibility of five pulse varieties to the development of A. obtectus, C. maculatus, and two mites (A. siro and C. berlesei), besides, the impact of ozone on pest control and on the quality of pulse seeds affected by infestation and ozone.

Infestation parameters of different pulse seed species to infestation with C. maculatus and A. obtectus

Data demonstrated how different legume seed infestation parameters, weight loss percentage, and SI were affected by C. maculatus and A. obtectus infestations. Regarding C. maculatus parameters, the cowpea (Dokki 126 variety) had the highest weight loss (4.70%), while the chickpea (Giza 531 variety) had the lowest (1.08%), making it the least preferred food for C. maculatus. As for A. obtectus parameters, the faba bean (Nubaria 1 variety) and chickpea (Giza 3 variety) had the lowest weight loss at 2.53% and 2.62%, respectively, while the common bean (Nebraska variety) had the highest weight loss, 4.38% (Figs. 1(a, b) and 2). Similar to those found by Gad and Abied (2019) [20], who reported that the weight-loss percentage of infested common bean seeds by A. obtectus was 5.88% after one month. Also, 12 weeks after the infestation of adults and eggs by C. maculatus, cowpea seeds showed the highest susceptibility of any pulse species to the infestation, with a ratio of 35.93 and 44.58% seed weight loss, respectively (Osman et al., 2015) [46].

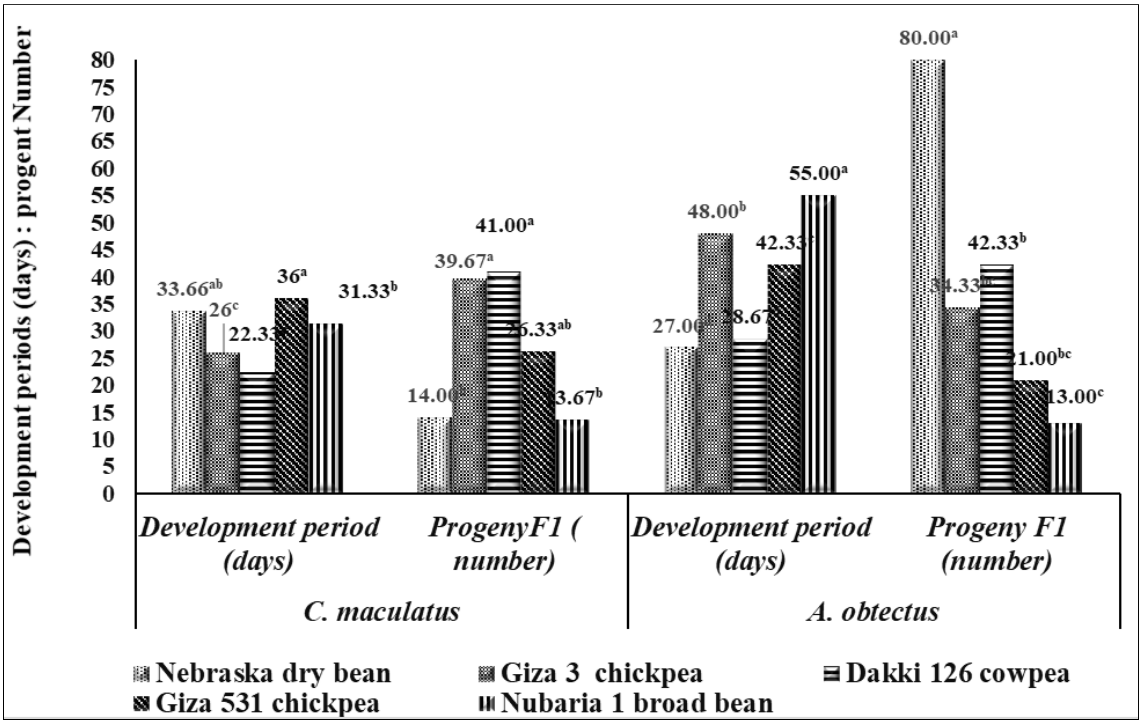


Values are means±SD (n=4) and means with different superscripts are significantly different at $p<0.05$.

Fig 1: Weight loss percentage (a), susceptibility index (b) of different pulse seeds infested with *C. maculatus* and *A. obtectus*.

In comparison between SI of different pulses, the results showed that cowpea (Dokki 126 variety) was the most preferred by *C. maculatus*. In contrast, the common bean (Nebraska variety) was selected by *A. obtectus*. There were differences in the susceptibility of pulse seeds to bruchid infestation. *C. maculatus* and *A. obtectus* caused the maximum damage to the natural host. However, the fact that chickpea and faba beans have coarser surfaces than cowpea and common beans may be why these seeds exhibited minimal damage (Verma *et al*. 2018; Tucić *et al*. 1997) [61, 62].

Fig. 2 reveals the mean developmental period (MDP) of *C. maculatus* and *A. obtectus* (from egg laying to adult) emergence and progeny on different types of pulse seeds. The findings showed a range in MDP across the legumes, with *C. maculatus* ranging from 22.33 to 36 days, while *A. obtectus* ranged from 27 to 55 days. Broad beans (faba bean) were the least preferred host for both insects, producing the fewest offspring (13.67 and 13.00 days) in the case of *C. maculatus* and *A. obtectus*, respectively, which indicated that faba bean seeds were not a suitable host for both beetles because of prolonged development, which produced the fewest offspring. This can be attributed to the higher hardness index and lower moisture content of faba bean seeds compared to those of the other pulses tested, as concluded by Mansouri *et al*. (2022) [38] and Gvozdenac *et al*. (2023) [25].

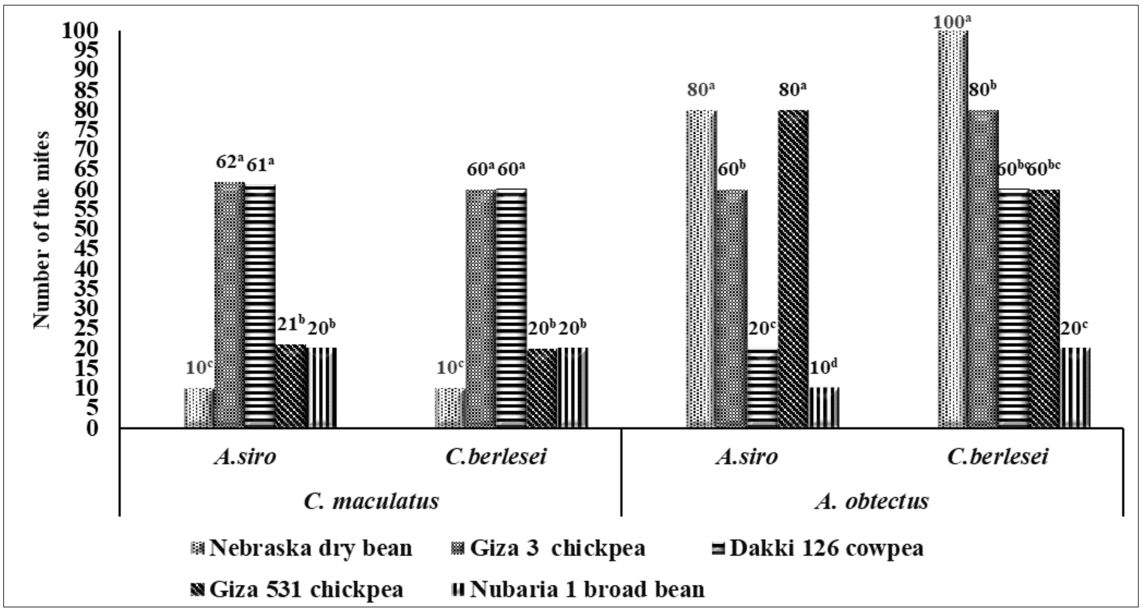


Values are means±SD (n=4) and means with different superscripts are significantly different at p<0.05

Fig 2: Development periods (days) and progeny number of different pulse seeds infested with *C. maculatus* and *A. obtectus.*

Figure 3 presents the infestation rate of two mites on different pulse seeds after being infested with *C. maculatus* and *A. obtectus*. The data indicated that *C. berlesei* mites are more widespread than *A. siro*, and common beans (Nebraska variety) were the most preferred host for both mites. These findings are consistent with those of Yassin *et al*. (2017) [63], who found that *C. berlesei* and A. siro, two gravidorous mites, were the most common and dominant in stored food products. Furthermore, Putatunda (2002) [49] found that the most prevalent and harmful mites in food products stored in India were *Leiodinychus parasiticus* (30%) and *A. siro* (27%).

Data from Table 1 showed that infestation with C. *maculatus* and A. *obtectus* affected seed germination percentage. The results showed that *C. maculatus* and *A. obtectus* caused deterioration in the germination percentage of different pulse seeds. Particularly in common beans and cowpea seeds, germination percentage decreased by 18-51**%** and 20-64%, respectively, compared with uninfested seeds. A similar finding has been reported by Huignard *et al*. (2011) [28], who stated that germination capacity is very low in infested seeds; damage ranges from 75 to 85% depending on the number of opercula per seed.



Values are means±SD (n=4) and means with different superscripts are significantly different at p<0.05.

Fig 3: Infestation rate of A. siro and C. berlesei mites on different legume after infested with C. maculatus and A. obtectus

Table 1: Germination percentage (%) of different pulses seed after infested with C. maculatus and A. obtectus

Pulses	Germination percentage (%)		
	Uninfested (control)	**Infested with C.maculatus**	**Infested with A.obtectus**
Common bean Nebraska	83.33±2.36^{a}	65.00±4.08^{a}	31.67±6.23^{c}
Cowpea Dokki 126	85.00±±4.08^{a}	21.67±6.24^{d}	65.00±8.16^{a}
Chickpea Giza 3	68.33±6.24^{b}	35.00±7.07^{c}	53.33±4.71^{b}
Chickpea Giza 531	73.33±0.70^{b}	48.33±4.71^{b}	53.33±4.71^{b}
Broad bean Nubaria 1	78.33±0.12^{b}	73.33±10.27^{a}	71.67±6.23^{a}

Values are means±SD (n=4) and means within a column with different superscripts are significantly different at $p<0.05$.

Efficacy of ozone gas application on different stages of A. obtectus

Table 2 shows the efficacy of ozone gas on different stages of A. obtectus. Results from the ozone gas evaluation showed that adult mortality increased with both concentration and exposure time. As well as the number of the first generation, the number resulting from exposure of immature stages decreased. In the adult stage, there was a complete mortality (100%) after the exposure time of 6 h at 500 ppm. On the other hand, the number of progeny (F1) resulting from eggs, larvae, and pupa exposed to different ozone gas concentrations decreased. The data indicated that pupae were more tolerant to ozone gas treatment than the egg and larval stages. After a 6-hour exposure period, it was evident that all immature stages were eliminated at both 400 and 500 ppm. The results generally agree with those of Abdelfattah *et al.* (2023) [1], who reported that as the duration of ozone exposure increased, the percentage of adult mortality increased and the percentage of all immature stages decreased in C. maculatus. Additionally, after 6 hours of exposure to the ozone gas, the reduction percentage for the first generation (F1) was 99.90% in the egg stage and 67.47% in the larva stage. The adult stage had a 100% death rate. At all inlet ozone gas concentrations, C. maculatus adult progeny decreased as exposure time to ozone gas increased (Abreu *et al.* 2022) [2].

Table 2: Effect of ozone gas concentrations on different stages of A. obtectus on mortality %, progeny (F1) and reduction % of emerged adults

Ozone gas concentration (ppm)	Exposure time (hour)	Stage						
		Adult	**Eggs**		**Larvae**		**Pupa**	
		Mortality %	**Progeny (F1)**	**Reduction %**	**Progeny (F1)**	**Reduction %**	**Progeny (F1)**	**Reduction %**
100	1	9±7.14^{d}	39.75±1.75^{b}	8.62	23.75±0.85^{b}	13.64	30.0±0^{b}	6.25
	2	15±5.19^{c}	36.25±1.10^{b}	16.67	20.50±0.64^{b}	25.45	27.50±0.65^{b}	14.06
	3	30±4.47^{e}	30.0±0.70^{b}	31.03	16.50±0.95^{b}	40	24.50±0.86^{b}	23.44
	4	36±2.83^{e}	23.0±1.58^{b}	47.13	12.50±1.04^{b}	54.55	20.0±0.0^{b}	37.50
	5	43±3.32^{d}	16.25±1.10^{b}	62.64	9.0±0.57^{b}	67.27	15.0±0.91^{b}	53.13
	6	49.49±5.20^{d}	11.50±1.19^{b}	73.56	6.0±0.40^{b}	78.18	10.50±0.28^{b}	67.19
200	1	18±4.47^{cd}	36.0±1.29^{c}	17.24	19.75±0.85^{c}	28.18	28.0±0.40^{c}	12.50
	2	23±3.32^{c}	32.50±1.04^{c}	25.29	17.25±1.25^{c}	37.27	25.50±0.64^{c}	20.31
	3	39±5.92^{d}	26.0±1.08^{c}	40.23	12.0±0.81^{c}	56.36	21.50±0.64^{c}	32.81
	4	50±4.47^{d}	17.0±1.08^{c}	60.92	5.25±0.62^{c}	80.91	15.75±0.85^{c}	50.78
	5	61±1.73^{c}	11.25±0.47^{c}	74.14	2.50±0.64^{c}	90.91	12.25±1.10^{c}	61.72
	6	69±3.32^{c}	6.0±0.40^{c}	86.21	1.0±0.0^{c}	96.36	9.0±0.0^{c}	71.88
300	1	27±3.23^{bc}	30.25±0.63^{d}	30.46	15.0±0^{d}	45.45	23.50±1.32^{d}	26.56
	2	35±3.32^{b}	24.25±1.10^{d}	44.25	11.25±1.25^{d}	59.09	20.25±0.47^{d}	36.72
	3	49±3.32^{c}	18.50±0.64^{d}	57.47	7.75±1.03^{d}	71.82	14.75±1.10^{d}	53.91
	4	61±5.91^{c}	12.75±0.85^{d}	70.69	3.0±0.70^{d}	89.09	12.25±0.25^{d}	61.72
	5	68±4.90^{c}	7.25±0.85^{d}	83.33	1.25±0.25^{cd}	95.45	7.50±0.95^{d}	76.56
	6	73±5.91^{c}	1.25±0.25^{d}	97.13	0.0±0^{c}	100	4.25±0.62^{d}	86.72
400	1	38±2.00^{ab}	24.0±0.70^{e}	44.83	13.0±1.29^{de}	52.73	20.75±0.47^{e}	35.16
	2	41±1.74^{b}	19.25±0.94^{e}	55.75	9.05±0.64^{e}	65.45	16.50±0.60^{e}	48.44
	3	60±0.0^{b}	14.50±0.50^{e}	66.67	4.75±0.75^{de}	82.73	11.50±1.19^{e}	64.06
	4	69±3.32^{b}	8.25±0.47^{e}	81.03	1.25±0.25^{e}	95.45	8.25±0.47^{e}	74.22
	5	76±4.89^{b}	2.75±0.47^{e}	93.68	0.0±0.0^{d}	100	5.75±0.85^{d}	82.03
	6	86±4.47^{b}	0.0±0.0^{d}	100	0.0±0.0^{c}	100	0.0±0.0^{e}	100
500	1	46±9.16^{a}	20.25±0.47^{f}	53.45	11.0±0.70^{e}	60.00	19.50±0.64^{e}	39.06
	2	54±7.21^{a}	16.75±0.47^{f}	61.49	7.0±0.81^{e}	74.55	13.75±0.75^{f}	57.03
	3	74±4.47^{a}	9.50±0.64^{f}	78.16	2.75±0.94^{e}	90	8.75±0.25^{f}	72.66
	4	81±3.32^{a}	3.0±0.70^{f}	93.10	0.0±0.0^{e}	100	5.75±0.47^{f}	82.03
	5	92±2.83^{a}	0.0±0.0^{f}	100	0.0±0.0^{d}	100	1.0±0.0^{e}	96.88
	6	100±0.00^{a}	0.0±0.0^{d}	100	0.0±0.0^{c}	100	0.0±0.0^{e}	100
Control (untreated)	-	0f	43.50±0.64^{a}	-	27.50±1.19^{a}	-	32.0±1.08^{a}	-

Values are means±SD (n=4) and means within a column with different suberscripts (for different doses) significantly different at $p<0.05$.

Efficacy of ozone gas application on A. siro and C. berlesei mites.

Results concerning the efficacy of ozonation on A. siro and C. berlesei mites are shown in Table 3. Data showed that mortality (%) increased with both concentration and exposure period. Complete mortality (100%) was observed after the exposure time of 5 hours at all concentrations of ozone gas. *A. siro* was more tolerant to ozone gas treatment than C. berlesei. Mahmoud *et al*. (2022) [36] indicated that ozone gas, particularly at concentrations of 200 ppm in just 4 hours, could inhibit the mites *Tyrophagus putrescentiae* (Schrank) (Astigmata: Acaridae) and *Rhizoglyphus robinimites.*

Table 3: Effect of ozone concentrations and exposure time on *A. siro* and *C. berlesi* mites.

Ozone concentration (ppm)	**Exposure time (hour)**	**Mortality (%)**	
		A. siro	***C. berlesei***
100	1	22.50 ± 5.59^{ed}	26.25 ± 4.14^{ed}
	2	41.25 ± 4.14^{dd}	45 ± 0.0^{dd}
	3	63.75 ± 9.60^{cd}	65 ± 9.35^{cd}
	4	77.50 ± 5.59^{bd}	78.75 ± 7.40^{bd}
	5	100 ± 0.0^{a}	100 ± 0.0^{a}
200	1	33.75 ± 4.15^{ec}	35 ± 5.0^{ec}
	2	58.75 ± 4.14^{dc}	63.75 ± 9.60^{dc}
	3	75 ± 6.13^{cc}	77.50 ± 5.59^{cc}
	4	83.75 ± 5.45^{bc}	90 ± 3.54^{bb}
	5	100 ± 0.0^{a}	100 ± 0.0^{a}
300	1	40 ± 3.53^{eb}	42.50 ± 7.5^{eb}
	2	72.50 ± 5.59^{db}	76.25 ± 4.15^{db}
	3	83.75 ± 4.15^{cb}	86.25 ± 4.14^{cb}
	4	90 ± 3.53^{bb}	93.75 ± 4.15^{bb}
	5	100 ± 0.0^{a}	100 ± 0.0^{a}
400	1	42.50 ± 5.59^{db}	45 ± 6.12^{db}
	2	81.25 ± 4.14^{ca}	82.50 ± 2.50^{cb}
	3	90 ± 3.53^{ab}	91.25 ± 6.50^{b}
	4	98.75 ± 2.16^{a}	100 ± 0.0^{a}
	5	100 ± 0.0^{a}	100 ± 0.0^{a}
500	1	53.75 ± 4.14^{ca}	55 ± 3.53^{ca}
	2	87.50 ± 5.59^{ba}	90 ± 0.0^{ba}
	3	96.25 ± 4.14^{a}	100 ± 0.0^{a}
	4	100 ± 0.0^{a}	$100\pm0\cdot0^{a}$
	5	100 ± 0.0^{a}	100 ± 0.0^{a}

Values are means±SD (n=4) and means within a column with different suberscripts (for different doses) and superscripts (for time per hours) significantly different at $p<0.05$.

LC_{50} and LC_{90} (concentration required to kill 50 and 90% of the population) values of ozone gas against different stages of *A. obtectus* beetle, *A. siro,* and *C. berlesei* mites are presented in Table 4. Results revealed that ozone gas was more effective for all species when concentrations were increased from 100 to 500 ppm. LC_{90} for the egg, larvae, and pupae exposed to ozone gas were 1161.40, 593.50, and 1197.19 ppm, respectively. The data showed that larvae were more sensitive to ozone gas than other stages and that the LC_{90} for A. siro and C. berlesei was 459.08 and 392.75 ppm, respectively. It is stated that A. siro is more tolerant to ozone than C. berlesei. Therefore, the results indicated that ozone gas could be used as a fumigant to control A. obtectus beetle, A. siro, and C. berlesei mites on common beans. It was observed that ozone gas was more effective against the tested species when the concentration and exposure period were increased (Gad *et al*., 2021; Abreu *et al*., 2022; Mahmoud *et al*., 2022) [2, 21, 36].

Table 4: LC_{50} and LC_{90} values with their confidence limits for different stages of *A.obtectus* and mites exposed to ozone for 3 hours

Pest	**Stages**	**LC_{50} (ppm)**	**LC_{90} (ppm)**	**Confidence limits (LC_{50})**		**Confidence limits (LC_{90})**		**Slope±**	**X^2**
				Lower	**Upper**	**Lower**	**Upper**		
A. obtectus	Eggs	221.99	1161.41	186.95	257.23	826.46	2048.38	1.78±0.24	3.88
	larvae	146.15	593.50	118.31	170.55	481.64	811.04	2.10±0.24	2.78
	Pupa	268.61	1197.19	233.99	308.29	869.99	2000.00	1.97±0.24	3.27
	Adults	257.73	1701.00	215.88	306.03	1638.50	3873.10	1.56±0.23	4.71
A. siro	Adults	61.14	459.08	24.86	91.02	336.95	886.50	1.46±0.31	0.72
C. berlesei	Adults	59.264	392.75	25.31	87.50	299.64	669.70	1.56±0.32	0.43

Effect of ozone gas on the germination of common bean

Data in Table 5 revealed that, compared to the control, the germination percentage of treated seeds with ozone gas decreased with increasing exposure time to 6 hours at all concentration levels (3-12%). The germination percentage of ozonated cowpea seeds was not significantly different from that of the control (Gad *et al*. 2021) [21].

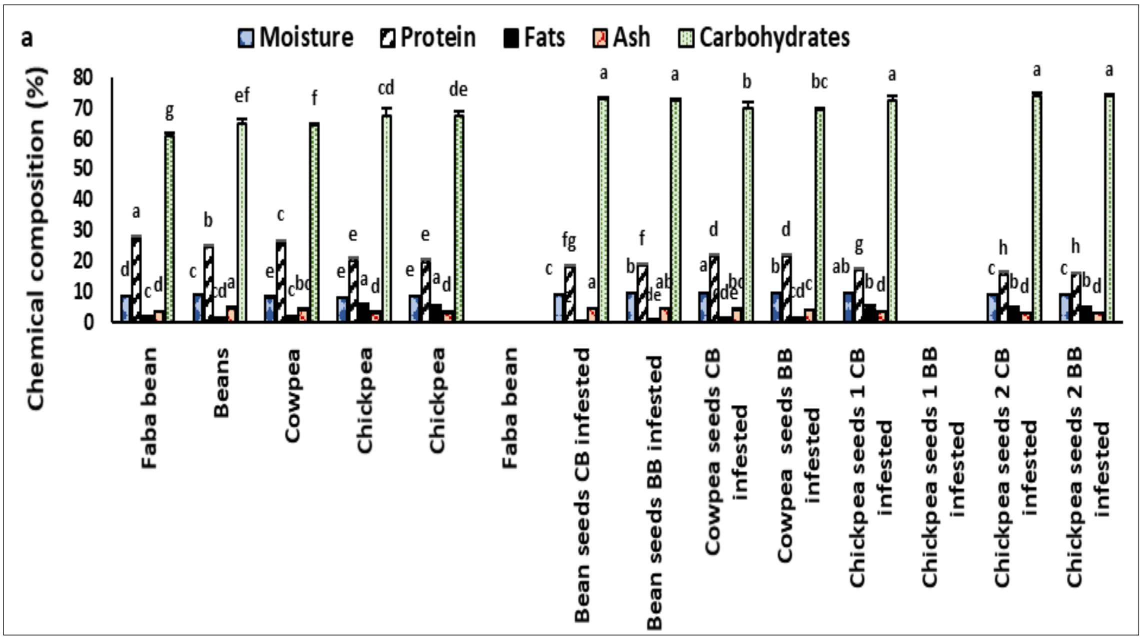
Table 5: Germination (%) of common bean seed treated with different ozone gas concentrations at 3 and 6 hours.

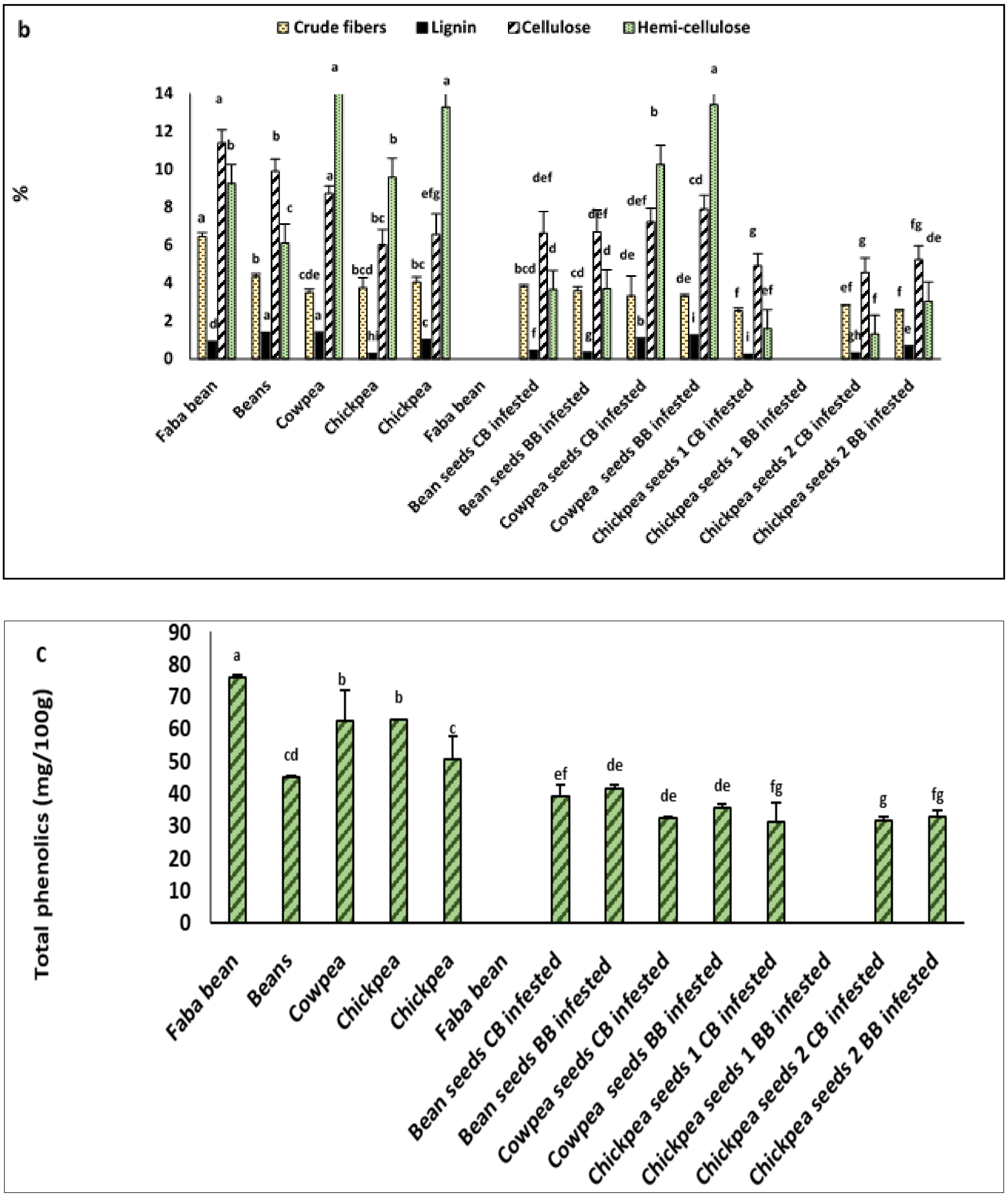
Ozone gas concentration (ppm)	Exposure time (h)	Germination (%)
100	3	90.00±0.0^{a}
	6	86.67± 2.36^{a}
200	3	88.33±2.36^{a}
	6	85.00± 0.00^{ab}
300	3	85.00±4.08^{ab}
	6	83.00± 2.36^{bc}
400	3	83.33±2.36^{bc}
	6	81.67±2.36 ^{c}
500	3	80.00±4.08^{c}
	6	78.33± 2.36^{d}
Control (untreated)	-	90.00±4.08^{a}

Values are means±SD (n=4) and means within a column with different superscripts are significantly different at $p<0.05$.

Proximate chemical composition, fiber components, and total phenolics of non-infected (control) and infested pulse seeds

The relationship between pulse type and nutrients was investigated to evaluate the sensitivity of the pulses to both beetle infestations. Fig. 4 shows the proximate chemical composition, fiber components, and total phenolics of different non-infested and infested pulse seeds. The results for infested pulses indicated decreases in protein, crude fiber, and fat content. However, the moisture content increased in infested seeds compared with control seeds, and the increase rate was higher in seeds infested with cowpea beetles than with common bean beetles. The results could be explained by sensitivity, selectivity, pulse type, and variety variation. The reduction in protein content was higher in infested bean seeds than in the control. Concerning fiber components (lignin, cellulose, and hemicellulose), the components decreased after beetle infestation, and this may be due to the secretion of digesting enzymes that break down the complex molecules into simple molecules. The primary determinants of interactions between bruchids and their host plants are seed morphology and chemistry. For instance, non-protein amino acids like l-canavanine are secondary metabolites that can act as chemical defences. The absence of l-canavanine in Phaseoleae species may contribute to their suitability for Callosobruchus species, whose range is restricted to Phaseoleae (Gatehouse *et al*. 1990) [23]. Mofunanya and Namgbe (2016) [41] and Nickhil *et al*. (2021) [44] reported that cowpea and bean beetles increase seed moisture content, negatively impacting seed quality. Besides, *C. maculatus* can destroy stored seeds, altering their amino acid profile and protein content. Szentesi (2021) [58] found that seed morphological factors (seed size, wrinkled seed shape, and higher seed coat percentage) and biochemical defence mechanisms may help inhibit beetle infestation. Even if the seeds are suitable for infestation, the thickness of the seed coat may prevent first-instar larvae from penetrating the seed. The phenolic content decreased in pulse seeds after infestation with both beetles, and the reduction was greater for seeds infested with cowpea beetles. Further, according to Baoua *et al*. (2015) [10], pulse seeds may be attacked by storage pathogens and pests, leading to quantitative and qualitative losses (such as reduced nutritional value and weight), contamination by mycotoxins, and the formation of off-odours.





The pulse types include Faba bean (Nubaria 1), Bean (Nebraska), Cowpea (Dokki 126), Chickpea 1 (Giza 531), and Chickpea 2 (Giza 3). Infected samples were exposed to either cowpea beetles (CB) (*Callosobruchus maculatus*) or bean beetles (BB) (*Acanthoscelides obtectus*). Values are means±SD (n=3) and means with different superscripts are significantly different at $p<0.05$.

Fig 4: Proximate chemical composition (a), fiber components (b), and total phenolics (c) of uninfested and infested pulse seeds with both beetles

Physical, chemical, cooking quality and sensory attributes of ozonated common bean seeds (the model for seed control)

Scanning Electron Microscopy (SEM)

The granules' functional qualities, required for food and industrial applications, are affected by their shape and size. The SEM images of the common beans, when exposed to ozone gas for 3 and 6 hours (at 500 ppm ozone dose), are shown in Fig. 5. Data recognized that the ozonated bean starch granules (Fig. 5b and 5c), after ozone treatment for 3- and 6-hours duration, were slightly less in size than the control bean (ranging from 13.12 to 24.52 µm and from 10.57 to 20.59 µm in width, respectively). The starch surface in both samples had some cracks. However, the control bean starch granules (untreated) have a relatively oval to spherical shape, with a width ranging from 10.78 to 26.40 µm (Fig. 5a). Bean starch granules are heterogeneous in size and vary from oval to kidney shapes (Granza *et al.* 2015) [24].

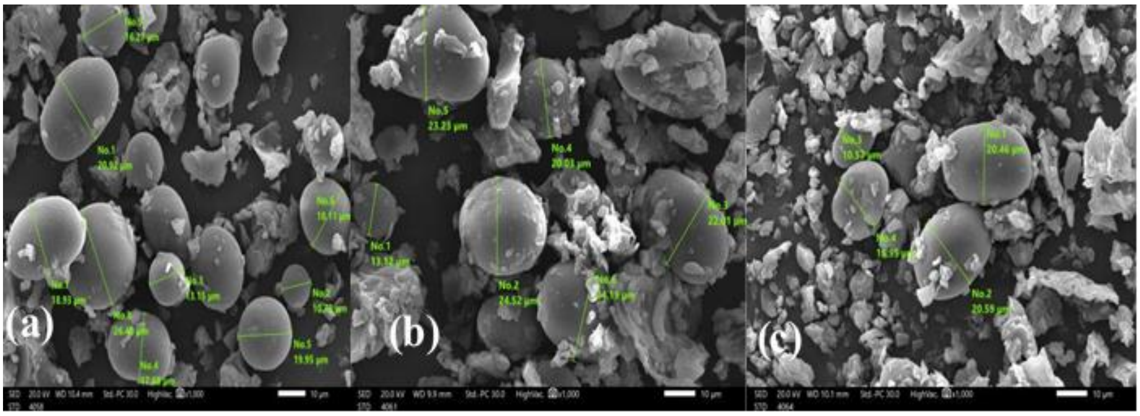


Fig 5: Scanning Electron Micrographs (SEM) of control (a), ozonated common bean for 3 (b) and ozonated bean for 6 hours (c) (1000 x magnification).

Fourier infrared spectroscopy (FTIR)

Fig. 6 displays the FTIR spectrum of the control and ozonated common bean samples. Peaks in the bean samples ranged between 400 and 3900 cm^{-1}. The transmittance percentage was higher in the control bean and decreased with increasing ozone gas exposure duration. This may be due to ozone's effect on certain chemical bonds. Nickhil *et al.* (2022) [43] stated that the peaks between 2700 and 3000 cm^{-1} indicate the presence of the C-H region. The C-H bonds in CH_3 and CH_2 groups appear at peaks at 2854 and 2928 cm^{-1}, respectively. Carboxyl components (ketones, aldehydes, and esters) are present in the range of 1500 to 2000 cm^{-1}. Ozone gas induced minor changes in some functional groups, as indicated by intensity and position, in chickpea seeds, and oxidation by ozone altered hydroxyl, H_2O, and ammonium groups.

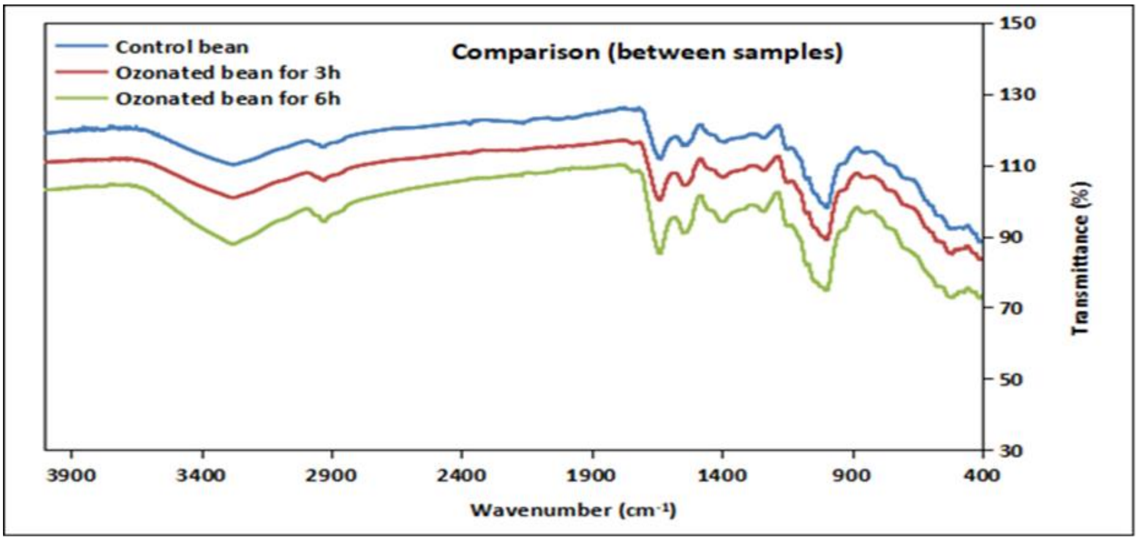


Fig 6: FTIR spectra of control and ozonated common bean samples.

Physicochemical properties of control and ozonated common bean

Table 6 presents the physical properties of the control and ozonated common beans, including 100-seed weight, density, water absorption after soaking, seed parts, and colour values. The results showed that exposure to ozone gas insignificantly decreased 100-seed weight and water absorption after soaking, in response to ozone gas treatment. Mishra *et al.* (2019) [40] observed that treated wheat grains with ozone gas decreased the seeds weight compared with control seeds. Conversely, the density values were insignificantly ($P>0.05$) increased after ozone gas exposure. Tiwari *et al.* (2010) [60] reported that ozone gas has a minimum or no influence on seed quality and may provide distinct advantages for seed processing while it also addresses growing concerns about the use of pesticides. Regarding the color values of ozonated bean seeds, the exposure to ozone gas significantly ($p<0.05$) increased the lightness (L^*) values of beans from 90.08 to 96.48. However, the redness (a^*) and yellowness (b^*) values were significantly decreased (from 1.43 to 0.52 and from 12.04 to 9.64, respectively). Marston *et al.* (2015) [39] found that the change in color of some pulse seeds is due to ozone gas exposure. The double bonds in their pigments can react with ozone gas, which may oxidize certain color-forming pigments. This oxidation can lead to the loss of some existing compounds, causing a significant decrease in the intensity of the yellow color.

Table 6 showed the same proximate chemical composition of common bean seed samples after 3 and 6 hours of exposure to ozone gas. The results indicated reductions in protein, moisture, crude fiber, and ash content, whereas fat and carbohydrate content were higher. The decrease in moisture content may be due to ozone oxidation. Mishra *et al.* (2019) [40] observed that ozonation treatment may affect the final product's nutritional value, and the decrease in protein content could be explained by ozone-induced protein degradation.

Concerning the total phenolics content (as gallic acid equivalent), the total phenolics content of ozonated bean seeds was significantly ($p<0.05$) decreased compared with the control. The same was found by Marston *et al.* (2015) [39] and Sachadyn-Król and Agriopoulou (2020) [51], who observed that ozone gas could oxidise some pigment compounds and may decrease their contents. The free radicals produced by ozone act as catalysts for the oxidation of phytochemicals and biologically active compounds, and their effect increases with higher ozone gas concentrations and longer contact times, which could explain the phenolic reduction.

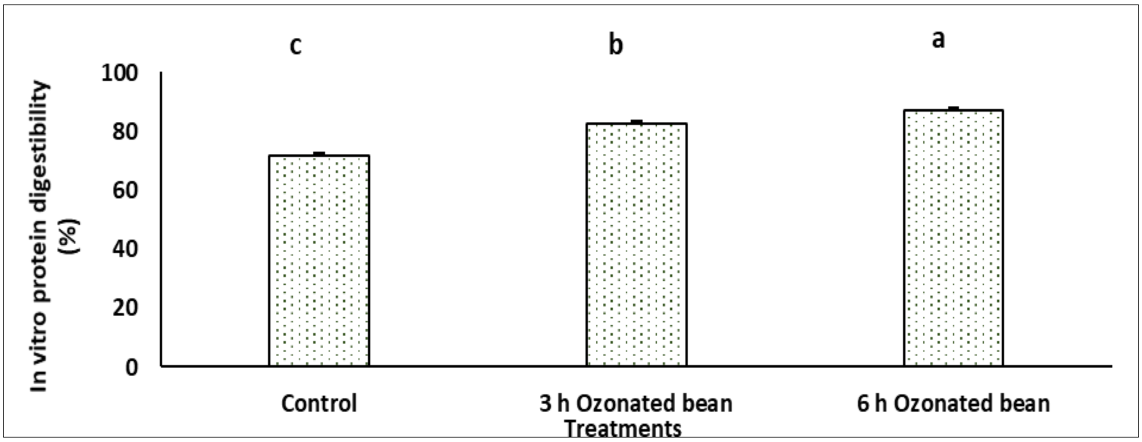
Table 6: Physicochemical characteristics of ozonated common bean seeds.

Parameters	Control	3 h Ozonated bean	6 h Ozonated bean
100-seeds weight (g)	42.20±1.07 ^{a}	41.33±0.40 ^{a}	41.18±0.31 ^{a}
Density (g/cm^3)	1.12±0.11 ^{a}	1.22±0.15 ^{a}	1.30±0.09 ^{a}
Water absorption after soaking (%)	131.37±0.98 ^{a}	131.12±2.57 ^{a}	129.46±2.27 ^{a}
	Seed parts		
Cotyledons (%)	90.60±0.31 ^{a}	90.72±0.22 ^{a}	91.01±0.10 ^{a}
Seed coat (%)	9.32±0.30 ^{a}	9.28±0.12 ^{a}	8.99±0.05 ^{a}
Cotyledons/seed coat ratio	9.74±0.35 ^{a}	9.78±0.25 ^{a}	10.12±0.06 ^{a}
	Color parameters		
*L**	90.08±0.622 ^{b}	95.85±0.16 ^{a}	96.48±0.17 ^{a}
*a**	1.43±0.13 ^{a}	0.55±0.04 ^{b}	0.52±0.05 ^{b}
*b**	12.04±0.48 ^{a}	9.68±0.24 ^{b}	9.64±0.11 ^{b}
Proximate chemical composition (%)*			
Moisture	8.89±0.05 ^{a}	8.63±0.01 ^{b}	8.54±0.04 ^{c}
Protein	24.53±0.66 ^{a}	24.21±0.56 ^{a}	23.64±0.76 ^{a}
Fat	1.47±0.05 ^{a}	1.56±0.09 ^{a}	1.62±0.04 ^{a}
Crude fibers	4.26±0.09 ^{a}	4.17±0.07 ^{a}	4.14±0.11 ^{a}
Ash	4.62±0.32 ^{a}	4.42±0.05 ^{a}	4.39±0.06 ^{a}
Carbohydrates	65.12±1.19 ^{a}	65.64±0.70 ^{a}	66.21±0.97 ^{a}
Total phenols (mg/100g)	44.96±0.40 ^{a}	43.02±0.67 ^{b}	39.92±0.95 ^{c}

*on the dry weight basis. Control= untreated seeds, ozonated= bean seeds exposure to ozone gas for 3 and 6 hours, *L**= Lightness, *a**= redness and *b**= yellowness. Values are means±SD (n=3) and means within a row with different superscripts are significantly different at $p<0.05$.

Protein digestibility

Fig. 7 demonstrates the *in vitro* protein digestibility of control and ozonated common bean samples. Protein digestibility increased significantly ($p<0.05$) after ozone gas treatment compared with the control. It could be explained by the influence of ozone on macromolecules, such as proteins. Seed ozonation alters protein morphology, making it easier to digest; this can be quite beneficial in the processing of nutritious food products (Nickhil *et al.*, 2022) [43]. Nath *et al.* (2014) [42] mentioned that ozone oxidizes amino acids and sulphydryl groups of peptides, proteins, and enzymes, leading to shorter peptides.



Values are means±SD (n=3) and means with different superscripts are significantly different at $p<0.05$.

Fig 7: *In vitro* protein digestibility of control and ozonated common bean

Cooking quality

The cooking quality of bean seeds was evaluated by determining the cooking time, weight gain, and total soluble or water-soluble solids (Table 7). The results showed that the ozone-treated bean had a significantly shorter cooking time ($p<0.05$) than the control. Bean exposure to ozone gas for 3 and 6 hours resulted in a significant ($p<0.05$) increase in weight absorption after cooking compared with thecontrol. Total soluble solids of control common bean seeds were found to be lower than ozonated beans, and this may be due to the influence of ozone gas on common bean nutrients. Tiwari *et al.* (2010) [60] stated that ozone gas treatments offer unique functionalities for processing many types of seeds. In terms of the effect of ozone on sensory attributes.

Table7: Cooking quality of ozonated common bean seeds.

Parameters	Control	3 h Ozonated bean	6 h Ozonated bean
Cooking time (min.)	55.67±1.15 ^{a}	46.33±1.16 ^{b}	44.00±1.73 ^{b}
Water absorption after cooking (%)	129.59±7.38 ^{a}	132.70±3.47 ^{a}	137.98±4.82 ^{a}
Total soluble solids (%)	8.36±1.17 ^{c}	10.09±0.44 ^{b}	11.90±0.04 ^{a}

Control= untreated bean seeds, ozonated= bean seeds exposure to ozone gas for 3 and 6 hours. Values are means±SD (n=3) and means within a row with different superscripts are significantly different at $p<0.05$.

Sensory properties

Table 8 presents the sensory acceptance scores of bean seeds after ozone exposure for colour, texture, odour, taste, and overall acceptability. The results revealed that the color, taste, odor, and overall acceptability of beans were not significantly different with ozone gas, except for the texture scores at 6 hours of ozone exposure, which were significantly different from the control. This indicates that, despite being treated with ozone, the sensory properties of bean seeds remained satisfactory. This aligns with Agriopoulou *et al.* (2022) [3], who observed that while ozonation can affect food quality characteristics, it does not necessarily lead to negative sensory changes, and may in some cases preserve acceptability

Table 8: Sensory properties of ozonated common bean seeds.

Parameters	Control	3 h Ozonated bean	6 h Ozonated bean
Color	8.10±0.39	8.11±0.65	8.50±0.53
Odor	8.80±0.63	8.50±0.53	8.30±0.95
Texture	8.80±0.35 ^{a}	8.30±0.67 ^{ab}	8.20±0.63 ^{b}
Taste	8.70±0.67	8.35±0.58	8.30±0.42
Over all acceptability	8.60±0.46	8.35±0.47	8.30±0.35

Values are means±SD (n=10) and means within a row with different superscripts are significantly different at $p<0.05$. The caption that where no letters are presented, there were no significant differences within rows.

Conclusion

Understanding the relationship between pest infestation impacts and the biochemical aspects of crop types could lead to lower infestations without compromising the qualities that contribute to seed value. The 500-ppm ozone gas used for 6 h of emissions was effective in rapidly controlling various stages of A. obtectus and mites. Ozone may be an effective fumigant for the prevention and control of pulse pests (beetles and mites). The ozonated bean seeds were attractive to consumers because they weighed less than control beans, and their moisture content decreased after ozone treatment, which is important for storage facilities. Ozone gas treatment increased fat and *in vitro* protein digestibility. Further, ozone gas increased the weight of cooked bean seeds and decreased cooking time, especially for beans exposed to 6 hours of ozone. The ozone gas treatment did not influence the taste or overall acceptability of cooked common beans. Ozone gas treatments are useful for preventing pulse pest infections and minimising the use of insecticides that affect consumer health while preserving pulse quality. These research directions would significantly contribute to establishing ozone treatment as a sustainable and effective alternative to conventional pesticides in pulse storage and preservation.

Declaration of competing interest

On behalf of all authors, the corresponding author states that there is no conflict of interest

Author contributions

Rasha A. Zinhoum conceptualization, investigation, resources, methodology, data curation, writing original draft, review & editing the final article. Azza A. Omran conceptualization, investigation, resources, methodology, formal analysis, data curation, writing original draft, review & editing the final article. Eman F. Ebian conceptualization, investigation, resources, data curation. Enas M.K. Kassem conceptualization, investigation, resources, methodology, and formal analysis. All of the authors read and approved the manuscript.

Data availability

All data have been provided in the manuscript.

Funding

No external funding was obtained for this research.

Statement of ethics consent

Ethical approval is not applicable, because this article does not contain any studies with human or animal subjects

References

1. Abdelfattah NAH, Marie AM, Omran AA. Influence of ozone gas on cowpea beetle (Callosobruchus maculatus) as well as seed technological characteristics. World Journal of Dairy and Food Sciences,2023:18(1):1–11. https://doi.org/10.5829/idosi.wjdfs.2023.01.11
2. Abreu AO, Faroni LDA, Silva MVA, Sousa AH, Alencar ER, Silva GN, *et al.* Ozone as an alternative fumigant for controlling Callosobruchus maculatus (F.) (Coleoptera: Chrysomelidae) in cowpea beans. Journal of Stored Products Research,2022:97:101969. https://doi.org/10.1016/j.jspr.2022.101969
3. Agriopoulou S, Sachadyn-Król M, Stamatelopoulou E, Varzakas T. Effect of ozonation and plasma processing on food bioactives. In: Jafari SM, Capanoglu E, editors. Retention of Bioactives in Food Processing, Food Bioactive Ingredients. Springer Nature, 2022, 547–577.
4. Angeles JGC, Villanueva JC, Uy LYC, Mercado SMQ, Tsuchiya MCL, Lado JP, *et al.* Legumes as functional food for cardiovascular disease. Applied Sciences,2021:11:5475. https://doi.org/10.3390/app11125475
5. Ahmed SS, Naroz MH, Abdel-Aziz SY, Awad MAR, Abdel-Shafy S. Morphological, molecular and

biological studies on common bean weevil Acanthoscelides obtectus (Say) in Egypt. Journal of Entomology,2019:16(1):30–38. https://doi.org/10.3923/je.2019.30.38

6. Akeson WR, Stahmann MA. Pepsin pancreatin digest index of protein quality evaluation. Journal of Nutrition,1964:83:257–261.
7. Amarowicz R. Legume seeds as an important component of human diet. Foods,2020:9(12):1812. https://doi.org/10.3390/foods9121812
8. American Association of Cereal Chemists. Approved Methods of the American Association of Cereal Chemists. 13th ed. St. Paul, Minnesota, 2002.
9. Association of Official Analytical Chemists. Official Methods of Analysis of AOAC International. 21st ed. Washington DC, 2019.
10. Baoua IB, Amadou L, Abdourahmane M, Bakoye O, Baributsa D, Murdock LL. Grain storage and insect pests of stored grain in rural Niger. Journal of Stored Products Research,2015:64:8–12. https://doi.org/10.1016/j.jspr.2015.04.007
11. Chidananda KP, Chelladurai V, Jayas DS, Alagusundaram K, White NDG, Fields PG, *et al*. Respiration of pulses stored under different storage conditions. Journal of Stored Products Research,2014:59:42–47. https://doi.org/10.1016/j.jspr.2014.04.006
12. Çınar Acar B. Evaluation of ozone effectiveness against Gram-positive and Gram-negative pathogens using different methods. Mustafa Kemal Üniversitesi Tarım Bilimleri Dergisi,2024:29(2):606–621. https://doi.org/10.37908/mkutbd.1437244
13. Deshpande VK, Makanur B, Deshpande SK, Adiger S, Salimath PM. Quantitative and qualitative losses caused by Callosobruchus maculatus in cowpea during seed storage. Plant Archives,2011:11(2):723–731.
14. Dobie P. The laboratory assessment of the inherent susceptibility of maize varieties to post harvest infestations by Sitophilus zeamais. Journal of Stored Products Research,1974:10:183–197. https://doi.org/10.1016/0022-474X(74)90006-X
15. Ekoja EE, Ogah BE. Efficacy of oils from nine plant species as protectants against infestation by Callosobruchus maculatus. World Journal of Advanced Research and Reviews,2020:7(3):7–15. https://doi.org/10.30574/wjarr.2020.7.3.0323
16. Espinal R, Higgins R, Wright V. Economic losses associated with Zabrotes subfuscintus and Acanthoscelides obtectus infestations of stored dry red beans (Phaseolus vulgaris). CEIBA,2004:45(2):107–119.
17. Finney DJ. Probit Analysis. 3rd ed. Cambridge University Press, 1971.
18. Fritz T. Germination and vigour test of cereals seed. Proceedings of the International Seed Testing Association, 1965.
19. Food and Drug Administration. Secondary direct food additives permitted in food for human consumption. Federal Register, 2001, 66.
20. Gad HA, Abied MK. Effect of some legumes on the biological parameters of Acanthoscelides obtectus. Egyptian Academic Journal of Biological Sciences A Entomology,2019:12(3):85–93. https://doi.org/10.21608/EAJBSA.2019.36231
21. Gad HA, Abo Laban GF, Metwaly KH, AlAnany FS, Abdelgaleil SAM. Efficacy of ozone for Callosobruchus maculatus and Callosobruchus chinensis untreated in cowpea seeds and its impact on seed quality. Journal of Stored Products Research,2021:92:101786. https://doi.org/10.1016/j.jspr.2021.101786
22. Gad HA, Al-Ayat AA, Hassuba MM, Abdelgaleil SA. Effectiveness of binary combinations of abamectin and deltamethrin with two inert dusts for the management of Trogoderma granarium on wheat grains. Journal of Stored Products Research,2023:100:102071. https://doi.org/10.1016/j.jspr.2022.102071
23. Gatehouse AMR, Minney BH, Dobie P, Hilder V. Biochemical resistance to bruchid attack in legume seed. In: Fujii K, Gatehouse AMR, Johnson CD, Mitchel R, Yoshida T, editors. Bruchids and Legumes: Economics, Ecology and Co-evolution. Kluwer, 1990, 241–256.
24. Granza AG, Travalini AP, Farias FO, Colman TAD, Schnitzler E, Demiate IM, *et al*. Effects of acetylation and acetylation–hydroxypropylation on the properties of starch from Carioca bean (Phaseolus vulgaris). Journal of Thermal Analysis and Calorimetry,2015:119(1):769–777. https://doi.org/10.1007/s10973-014-4092-9
25. Gvozdenac S, Ilic A, Vasic M, Tanaskovic S, Prvulovic D. Suitability of three different legumes for Acanthoscelides obtectus development and population growth. Journal of Central European Agriculture,2023:24(2):455–463. https://doi.org/10.5513/JCEA01/24.2.3826
26. Henderson CF, Tilton EW. Test with acaricides against the brown wheat mites. Journal of Economic Entomology,1955:48:157–161. https://doi.org/10.1093/jee/48.2.157
27. Hervet VA, Fields PG, Hamilton KD, Nadimi M, Paliwal J. Cold tolerance of Acanthoscelides obtectus. Journal of Stored Products Research,2023:104:102169. https://doi.org/10.1016/j.jspr.2023.102169
28. Huignard J, Glitho IA, Monge JP, Regnault-Roger C. Insectes ravageurs des graines de légumineuses. Editions Quae, 2011. https://doi.org/10.35690/978-2-7592-1656-7
29. Irudayaraj J, Yang H. Depth profiling of a heterogeneous food-packing model using step-scan Fourier transform infrared photoacoustic spectroscopy. Journal of Food Engineering,2002:55:25–33. https://doi.org/10.1016/S0260-8774(01)00225-4
30. Jat NR, Rana BS, Jat SK. Estimation of losses due to pulse beetle in chickpea. Bioscan,2013:8:861–863.
31. Kaur H, Gill RS, Kaur R. Correlation between biophysical seed characteristics of rice bean (Vigna umbellata) and development of Callosobruchus maculatus. Journal of Stored Products Research,2019:83:9–13. https://doi.org/10.1016/j.jspr.2019.05.010
32. Kaur K, Kaur P, Kumar S, Zalpouri R, Singh M. Ozonation as a potential approach for pesticide and microbial detoxification of food grains. Food Reviews International,2022:39(10):1–33. https://doi.org/10.1080/87559129.2022.2092129
33. Khare BP, Johari RK. Influence of phenotypic characters of chickpea cultivars on susceptibility to

Callosobruchus chinensis. Journal of Legume Research,1984:7(1):54–56.

34. Kumar R. Insect Pests of Stored Grain: Biology, Behavior, and Management Strategies. Apple Academic Press, 2017. https://doi.org/10.1201/9781315365695
35. Larmond E. Laboratory methods for sensory evaluation of food. Canadian Department of Agriculture, 1977.
36. Mahmoud RH, Abdel-Khalik AR, El-Shafei WKM. Comparison between two physical methods to control stored dates fruit mites. Egyptian Academic Journal of Biological Sciences,2022:14(1):149–158. https://doi.org/10.21608/EAJBSZ.2022.228058
37. Malik A, Gulati R, Duhan K, Poonia A. Tyrophagus putrescentiae as a pest of grains. Journal of Entomology and Zoology Studies,2018:6(2):2543–2550.
38. Mansouri SM, Naseri B, Bidar F. Oviposition preference and population growth of Callosobruchus maculatus on legumes. Journal of Stored Products Research,2022:99:102011. https://doi.org/10.1016/j.jspr.2022.102011
39. Marston K, Khouryieh H, Aramouni F. Evaluation of sorghum flour quality influenced by ozone treatment. Food Science and Technology International,2015:21:631–640. https://doi.org/10.1177/1082013214559311
40. Mishra G, Palle A, Srivastava S, Mishra HN. Disinfestation of stored wheat by ozone treatment. Journal of the Science of Food and Agriculture,2019:99:5008–5018. https://doi.org/10.1002/jsfa.9742
41. Mofunanya AAJ, Namgbe EE. Damage due to Callosobruchus maculatus infestation on cowpea. IOSR Journal of Agriculture and Veterinary Science,2016:9(12):96–101. https://doi.org/10.9790/2380-09120196101
42. Nath A, Mukhim K, Swer T, Dutta D, Verma N, Deka BC, *et al*. Application of ozone in food processing and packaging. Journal of Food Product Development and Packaging,2014:1:7–21.
43. Nickhil C, Mohapatra D, Kar A, Giri SK, Verma US, Muchahary S, *et al*. Gaseous ozone treatment of chickpea grains. Journal of Food Science,2022:87(3):5191–5207. https://doi.org/10.1111/1750-3841.16359
44. Nickhil C, Mohapatra D, Kar A, Giri SK, Verma US, Sharma Y, *et al*. Effect of gaseous ozone on disinfestation and quality of chickpea grains. Journal of Stored Products Research,2021:93:101823. https://doi.org/10.1016/j.jspr.2021.101823
45. Noack S, Reichmuth CH. Rechnerisches Verfahren zur Bestimmung von Dosiswerten. Mitteilungen aus der Biologischen Bundesanstalt,1978:185:1–49. https://doi.org/10.5073/20210624-110153
46. Osman MAM, Mahmoud MF, Mohamed KM. Susceptibility of pulse grains to Callosobruchus maculatus. Journal of Applied Plant Protection,2015:3:9–15. https://doi.org/10.21608/JAPP.2015.7708
47. Pandiselvam R, Thirupathi V, Mohan S, Vennila P, Uma D, Shahir S, *et al*. Gaseous ozone for control of Callosobruchus maculatus. Journal of Applied Entomology,2019:143(2):451–459. https://doi.org/10.1111/jen.12618
48. Pawar KR, Atkari VT, Pawar DA, Ukey PD. Ozone treatment: The green technology in food industry. Chapter, 2021, 1–13.
49. Putatunda BN. Mites in post-harvest storage of food grains. Plant Protection Bulletin,2002:54(1–2):25–27.
50. Riaz T, Jalil F, Najeeb A, Minhas T, Shakoori FR. Comparative effectiveness of insecticides against Trogoderma granarium. Journal of Stored Products Research,2024:105:102233. https://doi.org/10.1016/j.jspr.2023.102233
51. Sachadyn-Król M, Agriopoulou S. Ozonation as abiotic elicitation improving plant products. Molecules,2020:25:2416. https://doi.org/10.3390/molecules25102416
52. Silva MGC, Silva GN, Sousa AH, Freitas RS, Silva MS, Abreu AO, *et al*. Hermetic storage for controlling Callosobruchus maculatus. Journal of Stored Products Research,2018:78:27–31. https://doi.org/10.1016/j.jspr.2018.05.010
53. Silva-Filho R, Santos RHS, Tavares WS, Leite GLD, Wilcken CF, Serrão JE, *et al*. Rice-straw mulch reduces aphid populations. PLoS One,2014:9:e94174. https://doi.org/10.1371/journal.pone.0094174
54. Singleton VL, Rossi JA. Colorimetry of total phenolics. American Journal of Enology Viticulture,1965:16:144–158. https://doi.org/10.5344/ajev.1965.16.3.144
55. Sousa AH, Faroni LRDA, Pimentel MAG, Silva GN, Guedes RNC. Ozone toxicity to Sitophilus zeamais. Journal of Stored Products Research,2016:65:1–5. https://doi.org/10.1016/j.jspr.2015.11.001
56. Steel RGD, Torrie JH, Dickey DA. Principles Procedures of Statistics. McGraw-Hill,1997:352–358.
57. Stejskal V, Aulicky R, Kucerova Z. Pest control strategies in seed stores. Plant Protection Science,2014:50(4):165–173. https://doi.org/10.17221/10/2014-PPS
58. Szentesi Á. Seed coat effects on oviposition preference in Acanthoscelides obtectus. BMC Ecology and Evolution,2021:21:171. https://doi.org/10.1186/s12862-021-01892-9
59. Taha HA. Morphological and biological studies on mites associated with stored products. PhD Thesis, Al-Azhar University, 1985.
60. Tiwari BK, Brennan CS, Curran T, Gallagher E, Cullen PJ, O'Donnell CP, *et al*. Application of ozone in grain processing. Journal of Cereal Science,2010:51(3):248–255. https://doi.org/10.1016/j.jcs.2010.01.007
61. Tucić N, Mikuljanac S, Stojković O. Genetic variation in Acanthoscelides obtectus. Entomologia Experimentalis et Applicata,1997:85:247–256. https://doi.org/10.1046/j.1570-7458.1997.00255.x
62. Verma S, Malik M, Kumar P, Choudhary D, Jaiwal PK, Jaiwal R, *et al*. Susceptibility of Indian grain legumes to bruchids. International Journal of Entomology Research,2018:3(2):5–10.
63. Yassin EMA, Abdel-Khalik AR, Abdul-Aziz SA, Osman SA. Mites associated with stored hay in Egypt. Menoufia Journal of Plant Protection,2017:2(3):191–201. https://doi.org/10.21608/mjapam.2017.125928
64. Zhou J, Li M, Bai Q, de Souza TSP, Barrow C, Dunshea F, *et al*. Effects of processing methods on pulses phytochemicals. Food Reviews International,2024:40(4):1138–1195. https://doi.org/10.1080/87559129.2023.2212041