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Nutritional alterations and damages to stored chickpea in relation with the pest status of *Callosobruchus maculatus* (Chrysomelidae)

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ABSTRACT

The identification of substitute products for the replacement of wheat flours for paste and bread is a strategy which may help to overcome the wheat yield problems and to meet the huge consumption rates in Tunisia. In this respect, chickpea flour can provide good opportunities. Nevertheless, seed beetles were the major constraints to achieve this goal. This paper presents extensive data on the pest status of *Callosobruchus maculatus* infesting chickpea in relation to its damage on chickpea seeds and flour. Data on population dynamics, growth and demographic parameters, economic injury level (EIL) and impact on functional and physical properties under two food supply conditions were studied. Results revealed that *C. maculatus* is a major pest altering nutritional properties of stored chickpea. Our results showed that the food supplies influence the reproductive behavior and demographic traits of *C. maculatus* leading to significant impacts on seed germination, weight loss and functional and physical properties. Under food optimal conditions, fertility rates were 38.1 and 47.2% respectively for Amdoun 1 and Beja 1. Moreover, the intrinsic rate of increase r and the finite rate of increase λ reached, respectively, 0.057 and 1.06 ♀/♀/days for Amdoun 1 and 0.048 and 1.05 ♀/♀/days for Beja 1. On the other hand, results showed that germination reduction depended on *C. maculatus* infestation level. Moreover, this work pointed out the variability of EILs with host varieties. Results also revealed that *C. maculatus* seed infestation led to nutritional changes in the seeds. The proximate seed composition was significantly influenced by variety and seeds category. Increases in percentages of protein (33.05 and 22.53% for 53 Amdoun 1), moisture (10.80 and 10.67% for Amdoun 1) and ash have been observed in infested seeds; decreases were observed in percentages of crude fat, carbohydrates (47.96 and 58.69% for Amdoun 1) and nutritional values (355.90 and 367.51 kcal for Amdoun 1) for the same infested seeds.

Introduction

In Tunisia, wheat is mainly cultivated under rainfall conditions and subjected to severe and frequent drought. Thus, yields were variable and low (Latiri et al., 2010). In contrast, Tunisia has one of the highest rates of per capita consumption for wheat in North Africa. Consequently, almost half of the wheat consumed is imported (Hanson, 2016). Therefore, research on composite blends from locally products for the substitution of wheat flour is necessary. In these regards, since chickpea flour is an important source of food proteins and plays an

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important role in the traditional diet (Sfayhi and Kharrat, 2011; Du et al., 2014), it seems to be a potential candidate to replace wheat flour and could be easily integrated on small scale industries as value-add products.

During the last two decades, a national chickpea improvement program has significantly contributed to the increase in the production area and yield records (Khamassi et al., 2012; Amri et al., 2014). However, yield potential is seldom achieved due to biotic and abiotic stresses (Solh et al., 1994). Chickpea seeds are vulnerable, both in the field and in storage, to attack by seed-beetles. Beetles of the genus *Callosobruchus* are major storage pests of chickpea crops and cause considerable economic losses worldwide (Sharma and Thakur, 2014). The cowpea weevil *Callosobruchus maculatus* is the principal field-carry-over storage pest of pulses including cowpea, chickpea, green gram, black gram and red gram (Loganathan et al., 2011).

In Tunisia, few references are available in the literature regarding *C. maculatus*. All the available studies indicate that *C. maculatus* is among the major insect pests attacking chickpea (Jerraya, 2003; Haouel et al., 2015; Mabrouk and Belhadj, 2012). Nevertheless, the pest status, population dynamic, reproductive parameters and demographic traits in relation with sub-optimal and optimal food supply were not yet studied properly. In addition, the impact of *C. maculatus* on chickpea seeds losses, their physical and cooking characteristics and proximate composition were not determined.

Previous works reported that *C. maculatus* populations vary considerably in their host associations, life history and behavioural traits associated with these host plant differences (Messina and Slade, 1997; Kawecki and Mery, 2003; Fox et al., 2004). In this context, Allahvaisi et al. (2010) reported that *C. maculatus* is the most serious pest threat to stored cowpea and many other hosts of the Fabaceae family. Similarly, Iturralde-García et al. (2016) indicated that *C. maculatus* is the principal pest of the stored chickpea. Moreover, Ouali-N'goran et al. (2014) reported that the sex ratio showed no significant difference between *V. unguiculata* varieties and was shifted in favor of females. In contrast, other studies indicated that *C. maculatus* sex ratio followed the expected ratio of 1:1 on *C. arietinum* (Douiri et al., 2014) and *V. radiata* (Heidari et al., 2016).

Previous works indicated that *C. maculatus* is a major pests of various food legumes making the grains unsuitable for human consumption and marketing (Melo et al., 2015). Indeed, the neonate larvae penetrate the grains causing serious damage such as grain weight loss, and reductions in germination, seed viability and nutritional quality (Oke and Akintunde, 2013). In this regard, Okokon et al. (2004) reported that level of *C. maculatus* damage to cowpea reached 50% after six months storage. Moreover, the pest infestations also affect seed quality, market value and reduce cowpea seed viability to 2% after three months of storage (Ukeh et al., 2011). Additionally, several studies pointed out the impact of *C. maculatus* on its host's functional and organoleptic properties. In this respect, Funtua et al. (2012) reported that the foam capacity and stability of the non-infested *Phaseolus vulgaris* flour is better than that of the bean infested flour. Similarly, Oyeyinka et al. (2013) demonstrated that water and oil holding capacities were greater in non-infested *V. unguiculata* grains compared to infested ones.

Previous investigations clearly indicated that infestation by *C. maculatus* had deleterious effects on the composition, nutritional quality and sensory properties of cowpeas. For example, Ojimelukwe and Ogwumike (1999) revealed that the pest infestations reduced the soluble carbohydrates contents of *V. unguiculata* seeds.

Despite the huge number of studies conducted on *C. maculatus*, little or no information is available on its economic injury level (EIL) under storage conditions. Umeozor (2005) mentioned that the establishment of an EIL for *C. maculatus* on *V. unguiculata* is an important component for a reliable estimation of economic weight losses. Moreover, Pedigo et al. (1986) specified that EILs may offer an improved understanding of physiological responses to injury of a pest. Additionally, Schmale et al. (2003) stated that in storage systems, the economic-injury level is very low.

The present work aims to investigate for the first time (i) the reproductive parameters and demographic traits of *C. maculatus* reared on two chickpea varieties, (ii) the quantification of chickpea losses through *C. maculatus* attacks and the establishment of an EIL and (iii) the impact of *C. maculatus* on chickpea functional and physical properties.

Materials and methods

Insect rearing and seed materials

C. maculatus rearing colonies were initiated from infested chickpea seeds (*Cicer arietinum* L.) provided by the Field Crops' Laboratory, Tunis, Tunisia since 2011. Rearing was conducted on two Kabuli type chickpea varieties namely Amdoun 1 (Pedigree Be-sel-81-48) and Beja 1 (Pedigree INRAT 93-1), provided by the national chickpea improvement program germplasm collection, in glass bottles of 1 l in a growth chamber at 30 ± 5 °C, 65 ± 5% R.H. and 12:12 Light:Darkness photoperiod. These substrates were chosen because they are the most commonly grown chickpea varieties in Tunisia.

Two food supply regimes, 10 and 100 g chickpea seeds, for ten pairs of *C. maculatus* were used. These corresponded to suboptimal and optimal food supply conditions, respectively, for the rearing of *C. maculatus* (Haouel, 2017). The flightless-form was used for this study.

Reproductive parameters and life table study

In order to synchronize the age of eggs, at the beginning of trials, ten pairs of *C. maculatus* were transferred from the rearing colony on each chickpea variety. After 24 h, 100 laid eggs on seeds of each variety were used for experiments. The seeds with only a single egg were transferred into Petri dishes and were carefully checked daily until the emergence of adults. Incubation and larval periods and their mortality were recorded on each chickpea variety. Duration of adult longevity was also recorded daily until death of last female. After emergence of adults, each female with one male was placed into Petri dish containing 20 g (≈ 30 seeds) of each chickpea variety. The duration of ovipositon and post-oviposition periods as well as longevity, daily fecundity (eggs per reproduction day), total fecundity (eggs during reproduction period), sex ratio and emergence rate were recorded.

Demographic traits study

The life histories of all individuals developed from those 100 eggs (males, females and immature stages) were analyzed according to age-stage and two-sex life table theory (Chi, 1988). The age stage specific survival rate (S_{xj}) (with x = age in days and j = stage), the age stage specific fecundity (f_{xj}), the age-specific survival rate (l_x), the age-specific fecundity (m_x) and the population growth parameters namely: the net reproduction rate (R_o), the mean generation time (T), the intrinsic rate of increase (r), the finite rate of increase (λ), and doubling time (DT) were calculated according to (Win et al., 2011; Khanamani et al., 2013).

The age-specific survival rate (l_x) comprises both female and male, was calculated according to Chi and Liu (1985) as:

$$l_x = \sum_{j=1}^{k} s_{xj} \qquad \text{With k:the number of stages}$$

The age-specific fecundity (m_x) was calculated as:

$$m_x = \frac{\sum_{j=1}^{k} S_{xj} f_{xj}}{\sum_{j=1}^{k} S_{xj}}$$

The net reproduction rate (R_o) was calculated according to Carey

(1993) as:

$$Ro = \sum_{j=1}^{k} l_x m_x$$

The mean generation time (T) can be defined as the average length of time between when an individual is born and the birth of its offspring approximated by the following formula (Birch, 1948):

$$T = \frac{\sum x\, l_x m_x}{\sum l_x m_x}$$

The intrinsic rate of increase (*r*) also called the intrinsic rate of natural increase or the innate capacity for increase was after that estimated using Carey (1993) formula as:

$$r = \frac{Ln\ Ro}{T}$$

The doubling time (*DT*) defined as the number of days required by a population to double was as well calculated according to Carey (1993).

$$DT = \frac{Ln\ 2}{r}$$

The finite rate of increase (λ) characterized the number of female offspring per female per day calculated according to Carey (1993) as:

$$\lambda = e^r$$

Seed germination test

Germination tests were carried out following the methodology of the International Seed Testing Association (ISTA, 1999). Three replicates of 100 seeds were placed between filter papers in Petri dishes containing each 6 ml of distilled water. Petri dishes were incubated at 25 ± 1 °C in the dark for 7 days for seed germination. Germination test was realized on the two chickpea varieties with three *C. maculatus* infestation rates (0; 5 and 80%). Seedlings with normal and abnormal growth, as well as ungerminated seeds, were counted.

Seed weight losses

This trial was conducted over a storage period of six months, seed weight losses were performed each month. The experiment consists on placing five pairs of *C. maculatus* (< 24 h old) in glass bottles containing 100 g of healthy chickpea seeds from each variety. The test was replicated three times. The females laying their eggs were left until they died. After laying eggs, the dead adults were removed from bottles. Hatched eggs were allowed to develop until adult emergence.

Infested and healthy seeds were separated, cleaned, counted, and finally weighed after completion of adult emergence each month. Seed infestation and weight loss were computed by using the following formulae (Anonymous, 1988).

$$A\% = \frac{Nd}{Nd + Nu}{}^{*}100$$

$$B\% = \frac{WuNd - WdNu}{Wd(Nd + Nu)}{}^{*}100$$

With: A% percent damage, B% percentage weight loss, Wu weight of undamaged seeds, Wd weight of damaged seeds, Nu number of undamaged grains and Nd number of damaged grains.

Economic injury levels

Callosobruchus maculatus economic injury levels were determined for each chickpea variety under optimal and suboptimal food supply conditions for each storage month for a period of six months' storage duration. EILs were calculated according to Pedigo et al. (1986) formula:

$$EIL = \frac{C^{*}N}{V^{*}I}$$

where C = management cost per production unit expressed with Tunisian Dinars/kg, N = the number of pests causing injury, V = market value per production unit expressed with Tunisian Dinars/kg, and I = the percent weight loss.

It has been observed that *C. maculatus* has a host trophic preference toward chickpea varieties. Thus, we include the trophic preferences in our economic injury level study expressed by the use of two chickpea varieties (Amdoun 1 and Beja 1). We also use different food support conditions and storage periods (1–6 months) in the study to capture some of the dynamics of changing in weight losses in order to project future injury potential. Thus, this study evaluated the value of the management costs per production and market value per production.

Functional and physical properties

Seed characteristics: physical and cooking properties

Seed weight, volume, density, diameter, hydration capacity, hydration index, swelling capacity, swelling index and cooking time were evaluated for both healthy and infected seeds according to the method of Williams et al. (1988).

Proximate composition

Healthy and infested seeds from the two chickpea varieties were used for these trials. Dry matter, fat, ash, protein contents, soluble carbohydrates, nutritional value, soluble solids (Brix), pH, least gelation concentration (LGC) and gel texture properties of the powders of two chickpea varieties were determined by employing standard methods of analysis (AOAC, 1984). Briefly, the crude protein content (N × 4.38) of the samples was estimated by the macro-Kjeldahl method; the crude fat was determined by extracting a known weight of powdered sample with petroleum ether, using a Soxhlet apparatus; the ash content was determined by incineration at 600 ± 15 °C. Total carbohydrates were calculated by difference. Energy was calculated according to the following equation:

$$\text{Energy(kcal)} = 4 \times (\text{g protein}) + 3.75 \times (\text{g carbohydrate}) + 9 \times (\text{g fat})$$

Statistical analysis

For each reproductive, demographic, damage, functional and physical properties parameters, data were subjected to two-way ANOVA, with variety and food supply or seed category as main fixed factors plus a variety ∗ food supply or variety ∗ seed category interaction term. The means were separated using the Least Significant Difference (LSD) ($p < 0.05$). Differences in values of each variety to the food supply or seed category were tested by one-way ANOVA followed by Duncan test. Where necessary, data were transformed by common logarithm or square root to meet the assumptions of normality. All values given were the mean of three replications and were expressed as the mean ± standard deviation ($\overline{x}$ ± SD). Significant differences are reported as $p < 0.05$. Correlations analyses (Pearson's correlation coefficient) were established between damage parameters (seed damage, weight losses) and variety, food supply and storage period. All statistical analyses were performed using SPSS statistical software version 20.0.

Results

Reproductive parameters

Tables 1 and 2 summarizes results of the impact of two food supply supports (suboptimal and optimal) on reproductive parameters of *C. maculatus* reared on two chickpea varieties (Amdoun 1 and Beja 1). Results showed that immature development period, mean number of

Table 1
Biological parameters of *C. maculatus* (mean ± SD) reared on two chickpea varieties under two supplies supports.

Biological parameters	Amdoun 1 variety		Beja 1 variety	
	Suboptimal	Optimal	Suboptimal	Optimal
Adult longevity (days)	7.0 ± 1.0$^{a.A}$	6.7 ± 0.6$^{a.A}$	7.7 ± 0.6$^{a.A}$	7.0 ± 0.0$^{a.A}$
Oviposition period (days)	7.0 ± 0.0$^{a.A}$	6.3 ± 0.5$^{a.A}$	6.7 ± 0.6$^{a.A}$	6.0 ± 1.0$^{a.A}$
Immature development period (days)	33.0 ± 1.0$^{a.A}$	38.3 ± 1.5$^{b.A}$	32.0 ± 1.0$^{a.A}$	39.7 ± 0.6$^{b.A}$
Mean number of eggs/♀	41.0 ± 2.6$^{a.A}$	87.3 ± 3.8$^{b.B}$	34.3 ± 3.2$^{a.A}$	46.6 ± 5.6$^{b.A}$
Fertility rate (%)	19.6 ± 7.3$^{a.A}$	38.1 ± 9.9$^{b.A}$	13.2 ± 4.0$^{a.A}$	47.2 ± 2.8$^{b.A}$

For each chickpea variety, comparisons were made between suboptimal and optimal food supports for each biological parameter (lowercase letters). Between varieties, comparisons were made between supplies (either suboptimal or optimal) each biological parameter (uppercase letters). Means followed by the same letters are not significantly different at the 5% threshold (ANOVA).

Table 2
Variety and food supplies effects on longevity, oviposition period, immature development period, mean eggs per female and fertility rate of *C. maculatus*. $p < 0.05$ is highlighted in italic.

Variables	d.f.	Mean square	F	P	η^2 partial
Longevity					
Variety	1	0.85	1.80	0.22	0.18
Food supplies	1	0.85	1.80	0.22	0.18
Variety * food	1	0.18	0.20	0.67	0.02
Oviposition period					
Variety	1	0.33	0.80	0.39	0.09
Food supplies	1	1.33	3.20	0.11	0.29
Variety * food	1	0.00	0.00	1.00	0.00
Immature development period					
Variety	1	0.18	0.17	0.79	0.01
Food supplies	1	126.85	108.64	*< 0.001*	0.93
Variety * food	1	4.18	3.50	0.09	0.30
Mean eggs/♀					
Variety	1	1680.33	105.02	*< 0.001*	0.93
Food supplies	1	2581.33	161.23	*< 0.001*	0.95
variety * food	1	867.00	54.18	*< 0.001*	0.87
Fertility rate					
Variety	1	5.42	0.12	0.73	0.02
Food supplies	1	2067.84	46.83	*< 0.001*	0.85
variety * food	1	177.34	4.02	0.08	0.33

eggs and fertility rate were affected by food supply ($F_{1,8}$ = 108.64, p < 0.001 for immature development period; $F_{1,8}$ = 161.23, p < 0.001 for mean number of eggs; $F_{1,8}$ = 46.83, p < 0.001 for fertility rate); whereas both longevity and oviposition period were not ($F_{1,8}$ = 0.20, p = 0.67 for longevity and $F_{1,8}$ = 0.00, p = 1.00 for oviposition period).

Adults longevity and oviposition period were not affected by host variety (Table 1). Results showed that optimal food supplies significantly offered better performances for adults in terms of the immature development period (38.30 and 39.70 days for Amdoun 1 and Beja 1 respectively), the mean number of eggs per female (87.30 eggs/♀ for Amdoun 1 and 46.6 eggs/♀ for Beja 1) and fertility rate (38.10% for Amdoun 1 and 47.20% for Beja 1).

For more details, results in Table 2 revealed that 93% of variance of the immature development period is accounted for by food supplies, whereas, variety accounts only for 1% and the interaction variety * food supplies (V * F) accounts for 30%. In addition, the variance of the mean number of eggs per female is explained 95% by food supplies, 93% by variety and 87% by their interaction.

Our results showed that for the immature development period, the mean number of eggs per female and fertility rate, food supplies influences the most the reproductive behavior of *C. maculatus*.

Demographic parameters traits

The demographic parameters of *C. maculatus* reared on two chickpea varieties under two food supply regimes are illustrated in Tables 3 and 4. Results demonstrated that the food supply significantly affected MGR (exclusively for Beja 1 variety), MRE (for Amdoun 1 variety), MRL (for Beja 1 variety), ER (for Amdoun 1 variety), SR and GT (Table 3). Besides, the intrinsic rate of increase r, the finite rate of increase λ were significantly influenced by variety (r: $F_{1,8}$ = 12.75, p = 0.01; λ: $F_{1,8}$ = 12.72, p = 0.01); and food supplies (r: $F_{1,8}$ = 8.78, p = 0.02; λ: $F_{1,8}$ = 8.78, p = 0.02) and their interaction (V * F) (r: $F_{1,8}$ = 6.04, p = 0.04; λ: $F_{1,8}$ = 6.05, p = 0.04) (Table 4). Furthermore, doubling time DT varied significantly according to variety ($F_{1,8}$ = 13.23, p = 0.01) and food supplies ($F_{1,8}$ = 8.51, p = 0.02) (Table 4). Contrary to all above parameters, net reproductive rate Ro was not affected neither by variety, nor by food supplies or their interaction (Table 4). Indeed, for Amdoun 1 respective values were 9.17 and 10.16 ♀/♀/generation for suboptimal and optimal food supplies

Table 3
Demographic parameters of *C. maculatus* reared on two chickpea varieties under two supplies supports.

Demographic parameters	Amdoun 1 variety		Beja 1 variety	
	Suboptimal	Optimal	Suboptimal	Optimal
Mean growth rate MGR (%)	0.80 ± 0.04$^{a.A}$	0.84 ± 0.06$^{a.A}$	0.69 ± 0.15$^{a.A}$	0.88 ± 0.01$^{b.A}$
Mortality rate of eggs MRE (%)	85.98 ± 4.46$^{b.A}$	60.56 ± 10.62$^{a.A}$	79.67 ± 7.07$^{b.A}$	51.86 ± 3.38$^{a.A}$
Mortality rate of larvae MRL (%)	10.17 ± 2.59$^{b.A}$	4.58 ± 0.56$^{a.A}$	17.88 ± 6.58$^{b.A}$	5.66 ± 2.19$^{a.A}$
Emergence rate ER (%)	16.83 ± 6.38$^{a.A}$	47.18 ± 2.86$^{b.A}$	12.53 ± 3.76$^{a.A}$	37.66 ± 10.29$^{b.A}$
Sex-ratio SR	0.51 ± 0.02$^{a.A}$	0.74 ± 0.12$^{b.A}$	0.58 ± 0.04$^{a.A}$	0.85 ± 0.05$^{b.A}$
Net reproductive rate Ro (♀/♀/generation)	9.17 ± 0.66$^{a.A}$	10.16 ± 1.58$^{a.A}$	8.51 ± 0.71$^{a.A}$	8.81 ± 0.78$^{a.A}$
Generation time GT (days)	40.00 ± 1.00$^{a.B}$	44.67 ± 1.15$^{b.A}$	38.67 ± 1.52$^{a.A}$	45.33 ± 2.08$^{b.B}$
Intrinsic rate of increase r (♀/♀/days)	0.049 ± 0.001$^{a.A}$	0.057 ± 0.004$^{b.B}$	0.047 ± 0.001$^{a.A}$	0.048 ± 0.001$^{a.A}$
Doubling time DT (days)	13.97 ± 0.46$^{b.A}$	12.04 ± 0.89$^{a.A}$	14.45 ± 0.54$^{a.A}$	14.23 ± 0.55$^{a.B}$
Finite rate of increase λ (♀/♀/days)	1.05 ± 0.01$^{a.A}$	1.06 ± 0.01$^{b.B}$	1.05 ± 0.01$^{a.A}$	1.05 ± 0.01$^{a.A}$

For each chickpea variety comparisons were made between suboptimal and optimal food supports for each biological parameter (lowercase letters). Between varieties comparisons were made between supplies (either suboptimal or optimal) each biological parameter (uppercase letters). Means followed by the same letters are not significantly different at the 5% threshold (ANOVA).

Table 4
Variety and food supplies effects on demographic parameters of *C. maculatus*. $p < 0.05$ is highlighted in italic.

Variables	d.f.	Mean square	F	P	η2 partial
MGR					
Variety	1	0.00	0.42	0.53	0.05
Food supplies	1	0.03	4.67	*0.06*	0.37
Variety * food	1	0.02	2.31	0.17	0.22
MRE					
Variety	1	57.92	4.20	0.07	0.34
Food supplies	1	238.03	17.25	*< 0.01*	0.68
Variety * food	1	33.09	2.40	0.16	0.23
MRL					
Variety	1	168.96	3.48	0.10	0.30
Food supplies	1	2125.82	43.76	*< 0.01*	0.85
Variety * food	1	4.27	0.09	0.77	0.01
ER					
Variety	1	143.21	3.39	0.10	0.30
Food supplies	1	2308.04	54.60	*< 0.01*	0.87
Variety * food	1	20.38	0.48	0.51	0.06
SR					
Variety	1	0.03	5.06	0.05	0.39
Food supplies	1	0.18	35.19	*< 0.01*	0.81
Variety * food	1	0.00	0.17	0.69	0.02
Ro					
Variety	1	3.01	2.94	0.12	0.27
Food supplies	1	1.25	1.22	0.30	0.13
Variety * food	1	0.37	0.36	0.57	0.04
GT					
Variety	1	0.33	0.15	0.71	0.02
Food supplies	1	96.33	42.81	*< 0.01*	0.84
Variety * food	1	3.00	1.33	0.28	0.14
r					
Variety	1	0.00	12.75	*0.01*	0.61
Food supplies	1	0.00	8.78	*0.02*	0.52
Variety * food	1	0.00	6.04	*0.04*	0.43
DT					
Variety	1	5.39	13.23	*0.01*	0.62
Food supplies	1	3.47	8.51	*0.02*	0.52
Variety * food	1	2.18	5.35	0.05	0.40
λ					
Variety	1	0.00	12.72	*0.01*	0.61
Food supplies	1	0.00	8.78	*0.02*	0.52
Variety * food	1	0.00	6.05	*0.04*	0.43

against respectively 8.51 and 8.81 ♀/♀/generation for Beja 1 (Table 3).

Age-specific survival rate (l_x) and fecundity (m_x) at age of adult emergence of *C. maculatus* reared on two chickpea varieties and two food supply supports were represented in Fig. 1. Results showed that *C. maculatus* could successfully survive and reproduce under the two food supply conditions. Under optimal conditions, fecundity (m_x) was significantly greater on Amdoun 1 ($m_x = 0.29$) than on Beja 1 ($m_x = 0.18$). Statistical analysis revealed significant differences regarding this parameter after 36 days ($t = 5.21$, df = 5, $p = 0.003$). Results revealed that the highly acceptability of Amdoun 1 variety by *C. maculatus* could be attributed to food and oviposition preferences toward this host (Fig. 1).

Thus, population growth parameters (demographic parameters) were more dependent on quantity of food (food supply conditions) than on host variety

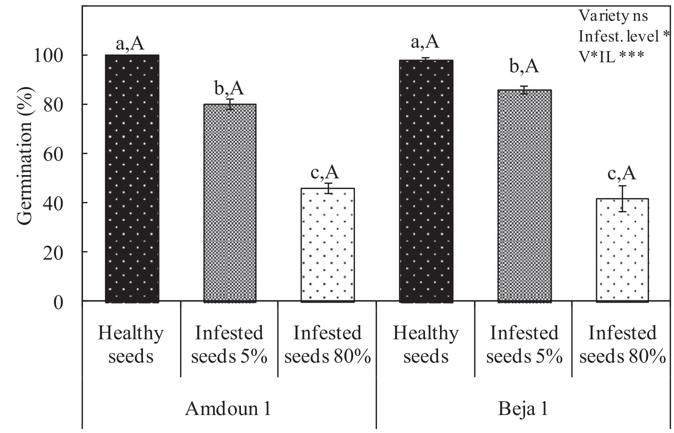

Fig. 2. Profiles of germination rate of chickpea seeds infested by *C. maculatus*. Different letters indicate significant differences (at $p < 0.05$) among infested levels for each chickpea variety (lowercase letters) and among varieties for each infested levels (uppercase letters). Each value is the mean ± SE of three replicate. ns, not significant at $p > 0.05$, $^{*}p < 0.05$,$^{***}p < 0.001$.

Damage of C. maculatus

Impact on seed germination

Data on germination of chickpea seeds under two infestation rates by *C. maculatus* (5 and 80%) was reported in Fig. 2. Results indicated that *C. maculatus* attacks affected the seed germination. Indeed, for each variety, significant differences were observed in germination rates between infested and non infested seeds (variety: $F_{1,12} = 0.01$, $p = 0.93$; infestation level: $F_{2,12} = 722.95$, $p < 0.001$ and their interaction V * IL: $F_{2,12} = 5.91$, $p = 0.02$). Moreover, germination reduction depended on *C. maculatus* infestation level. In fact, significant differences were obtained between germination of seeds infested at 5 and 80% for each variety (Fig. 2).

Impact on weight loss

The effects of *C. maculatus* attacks on seed damage and weight loss under two food supply regimes over a six month storage period are

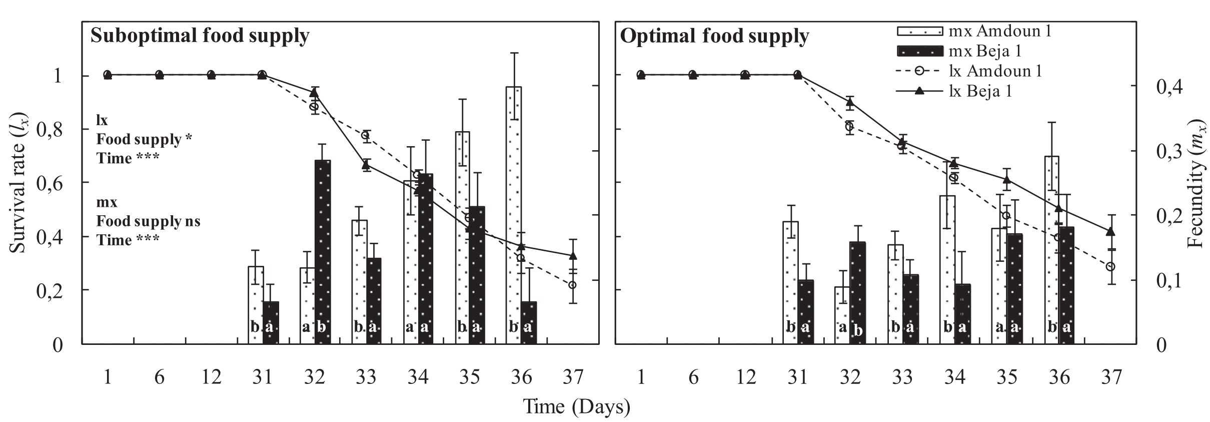

Fig. 1. Age-specific survival rate (l_x) and fecundity (m_x) of *C. maculatus* reared on two chickpea varieties under two food supply supports. Different letters indicate significant differences (at $p < 0.05$) among variety. Each value is the mean ± SE of three replicate. ns, not significant at $p > 0.05$, $^{*}p < 0.05$,$^{***}p < 0.001$.

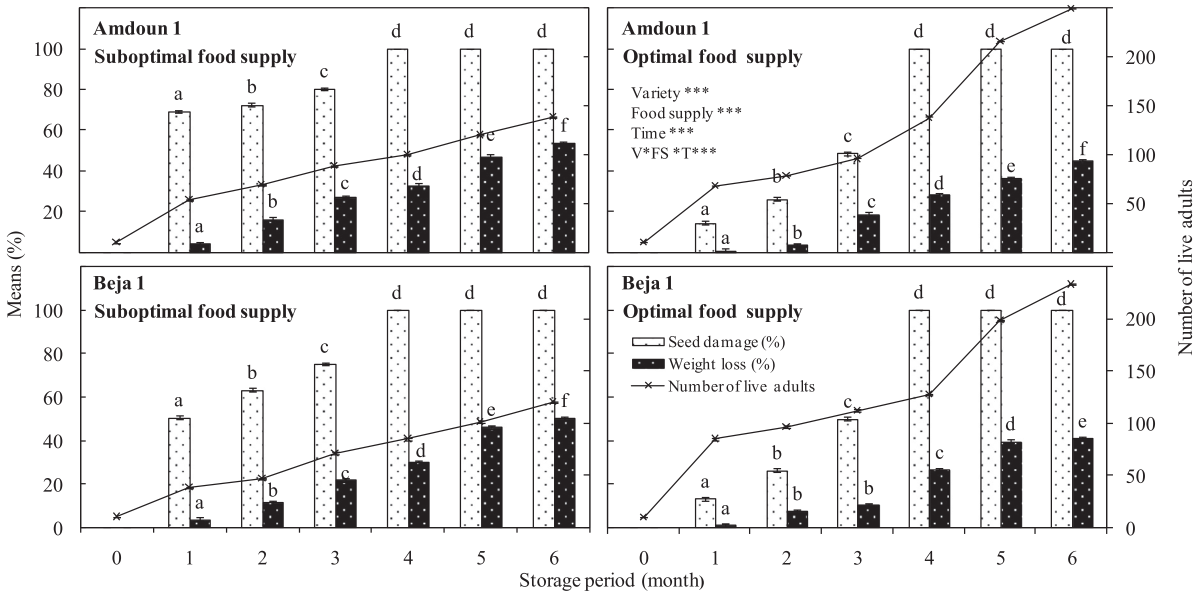

Fig. 3. Number of adults, seed damage (%) and weight loss (%) caused by *C. maculatus* reared in two chickpea varieties under two food supply regimes over a six month storage period. Different letters indicate significant differences (at $p < 0.05$) among storage period for each parameter (seed damage, weight loss) by Duncan test. Each value is the mean ± SE of three replicate. ns, not significant at $p > 0.05$, *$p < 0.05$, ***$p < 0.001$.

illustrated in Fig. 3. Food supply conditions have significant effects on seed damage ($F_{1,56} = 3309.53$, $p < 0.001$), weight loss ($F_{1,56} = 184.36$, $p < 0.001$) and population density of adults ($F_{1,56} = 9720.40$, $p < 0.001$). Moreover, varieties have significant effects on the seed damage ($F_{1,56} = 62.42$, $p < 0.001$), the weight loss ($F_{1,56} = 16.05$, $p < 0.001$) and the population densities of adults ($F_{1,56} = 233.30$, $p < 0.001$). Additionally, storage period significantly explained variation in seed damage ($F_{6,56} = 10{,}209.80$, $p < 0.001$), weight loss ($F_{6,56} = 988.77$, $p < 0.001$) and population density of adults ($F_{6,56} = 8805.93$, $p < 0.001$). Statistically significant differences between storage period-population densities of adults, seed damage-weight loss were obtained. Besides, we noticed that throughout the first three months of storage, the percentages of seed damage were much higher under suboptimal food supply conditions for both varieties (Fig. 3). Thus, weight losses of seeds are likely to increase much more quickly (weight losses percentages were respectively 27.36%, 22.00% for Amdoun 1 and Beja 1 after three months of storage).

Correlations analyses between damage parameters (seed damage, weight losses) and variety, food supply and storage period were recorded in Table 5. A highly positive correlation was recorded between the storage period and number of live adults ($r = 0.86$, $p < 0.001$), seed damage ($r = 0.89$, $p < 0.001$) and weight loss ($r = 0.96$, $p < 0.001$). However, a significant negative correlation was observed between seed damage and food supply (− 0.22, $p = 0.46$). Up four months of storage, increase in *C. maculatus* population was more dependent on food quantity rather than food variety.

Table 5
Correlations analyses between damage parameters and variety, supplies and storage period.

	Variety	Food supplies	Storage period	Seed damage	Weight losses
Seed damage	− 0.03	− 0.22*	0.89**		
Weight losses	− 0.05	− 0.17	0.96**	0.88**	
Live adults	− 0.06	0.37**	0.86**	0.72**	0.75**

* Significant at 5% level.
** Significant at 1% level.

Economic injury level

Results of EIL study were illustrated in Fig. 4. Results showed that the EILs depended on chickpea varieties and food supply conditions. The respective values were 25.2 and 39.8 insect/kg for Amdoun 1 and Beja 1 under suboptimal food supply conditions, while, these values reached 63.2 and 133.4 insect/kg under optimal food supply conditions.

Functional and physical proprieties

Impact of C. maculatus attacks on seed properties

The morphological characteristics and physical properties of infested chickpea seeds were presented in Table 6. Results indicated that seed sizes were not affected by *C. maculatus* attacks for both varieties (variety: $F_{1,8} = 1.74$, $p = 0.22$; seed category: $F_{1,8} = 0.12$, $p = 0.74$). Moreover, variety have significant effects on seed volume ($F_{1,8} = 7.44$, $p = 0.03$), while seed category (infested and healthy) was not a significant factor affecting seed volume ($F_{1,8} = 0.02$, $p = 0.89$).

Contrarily, all physical parameters were influenced. Seed weight, seed density, cooking time, hydratation capacity and swelling capacity were significantly different among varieties and seeds category.

Physico-chemical characteristics

Impact of C. maculatus attacks on proximate seed composition. The proximate composition of the seed flours from different chickpea varieties infested by *C. maculatus* were presented in Table 7. Results revealed that nutritional changes in the seeds infested by *C. maculatus* have occurred. In infested seeds, increases in percentages of protein, moisture and ash were observed, while decreases were noticed in percentages of crude fat, carbohydrate and nutritional values (Table 7).

The proximate seed composition was significantly influenced by variety and seed category. All studied parameters as protein (variety $F_{1,8} = 187.96$, $p < 0.001$; seed category: $F_{1,8} = 293.06$, $p < 0.001$), moisture (variety $F_{1,8} = 45.40$, $p < 0.001$; seed category: $F_{1,8} = 61.56$, $p < 0.001$), ash contents (variety $F_{1,8} = 7.16$, $p = 0.03$; seed category: $F_{1,8} = 99.66$, $p < 0.001$), carbohydrate (variety $F_{1,8} = 169.16$, $p < 0.001$; seed category: $F_{1,8} = 423.90$, $p < 0.001$) and nutritional values (variety $F_{1,8} = 13.68$, $p < 0.001$; seed category: $F_{1,8} = 367.87$, $p < 0.001$) were also affected by the interaction

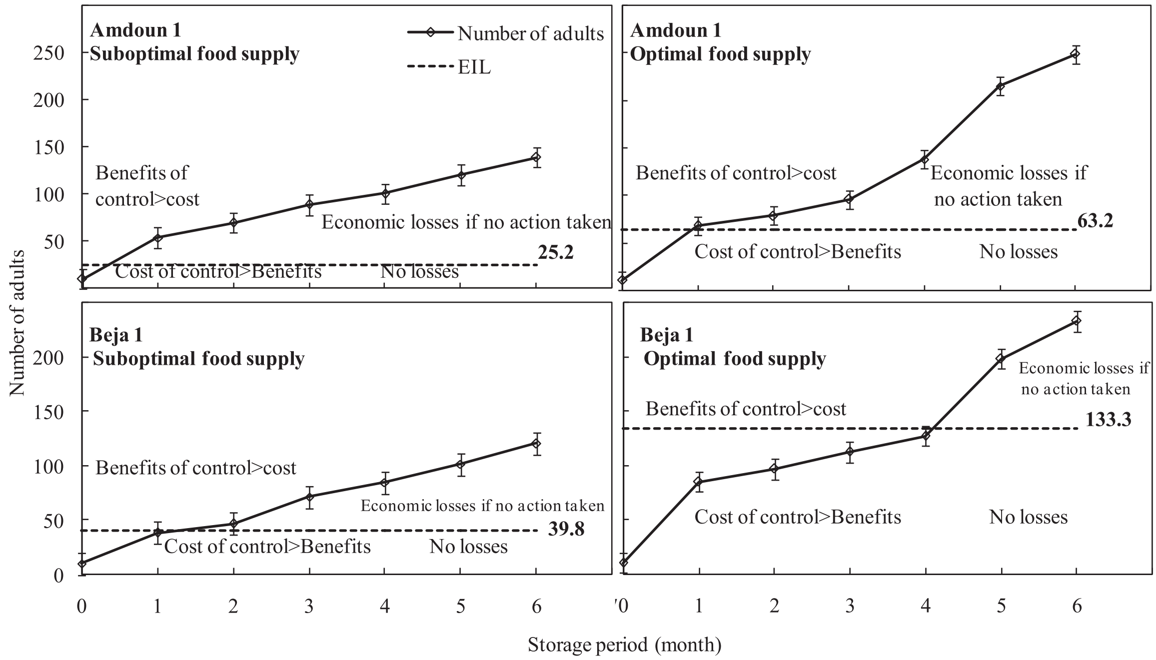

Fig. 4. Graphic representation of the Economic-injury Level (EIL) of *C. maculatus* and its relationship to economic loss, benefits, and costs. Values are means of three replications ± SD.

between variety and seed category. Only the crude fat was not significantly affected by variety and their interaction (variety $F_{1,8} = 0.13$, $p = 0.73$; their interaction $F_{1,8} = 4.46$, $p = 0.68$).

Impact of C. maculatus attacks on pH and soluble solids. The pH values of chickpea flours varied between infested and healthy seeds for each variety and between infested seeds for both varieties. Values ranged from 6.05 to 6.13 for infested seeds against 6.33 to 6.51 for healthy seeds (Table 8). Significant differences were found between healthy and infested seeds for each variety (variety $F_{1,8} = 3.35$, $p = 0.10$; seeds category: $F_{1,8} = 119.77$, $p < 0.001$).

Moreover, regarding soluble solid, Brix values varied significantly according to seed category (infested, healthy) rather than according to varieties. It ranged between 23.67 and 32.83 in infested seeds versus 34.00 and 35.67 in healthy seeds for Beja 1 and Amdoun 1 respectively (variety $F_{1,8} = 21.25$, $p < 0.001$; seeds category: $F_{1,8} = 10.84$, $p = 0.01$) (Table 8).

Impact of C. maculatus attacks on functional properties. No significant impacts of *C. maculatus* attacks were reported either on water and oil absorption index or on water solubility. Statistical analyses did not reveal any differences between infested and healthy flours (WAI: $F_{1,8} = 2.47$, $p = 0.15$; OAI: $F_{1,8} = 0.43$, $p = 0.53$; WSI: $F_{1,8} = 0.23$, $p = 0.65$) and between variety (WAI: $F_{1,8} = 3.98$, $p = 0.08$; OAI: $F_{1,8} = 0.09$, $p = 0.77$; WSI: $F_{1,8} = 2.42$, $p = 0.16$) (Fig. 5, A).

Results pointed out an increase of foaming capacity (FC) of infested flours compared to the control (Fig. 5, B). The FC values (percentage of entrapped gas) of infested flours varied between of 50.67 and 48.67% against 45 and 47% for non infested flours of Amdoun 1 and Beja 1 varieties, respectively. The foaming capacity was not significantly influenced by variety or seeds category (Amdoun 1: $F_{1,4} = 6.28$, $p = 0.07$; Beja 1 $F_{1,4} = 2.50$, $p = 0.19$).

Regarding foaming stability property, a decrease in foam volume as a function of time was observed for the two chickpea varieties flours (Fig. 5, C). Significant statistical differences were observed between seeds category (infested and healthy flours) and between variety (variety: $F_{1,24} = 5.00$, $p = 0.04$; seeds category: $F_{1,24} = 14.45$, $p = 0.001$; time: $F_{2,24} = 558.53$, $p < 0.001$).

Discussion

Despite the fact that the cowpea seed beetle *C. maculatus* is considered as the most important pest of stored food legume worldwide, this paper reported the first exhaustive investigation on the pest status,

Table 6
Impact of *C. maculatus* attacks on physical and cooking characteristics of chickpea seeds.

	Amdoun 1 variety		Beja 1 variety	
	Infested seeds	Healthy seeds	Infested seeds	Healthy seeds
Morphological characteristics				
Seed volume (ml)	$65.42 \pm 4.75^{b.A}$	$63.25 \pm 1.76^{a.A}$	$60.23 \pm 0.91^{b.A}$	$58.57 \pm 0.86^{a.A}$
Seed size (mm)	$6.95 \pm 0.04^{a.A}$	$6.95 \pm 0.30^{a.A}$	$6.93 \pm 0.03^{a.A}$	$6.91 \pm 0.62^{a.A}$
Physical parameters				
Seed weight (g/100 g)	$29.82 \pm 0.11^{b.A}$	$31.40 \pm 0.34^{a.A}$	$31.15 \pm 0.11^{b.B}$	$33.27 \pm 0.80^{a.B}$
Seed density (g/ml)	$0.45 \pm 0.31^{a.A}$	$0.49 \pm 0.24^{b.A}$	$0.52 \pm 0.01^{a.B}$	$0.57 \pm 0.02^{b.B}$
Cooking time (min)	$115.66 \pm 1.53^{a.B}$	$120.66 \pm 3.78^{b.B}$	$74.66 \pm 4.51^{a.A}$	$80.66 \pm 3.78^{b.A}$
Hydratation capacity (g/seed)	$0.44 \pm 0.06^{a.B}$	$0.66 \pm 0.15^{b.B}$	$0.30 \pm 0.05^{a.A}$	$0.32 \pm 0.03^{a.A}$
Swelling capacity (ml/seed)	$0.41 \pm 0.03^{a.A}$	$0.56 \pm 0.02^{b.B}$	$0.40 \pm 0.01^{b.A}$	$0.33 \pm 0.40^{a.A}$

For each chickpea variety, comparisons were made between infested and healthy seeds for each parameter (lowercase letters). Between varieties, comparisons were made among infested seeds, healthy seeds for each parameter (uppercase letters). Means followed by the same letters are not significantly different at the 5% threshold (ANOVA).

Table 7
Impact of *C. maculatus* attacks on proximate composition (%) of chickpea flour.

	Amdoun 1 variety		Beja 1 variety	
	Infested flour	Healthy flour	Infested flour	Healthy flour
Protein (%)	33.05 ± 0.62$^{b.B}$	22.53 ± 0.93$^{a.A}$	23.77 ± 0.17$^{b.A}$	21.81 ± 0.57$^{a.A}$
Moisture (%)	10.80 ± 0.20$^{b.A}$	10.67 ± 0.26$^{a.A}$	12.71 ± 0.31$^{b.B}$	10.64 ± 0.17$^{a.A}$
Ash (%)	4.64 ± 0.15$^{b.B}$	3.36 ± 0.11$^{a.A}$	4.06 ± 0.31$^{b.A}$	3.44 ± 0.09$^{a.A}$
Crude fat (%)	3.53 ± 0.25$^{a.A}$	4.73 ± 0.15$^{b.A}$	3.28 ± 0.19$^{a.A}$	4.91 ± 0.70$^{b.A}$
Carbohydrates (%)	47.96 ± 0.58$^{a.A}$	58.69 ± 0.85$^{b.A}$	56.16 ± 0.05$^{a.B}$	59.19 ± 0.53$^{b.A}$
Nutritional value (kcal)	355.90 ± 1.57$^{a.B}$	367.51 ± 1.40$^{b.A}$	349.29 ± 1.22$^{a.A}$	368.22 ± 1.29$^{b.A}$

For each chickpea variety, comparisons were made between infested and healthy flour for each parameter (lowercase letters). Between varieties, comparisons were made among infested flour, healthy flour for each parameter (uppercase letters). Means followed by the same letters are not significantly different at the 5% threshold (ANOVA).

Table 8
Impact of *C. maculatus* on pH and soluble solid (Brix) of chickpea flour.

	Amdoun 1 variety		Beja 1 variety	
	Infested flour	Healthy flour	Infested flour	Healthy flour
pH	6.13 ± 0.01$^{a.B}$	6.33 ± 0.02$^{b.A}$	6.05 ± 0.02$^{a.A}$	6.51 ± 0.09$^{b.A}$
Brix	32.83 ± 0.76$^{a.A}$	35.67 ± 0.58$^{b.A}$	23.67 ± 2.31$^{a.A}$	34.00 ± 1.00$^{b.A}$

For each chickpea variety, comparisons were made between infested and healthy flour for each parameter (lowercase letters). Between varieties, comparisons were made among infested flour, healthy flour for each parameter (uppercase letters). Means followed by the same letters are not significantly different at the 5% threshold (ANOVA).

population dynamic, growth and demographic parameters, economic injury level and the impact on nutritional proprieties of *C. maculatus* reared on two Tunisian chickpea varieties.

Results revealed that *C. maculatus* is a major beetle pest on stored chickpea in Tunisia. These findings are consistent with those reported by Kedia et al. (2015) and Iturralde-García et al. (2016) indicating that *C. maculatus* is the main pest of stored chickpea. Previous works reported that *C. maculatus* reproductive parameters varied according to host plants. In this respect, Obopile et al. (2011) specified that percentage of adult emergence, oviposition and developmental period depended on cowpea landraces. Moreover, highest mean egg counts and high percent of adult emergence were obtained on chickpea compared to other legume seeds (Swella and Mushobozy, 2009). Besides, previous studies pointed out that demographic parameters varied considerably according to food hosts. In this context, Heidari et al. (2016) and Modarres-Najafabadi et al. (2014) showed that the net reproductive rates (Ro) of *C. maculatus* were 38.70 and 79.98 for offspring reared on *Vigna radiata* (L.); toward 14.90 and 49.90 for offspring reared on *Vigna unguiculata* (L.) (Bellows, 1982; Credland, 1986) and 6.82 for offspring reared on *Cicer arietinum* (Douiri et al., 2014). Moreover, the values for intrinsic rate of increase (r) were 0.09 day^{-1} on *V. radiata* against 0.02 day^{-1} on *C. arietinum* and *V. unguiculata* (Douiri et al., 2014; Heidari et al., 2016).

The comparative study of the demographic traits between the two chickpea varieties and two food supply conditions performed in our study revealed that the parameters: intrinsic rate of increase (r), doubling time (DT) and finite rate of increase (λ) highly correlate with the preferred chickpea variety Amdoun 1, whereas means growth rate, larvae and eggs mortalities, adults' emergence, sex ratio and generation time correlated with food supply conditions. Thus, we can conclude that food supplies interfere with individual and population demographic traits in one-dimensional manner, resulting in the fact that if individual fitness adaptive traits increased, the population size and reproductive and demographic performances increased. These findings agree with those reported by Metz et al. (2008). In addition, our results are in accordance with those reported by Bull and Bonsall (2008) who indicated that the interaction between environmental limitation and population regulation can affect the dynamics and abundance of populations. Moreover, Ferriere and Legendre (2013) indicated that population viability is determined by the interplay of environmental influences and individual phenotypic traits shaping life histories and behavior. In this respect, Vamosi and Lesack (2007) and Schirmer et al. (2008) indicated that factors that may result in suboptimal conditions include an insufficiency of quantity or a low quality of food and space.

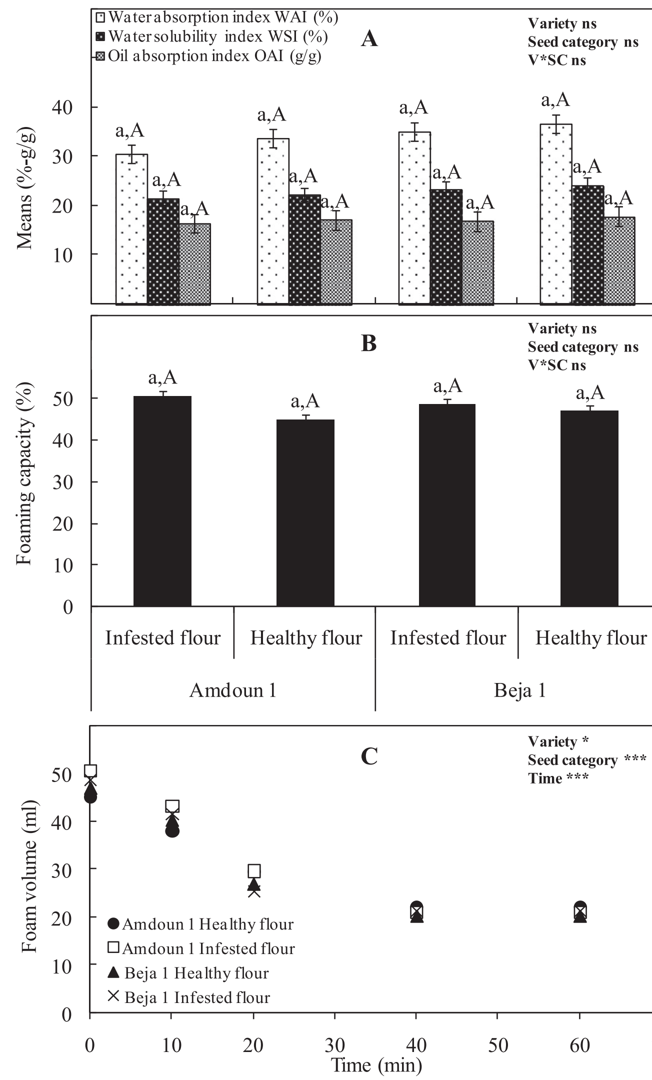

Fig. 5. Impact of *C. maculatus* attacks on water and oil absorption index, water solubility index (A); foaming capacity of flour (B) and foaming stability (C). Different letters indicate significant differences (at $p < 0.05$) among seed category (infested and healthy flour) for each variety (lowercase letters), and among varieties for each seed category (uppercase letters). Each value is the mean ± SE of three replicate. ns, not significant at $p > 0.05$, $^{*}p < 0.05$, $^{***}p < 0.001$.

Moreover, Shorrocks (1970) showed that resource limitation results in slower growth and smaller body sizes. Furthermore, Smallegange and Tregenza (2008) reported that with increasing larval density and limited food availability, *C. maculatus* females showed a reduction in their growth rate when food was more limited, emerging smaller after the same time.

On the other hand, *C. maculatus* is one of the most destructive pest species of many food legumes with larvae being internal feeders. Our study demonstrated that in case of heavy infestations, this beetle induced considerable weight losses and impairment of germination in chickpea damaged seeds. Previous works indicated that cowpea weevil infestations reduced seed weight considerably (Sarwar, 2012).

In Tunisia, chickpea production has significantly increases in the last years despite the major biotic constraints including insect pests. However, no economic studies were undertaken to assess the injury of these pests on yield and grain quality. Earlier works by Stern et al. (1959), Pedigo et al. (1986) and Mi et al. (1998) have reported that a basic component of decision making in pest management is the economic injury level. In this respect, Haouel-Hamdi et al. (2017) showed that EIL depended on food hosts. The respective value was 83 insect/kg for lentil. Thus, this study will provide reasons for farmers and food legume conditioners to make a decision to take a control action against *C. maculatus* during storage. Moreover, this work pointed out the variability of EILs with host varieties. Consequently, a complementary study on the Economic Threshold is required for a best postharvest management of *C. maculatus* populations.

Besides, this work pointed out to one consistent finding that the physicochemical proprieties of the chickpea substrate on which larvae feed affects the infestation level of this food. Indeed, protein, moisture and ash contents increased in infested seeds, while, crude fat, carbohydrate and nutritional values decreased in infested seeds. A similar trend has been observed by Bamaiyi et al. (2006) and Mbah and Silas (2007) which reported an increase in protein, moisture and ash contents with severity of infestation and a decrease of crude fat and carbohydrate on cowpea. This decrease in carbohydrate with the severity of infestation may be a result of the feeding activities of the larvae buried deep in the seeds; and the increase in protein content with the severity of infestation may be due to the eggs, egg cases, excretory products left behind on removal of larval, pupal and adult stages of *C. maculatus* before analysis (Mbah and Silas, 2007).

Functional properties including solubility, water and oil holding capacity, foaming capacity and stability, emulsifying activity and gel formation are not only important in the preparation processing and storage behavior of food systems but also they affect the sensory, nutritional and textural attributes of end products (Ghribi et al., 2015).

This study provides reasons for farmers, traders and industrials to make a decision to take a control action against *C. maculatus* during storage in order to preserve the nutritional value of chickpea flour for a potential application to substitute the wheat flour.

Conflict of interest

I hereby attest that all authors have no conflict of interest of any authority or persons in the field of our work in national and international levels.

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