J. Crop Prot. 2018, 7 (3): 273-281

Research Article

Laboratory evaluation of six improved cowpea *Vigna unguiculata* varieties for susceptibility to seed beetle, *Callosobruchus maculatus* (Coleoptera: Chrysomelidae) in Storage

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Abstract: Laboratory experiments were carried out to evaluate the susceptibility of six improved cowpea varieties to seed beetle, *Callosobruchus maculatus* (Coleoptera: Chrysomelidae) attack at a temperature of 29 ± 2 °C and a relative humidity of 65 ± 3%. Fifty grams of each variety were weighed in Kilner jars (250ml) and infested with three females and two males of *C. maculatus* that were 1-2 days old. Data recorded were the physical characteristics of the cowpea varieties, number of eggs laid, percentage egg survival, number of emerged adults, duration of emergence (DE), mean developmental period (MDP), percentage seed weight loss, percentage seed damage and susceptibility index. The experiment was a completely randomised design with three replications. Results showed that significant differences ($p < 0.05$) existed in DE and MDP among the cowpea varieties studied. IT90K-76, IT98K-131, IT11D-15-21 and IT07K-299-6 performed significantly better in duration of emergence than IT10K-866-1 and TVx 3236. IT98K-131-1 performed significantly better in median developmental period (26.67 days) than other varieties except for IT90K-76 (22.67 days). Results showed that IT90K-76, IT10K-866-1 and IT98K-131-1 were moderately resistant while IT07K-299-6, IT11D-15-21 and TVx 3236 were susceptible. This study recommends IT90K-76 because it performed appreciably better in these parameters than the other cowpea varieties when infested with *C. maculatus* in storage.

Keywords: Grains, insect activity, non-chemical control, resistance, storage

Introduction

Cowpea, *Vigna unguiculata* (L.) Walpers, is a common food crop throughout Nigeria but particularly in the middle belt and drier northern regions (Ojuederie *et al.*, 2009; Agbogidi and Egho, 2012). The crop is considered as of nutritional and health value to man and livestock (Abebe *et al.*, 2005; Agbogidi, 2010; Ghaly and Alkoaik, 2010). Cowpea feeds millions of people in developing countries with an annual world-wide production estimated around 4.5 metric tons on 12-14 million ha (Diouf, 2011). Cowpea provides a significant amount of calories; it is a good source of vitamins and minerals and provides a significant amount of dietary protein (Sule *et al.*, 2014).

Storage insect pests (e. g., *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae) cause serious losses of leguminous crops in both quality and quantity, particularly in the tropics and sub-tropics (Kenemi *et al.*, 2011).

Handling Editor: Saeid Moharramipour

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Received: 20 September 2017 Accepted: 5 June 2018
Published online: 15 July 2018

The larvae feed on the seed contents (Ali *et al*., 2004; Swella and Mushobozy, 2007) and develop inside the seeds and emerge as adults. Larval emergence holes (windows) in seeds are symptomatic of infestations in storage. Infestation starts on the field and continues in storage where heavy damage is done. The pest generates exceedingly high levels of infestation even when they pass only one or two generations on the host plant (Amusa *et al*., 2014).

In Nigeria, consequent upon the damage and losses associated with behavioural activities of *C. maculatus* on stored cowpea seeds, farmers employ the use of synthetic insecticides which is often accompanied by misuse, prohibitive cost, environmental hazards, detrimental effects on the user and insecticide resistance. In the developed countries, conventional fumigation technology is currently being scrutinized for many reasons such as ozone depletion potential of methyl bromide and carcinogenic concerns with phosphine (Adedire *et al*., 2011). Varietal susceptibility is a potential challenge to food security. This study was therefore initiated to evaluate the susceptibility of six improved cowpea varieties to seed beetle, *C. maculatus* in storage.

Materials and Methods

Study Location

The study was conducted in the laboratory of the Department of Crop Protection, Faculty of Agriculture, University of Ilorin, Ilorin, Nigeria at a temperature of 29 ± 2 °C and a relative humidity of 65 ± 3%.

Insect culture

Adults of *C. maculatus* were collected from infested cowpea seeds at the Insectary of the Nigerian Stored Products Research Institute (NSPRI) Headquarters, Ilorin, Nigeria and then introduced into dry, susceptible cowpea seeds bought from a local market in Ilorin. At 7 days after infestation, all parent beetles were removed from the jars. Freshly emerged adults of similar age were used for the experiment.

Physical assessment of the seeds

The physical traits such as seed length, breadth and width were measured using vernier calliper while seed colour and seed coat texture were assessed by observation and physically. The mean seed weight per cowpea variety was calculated using the method of Maina *et al.*, (2012). The seed dimensions were determined by dividing the readings of ten randomly selected seeds by ten.

Source and preparation of cowpea varieties

Six cowpea varieties used for this study (IT10K-866-1, IT90K-76, IT07K-299-6, IT11D-15-21, IT98K-131-1 and TVx 3236) were obtained from the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. The cowpea varieties were disinfested by placing them in a deep freezer at -20 ± 2 °C for 48 hours to kill off any immature stages of insects prior to the experiment. The cowpea varieties were placed on a laboratory table and allowed to thaw for 48 hours prior to use.

Experimental procedure

No-choice test method was adopted for the study. Fifty grams of each cowpea variety were weighed into 250ml Kilner jars and infested with three females and two males of 1-2 days old adults of *C. maculatus.* Each cowpea variety introduced into the jars was arranged in completely randomised design with three replications. The jars were covered with muslin cloth and fixed with rubber band to allow for aeration and prevent insect escape. On the 5^{th} day, the number of eggs laid on twenty seeds per replicate was counted and mean number of eggs laid on each variety was determined. Other data recorded included: percentage egg survival, duration of adult emergence, median developmental period and number of emerged adults. The study was terminated on the 90^{th} day when all insects were removed and the percentage seed weight loss, percentage seed damage and susceptibility index were calculated.

Percentage egg survival
The percentage survival of eggs was calculated by the number of eggs laid expressed as a percentage of the number of emerged adults:

$$Egg\ survival\ (\%) = \frac{No.\ of\ eggs\ laid}{No.\ of\ emerged\ adults} \times 100$$

Duration of emergence
Duration of emergence was taken as the difference between initial day of emergence and final day of emergence.

Median developmental period
The median development period was calculated as the time (in days) from the middle of the oviposition period to the emergence of 50% of the F_1 progeny (Dobie, 1977).

Adult emergence
At twenty (20) days after infestation (DAI), live and dead beetles were removed and discarded to prevent overlap with first generation. Thereafter, inspection of each container took place on daily basis and counting of freshly emerged adults continued until no further emergence was noted at 37 days after infestation.

Percentage seed weight loss
The contents of each container were sieved to remove dust, frass and insects. All insects were removed and the seeds were sorted into damaged and undamaged seeds.
Percentage seed weight loss was determined using the count and weight method of Gwinner *et al.* (1996).

$$Seeds\ weight\ loss\ (\%) = \frac{(Wu \times Nd) - (Wd \times Nu)}{Wu \times (Nd + Nu)} \times 100$$

Where
Wu = Weight of undamaged seed,
Nu = Number of undamaged seed,
Wd = Weight of damaged seed, and
Nd = Number of damaged seed.

Percentage seeds damaged were calculated according to the method described by Lephale *et al*. (2012).

$$Seeds\ damage\ (\%) = \frac{Number\ of\ seeds\ damaged}{Total\ number\ of\ seeds} \times 100$$

Susceptibility index
The susceptibility index was calculated using the method of Dobie (1974). This involves the number of F_1 progeny and the length of median development period.

$$Susceptibility\ Index\ (SI) = \frac{Log_e F_1}{MDP} \times 100$$

Where
$Log_e F_1$ = Natural logarithm of the total number of F_1 progeny emerged
MDP = Median developmental period.
The Dobie index was used to classify the cowpea varieties into susceptibility groups (Dobie, 1974):
Dobie index of 0 to 4 classified as resistant;
Dobie index of 4.1 to 7.0 moderately resistant;
Dobie index of 7.1 to 10.0 susceptible; and
Dobie index of ≥ 10.1 classified as highly susceptible.

Data analysis

Data obtained were subjected to analysis of variance to determine significant differences and the means were separated using Tukey Honest Significant Difference test at 5% level of significance.

Results

Table 1 shows that there was no significant difference ($p > 0.05$) in the parameters examined among the cowpea varieties studied except duration of emergence and median developmental period. The highest mean number of eggs laid (11.67 eggs/20 seeds) was recorded on IT07K-299-6 from which mean number of emerged adults recorded was 53.67; though the highest mean number of emerged adults was observed on TVx 3236 (54.33). The least mean number of eggs laid (3.00 eggs/20 seeds) was recorded on IT10K-866-1 from which least mean number of emerged adults was 13.00. The mean number of emerged adults of *C. maculatus* from all the cowpea varieties varied, although this was not significantly different ($p > 0.05$).In this study, percentage egg survival tended to be lower in the moderately resistant varieties (IT90K-76, IT10K-866-1 and IT98K-131-1) than in the susceptible varieties. Thus the most susceptible variety (IT11D-15-21) had the highest percentage egg survival (50.00) compared to other varieties.

Table 1 Mean number ± SE of eggs laid, eggs survival, emerged adults, duration of emergence and median developmental period, weight loss, seeds damage and susceptibility indices/ranking of cowpea varieties.

Variety	NEL	PES (%)	MEA	DE (days)[1]	MDP (days)[1]	Seed Wt. Loss (%)	Seed Damage (%)	SI	Status
IT90K-76	8.67 ± 3.48	23.22 ± 4.49	35.00 ± 10.69	18.67 ± 0.67[a]	22.67 ± 1.45[ab]	0.13 ± 0.60	4.04 ± 2.06	6.67 ± 0.52	MR
IT10K-866-1	3.00 ± 1.00	24.94 ± 3.49	13.00 ± 7.51	5.67 ± 2.40[c]	18.33 ± 0.88[b]	0.47 ± 0.25	8.98 ± 2.27	5.43 ± 1.54	MR
IT98K-131-1	11.00 ± 2.08	26.65 ± 11.88	30.67 ± 12.41	16.67 ± 2.33[ab]	26.67 ± 1.45[a]	0.72 ± 0.28	10.13 ± 3.91	5.16 ± 0.69	MR
IT11D-15-21	11.00 ± 2.08	50.00 ± 19.47	43.67 ± 3.33	10.00 ± 0.00[abc]	20.00 ± 1.00[b]	0.48 ± 0.21	11.50 ± 0.37	8.22 ± 0.35	S
IT07K-299-6	11.67 ± 4.37	41.60 ± 17.92	53.67 ± 30.78	9.33 ± 2.91[abc]	19.33 ± 0.88[b]	4.72 ± 3.96	26.75 ± 18.44	7.35 ± 2.16	S
TVX-3236	7.67 ± 3.28	30.40 ± 6.56	54.33 ± 36.12	7.00 ± 2.52[bc]	19.33 ± 0.33[b]	0.34 ± 0.10	13.71 ± 8.70	7.62 ± 2.07	S
	ns	ns	ns			ns	ns	ns	

[1] Means followed by different superscript(s) in the same column are significantly different at p = 0.05 using HSD Tukey test.
NEL: Number of eggs laid, PES: Percentage egg survival, MEA: Mean number of emerged adults, DE: Duration of emergence MDP: Median developmental period, SI: Susceptibility index, MR: Moderately resistant, S: susceptible, ns = Not significant.

The shortest duration of emergence and mean developmental period occurred on the IT98K-76 and produced lowest number of emerged beetles with the lowest seed weight loss and seed damage. The longest developmental period occurred on IT98K-131-1 followed by IT98K-76. High number of emerged adults caused high seed weight loss and seed damage, which was a clear indication of varietal susceptibility to *C. maculatus* (Table 1). There was no significant difference ($P > 0.05$) between the percentage seed weight loss and percentage seed damage of the cowpea varieties evaluated 90 days after infestation (Table 1). The least percentage seed weight loss (0.13) and seed damage (4.04) were observed on IT90K-76 while IT07K-299-6 recorded the highest percentage of seed weight loss (4.72) and seed damaged (26.75%) respectively.

There were no significant differences ($p > 0.05$) among the seed dimensions of the six cowpea varieties studied. TVx 3236 recorded the biggest seed weight (0.18g) and shortest length (4.45 mm) while IT98K-131-1 recorded the longest seed length (4.65 mm). IT10K-866-1 and IT07K-299-6 recorded the smallest seed weight (0.15g). IT07K-299-6 and IT10K-866-1 recorded the broadest seed breadth (4.68 mm) while IT98K-131-1 recorded the shortest seed breadth (4.23mm). It was observed that the cowpea varieties had rough seed coat texture except IT11D-15-21 which was observed to bear smooth coat texture. IT90K-76, IT98K-131- 1 and IT11D-15-21 were brown while IT10K-866-1, IT07K0299-6 and TVx 3236 were white.

Mean duration of emergence and median developmental period were significantly different ($p < 0.05$) among the six cowpea varieties studied (Table 1) with the highest mean values of the parameters recorded on IT90K-76 and IT98K-131-1, respectively. However, the lowest mean value of both parameters was observed on IT10K-866-1. IT90K-76 performed significantly better in duration of emergence (18.67 days) than IT10K-866-1 (5.67 days) and TVx 3236 (7.00 days). IT98K-131-1 performed significantly better in median developmental period (26.67 days) than other cowpea varieties except IT90K-76 with median developmental period of 22.67 days.

Discussion

Results revealed that breeders have developed cowpea varieties that are prone to *C. maculatus* infestation and the need to establish degree of susceptibility among the cowpea varieties to the seed beetle. This study therefore observed that IT90K-76 was the least preferred by the storage insect pest on the grounds of least number of eggs laid, emerged adults, lowest percentage egg survival and longest duration of emergence coupled with the susceptibility rating that considered it one of the moderately resistant cowpea varieties. Susceptibility emerges as a mechanism to maximize losses caused by *C. maculatus* during storage particularly under no intervention after infestation. The higher mean numbers of emerged adults in the susceptible varieties (IT11D-15-21, IT07K-299-6 and TVx 3236) were a consequence of the larger percentage egg survival. It could therefore be deduced that susceptible varieties favoured the development of larger number of emerged adults. The results agreed with the previous report (Garcia-Lara *et al.*, 2004) that progeny tended to be higher in the susceptible than in the resistant varieties.

The physical characteristics of seeds can determine the acceptability for oviposition but may not be related to the antibiotic nature of the seed (Messina and Renwick, 1985). Seed properties including: seed test a colour, mass, and size generally do influence the susceptibility of cowpea seeds to *C. maculatus* in storage (Maina and Lale, 2005; Maina and Dlamini, 2009). In this study, the rough seeds of IT07K-299-6 had higher number of egg load than the smooth seeds of IT11D-15-21. Nwanze *et al*. (1975) reported that rough seeds were less acceptable to *C. maculatus* than smooth ones. Murdock *et al*. (1997) indicated that varieties with smooth and glossy seed coat constantly were less preferable and therefore more resistant than rough seeded varieties. However, this present study revealed that though the highest number of eggs was laid on a rough seeded variety (IT07K-299-6), the only smooth variety present (IT11D-15-21) showed higher number of eggs laid than some of the rough varieties present. Hence, the preference for oviposition may not be attributed to the seed coat nature. Edde and Amatobi (2003) report indicated that seed coat plays no role in the resistance of cowpea to bruchid infestation.

This study has shown that moderately resistant cowpea varieties were recognised irrespective of seed size. Lephale *et al*. (2012) reported that the larger grains supply more food and space for insect growth and that the smaller grains or grains with less mass offer more resistance to pest infestation than larger grains. Results were similar to previous findings that seed properties including seed test a colour, mass, size and moisture content generally did not influence the susceptibility of cowpea seeds to *C. maculatus* in storage (Maina and Lale, 2005; Maina and Dlamini, 2009).

There was no significant difference in the percentage seed damage of the cowpea varieties and majority showed a low percentage seed damage however, the three cowpea varieties (TVX-3236, IT07K-299-6 and IT11D-15-21) with higher percentage seed damage were indicated by susceptibility index to be susceptible to *C. maculatus* infestation.

The susceptibility of the cowpea varieties to the insect pest was significantly influenced by duration of adult emergence and mean developmental period. It has been previously reported that chemical and nutritional compositions of grains were important primarily in resisting insect attack and damage (Dobie, 1974). In another investigation, percent grain damage ranging from 1.94 to 28.57 have been reported on selected maize genotypes screened for resistance to *S. zeamais* (Nwosu *et al.*, 2015). Susceptibility is indicated as the potential rate of increase of a pest population. According to the susceptibility index (Dobie, 1974), varieties IT11D-15-21, IT07K-299-6 and TVX-3236 were classified as susceptible, and IT90K-76, IT10K-866-1 and IT98K-131-1 as moderately resistant. TVX-3236 has the highest adult emergence. This is similar to the report made by Amusa *et al*. (2014) who also made the same assertion that the variety had the least tolerance to *C. maculatus*. The cowpea varieties showed that they are adequate for attack by *C. maculatus* and this can be observed in the absence of resistance to the insect pest among the cowpea varieties. The observed differences were very likely to be due to variations in the composition or levels of chemical substances that either deter or stimulate bruchid oviposition and/or feeding in these seeds (Gatehouse *et al.*, 1979). The range of susceptibility indices obtained was similar to the report of Siwale *et al.* (2009)who recorded a susceptibility index range of 0.77 to 7.11.The differences in susceptibility could be attributed to the variability in physical characteristics of the varieties, insect behaviour, genotype and environmental effects. Duarte *et al*. (2005) reported that genotype had a much larger influence on grain quality parameters than environment. In this study, the improved cowpea varieties were highly prone to postharvest infestation by *C. maculatus*. The susceptibility ranking according to Dobie (1974) indicates that IT90K-76, IT10K-866-1 and IT98K-131-1 were moderately resistant while IT11D-15-21, IT07K-299-6 and TVX-3236 were susceptible. On a comparative scale however, IT98K-131-1 was the moderately resistant with a susceptibility index of 5.16 while IT11D-15-21 was the susceptible with a susceptibility index of 8.22 followed by TVx 3236 and IT07K-299-6 with susceptibility indices of 7.62 and 7.35 respectively. However, since the cowpea varieties studied have not been able to confer complete resistance against cowpea beetle, there is therefore the need for more detailed studies on the genetic composition.

There is need for more detailed studies on the genetic composition of IT90K-76 to determine the inherent factors responsible for response of *C. maculatus* to the variety. This study shows that improved varieties are susceptible to *C. maculatus* attack at varying degrees, hence improved varieties that are resistant to the insect should be developed.

Acknowledgements

Authors are grateful to International Institute of Tropical Agriculture (IITA), Ibadan and Nigerian Stored Products Research Institute (NSPRI), Headquarters, Ilorin, for their support and cooperation.

Funding Acknowledgement

This research receives no specific grant from any funding agency in the public, commercial or not-for-specific- sectors.

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ارزیابی آزمایشگاهی شش رقم لوبیا چشم‌بلبلی *Vigna unguiculata* به سوسک چهارنقطه‌ای حبوبات *Callosobruchus maculatus* (Coleoptera: Chrysomelidae) در انبار

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دریافت: ۲۹ شهریور ۱۳۹۶؛ پذیرش: ۱۵ خرداد ۱۳۹۷

چکیده: ارزیابی‌های آزمایشگاهی به‌منظور بررسی حساسیت شش رقم لوبیا چشم‌بلبلی به سوسک چهارنقطه‌ای حبوبات *Callosobruchus maculatus* (Coleoptera: Chrysomelidae) در دمای ۲ ± ۲۹ درجه سلسیوس و رطوبت نسبی ۳ ± ۶۵ درصد انجام گرفت. پنجاه گرم از هر رقم در ظرف شیشه‌ای در بسته به حجم ۲۵۰ میلی‌لیتر قرار داده شد و توسط ۳ حشره ماده و ۲ حشره نر یک تا دو روزه سوسک چهارنقطه‌ای حبوبات آلوده شدند. سپس حصوصیات فیزیکی لوبیا چشم‌بلبلی، تعداد تخم‌های گذاشته شده، درصد تخم‌های زنده، تعداد حشرات کامل ظاهر شده، طول دوره ظهور (DE)، میانگین دوره رشد (MDP)، درصد کاهش وزن دانه، درصد خسارت به بذر و شاخص حساسیت ثبت گردید. آزمایش در قالب طرح کاملاً تصادفی و در سه تکرار انجام شد. نتایج نشان داد که اختلاف معنی‌داری (p, 0.05) میان ارقام برای DE و MDP وجود دارد. ارقام IT90K-76، IT98K-131، IT11D-15-21 و IT07K-299-6 از نظر طول دوره ظهور به‌طور معنی‌دار کارایی بهتری نسبت به ارقام IT10K-866-1 و TVx 3236 داشتند. رقم IT98K-131-1 (۲۶/۶۷ روز) به‌جز رقم IT90K-76 (۲۲/۶۷ روز) نسبت به سایر ارقام طول دوره رشد بهتری داشت. نتایج نشان داد که ارقام IT90K-76، IT10K-866-1 و IT98K-131-1 دارای مقاومت متوسط و ارقام IT07K-299-6، IT11D-15-21 و TVx 3236 حساس بودند. در این پژوهش می‌توان رقم IT90K-76 را توصیه نمود زیرا رقم فوق از نظر پارامترهای مطالعه شده در واکنش به سوسک چهارنقطه‌ای حبوبات بهتر بود.

واژگان کلیدی: بذور انباری، فعالیت حشره، کنترل غیرشیمیایی، مقاومت، انبار