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Research Article Morphological, Molecular and Biological Studies on Common Bean Weevil *Acanthoscelides obtectus* (Say) in Egypt

¹Sayeda Sayed Ahmed, ¹Magda Hanna Naroz, ¹Sahar Yassin Abdel-Aziz, ¹Mona Abel-Rahman Awad and ²Sobhy Abdel-Shafy

¹Department of Economic Entomology and Pesticides, Faculty of Agriculture, Cairo University, Giza, Egypt ²Department of Parasitology and Animal Diseases, Veterinary Research Division, National Research Centre, Dokki, Giza, Egypt

Abstract

Background and Objective: The bean weevil, *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae) is a serious Neotropical origin insect pest on kidney beans, *Phaseolus vulgaris* L. and other legume seeds. The objective of this study was to discriminate *A. obtectus* through morphological, molecular and biological studies. **Materials and Methods:** All stages of *A. obtectus* were morphologically described and their biology was performed. The adults of both *A. obtectus* and *Callosobruchus chinensis* were characterized by molecular analysis. **Results:** All the developmental stages were morphologically described. The morphological description of antennae, legs and wings of the adults indicated that the insect is *A. obtectus*. The partial sequence of COI gene of *A. obtectus* revealed 99% similarity with *A. obtectus* that previously recorded in GenBank and located in a separate glade in the phylogenetic tree. The biological parameters such as eggs per female, hatchability and durations of the developmental stages were also estimated. **Conclusion:** The morphological description, the partial sequence of COI gene and the biological parameters discriminated *A. obtectus* obviously from *C. chinensis*.

Key words: Beetles, bruchidae, bean weevil, Acanthoscelides obtectus, Phaseolus vulgaris L., legume seeds, kidney beans, Callosobruchus chinensis

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Corresponding Author: Sobhy Abdel-Shafy, Department of Parasitology and Animal Diseases, Veterinary Research Division, National Research Centre, Dokki, Giza, Egypt Tel: +202 333 717 28 Fax: +202 333709 31

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Actual human population growth and global climate change may affect the food reserves and the availability of seeds for growing plants in the near future. Post-harvest loss caused by pests may exceed 20% in poorly developed and tropical countries due to inadequate management practices and environmental conditions that allow rapid reproduction of pests, especially in developing countries¹. Bruchids, commonly known as pulse beetles are a serious threat to legumes worldwide. Many of the bruchid species have crossed the geographical boundaries and have become cosmopolitan in distribution through human-mediated migrations and import/export of food grain. This made these pest species highly adaptive and hence is distributed from temperate to tropical climates. They infest seeds of many grain legumes, both in the field and in storage. The Neotropical origin bean weevil, Acanthoscelides obtectus (Say) (Coleoptera: Bruchinae) is a serious pest of kidney beans, Phaseolus vulgaris L., P. lunatus L. and other legume seeds in Africa^{2,3}, Australia⁴, Europe^{5,6}, America^{7,8}, the Mediterranean area^{9,10} and various other parts of the world¹¹. A. obtectus infests the different hosts in the fields and stores and rendered unfit for consumption¹²⁻¹⁶. Populations of *A. obtectus* are most commonly detected in stores of dried legumes and their life cycle appears well adapted for reproduction in a storage environment. The females lay eggs in clusters under or nearby a single seed. The first instar larvae burrow into a seed where the beetles spend their larval and pupal stages. The final instar larvae excavate chambers just below the seed and presence of a larva may be detected by a small "window"¹⁷⁻¹⁹. This insect causes damage by reducing the mass and/or volume, reducing the physiological quality and germination capacity, increasing the temperature and water content of the seeds²⁰. Unlike most of the other bruchids, A. obtectus Say's reproductive cycle is continuous, without imaginal diapause for temperatures between 14-35°C and it attacks the beans in fields as well as stored seeds²¹. Larvae feed on beans and cause losses more than 30% of stored products²². This insect completes its entire life cycle within stored dry beans without returning to the field²¹. A. obtectus may adapt to several leguminosae: its host-plant, P. vulgaris L. and some non-host plants such as Vigna unquiculata (Walp.), Cicer arietinum L. and Vicia faba L.²³.

During investigation heavy infested *P. vulgaris* seeds by weevils, this study expected to find *C. chinensis* but some morphological features were different. Therefore, the objectives of this study were: (1) Discriminating of *A. obtectus* morphologically, (2) Characterizing of *A. obtectus* molecularly

by the Polymerase Chain Reaction (PCR), sequence analyses and phylogenetic tree targeting the gene cytochrome c oxidase subunit I (COI) and 3) Studying the biology of the insect reared on phaseolus seeds grown in Egypt under laboratory conditions.

MATERIALS AND METHODS

Insect culture: The *Acanthoscelides obtectus* strain was collected from infested kidney beans obtained from field that belongs to Giza Governorate, Egypt during the summer season 2016. The insect was maintained on kidney bean seeds *Pharseolus vulgaris* under $27\pm1^{\circ}$ C, $65\pm5\%$ relative humidity and L16: D8 photoperiod.

Morphological description: Eggs were examined to identify the shape and size with the aid of a stereo microscope. The eggs were mounted in Hoyer's solution as described by Sharkawi²⁴, precise description of the egg stage was given. Continuous daily dissections of infested seeds collected after harvesting carried out to study the larval morphological characteristics. The criteria used to determine the larval instars were the head capsule measurements of each instar (10 larvae/instar) by using temporary mounting in Hoyer's solution. A number of infested kidney beans were examined to follow up the development of pre-pupae and pupae. The criteria used to determine the adult measurements were the body measurements of 15 adults (males and females). A stereo microscope was used to examine adults of *A. obtectus* which were selected randomly.

Molecular characterization: The DNA was isolated using ZR Genomic DNATM-Tissue MiniPrep (Zymo Research, USA). According to the manufacturer's manual, cytochrome oxidase subunit I (COI) gene²⁵. Folmer region was amplified using My TagTM Red DNA Polymerase (BioLine, UK). The primers used for the amplification of the COI gene were 5'-TCA ACC AAC CAC AAA GAC ATT-3' and FishR1 5'-TAG ACT TCT GGG TGG CCA AAG AAT CA-3'. The thermal program consisted of initial step of 2 min at 54°C followed by 35 cycles of 40 sec at 94°C, 40 sec at 54°C and 1 min at 72°C followed in turn by final extension of 10 min at 72°C. The PCR products were visualized on 1.2% agarose gels, purified and sequencing. Amplicons were purified using DNA clean and concentrator TM-25. Products were labelled using the BigDye Terminator V.3.1 Cycle sequencing kit (Applied Biosystems, Inc.) and sequenced bidirectionally using ABI 3730 Automated Sanger sequencer (Macrogen, Inc.). Sequence was evaluated,

assembled and aligned using Geneious V8.1 software. Refined sequence was used to identify the species using DNA related database (BOLD and BLASTn). Phylogenetic analysis was performed using Mega²⁶ version 5.1.

Biological determination: The biology of A. obtectus was conducted in an incubator under the optimum constant laboratory conditions of $27\pm1^{\circ}$ C and $65\pm5\%$ R.H. Twenty newly laid eggs were carefully transferred to glass vials $(4 \times 10 \text{ cm})$. Ten vials (replicates) were used and checked daily until egg hatching. The incubation period and hatchability percentages were calculated. Furthermore, three glass jars of one pound capacity each were provided with sterilized kidney beans at a rate of 1/2 kg/jar. Every jar was infested by newly laid eggs obtained from the stock culture at a rate of 4000 eggs. After 3 days, random sample of 10 seeds was taken daily from every jar and immersed in water about 20 min and then carefully examined under a stereo microscope then carefully dissected to collect larvae, pre-pupae and/or pupae. Seeds daily dissection continued until all larvae transformed into pupae. Collected larvae and pre-pupae were preserved in 70% ethyl alcohol and glycerol (1:1 by volume). Preserved larvae and pre-pupae were separately mounted on slides using Hoyer's media and examined under a stereo microscope. Measurements of body length, length and width of the head capsules were taken. Moreover, 20 fresh pupae (<24 h old) were removed carefully from the dissected seeds and transferred into glass vials (4×10 cm). Ten vials (replicates) were, incubated under the previously mentioned optimum conditions and checked daily until adult emergence to record the pupal duration. Additionally, 10 pairs (one female and one male each) of the newly-emerged adults were

individually transferred into glass vials each measuring 4×10 cm. Vials were examined daily to remove the eggs using sieving until the females died. Eggs were kept into Petri dishes and pre-oviposition, oviposition and post- oviposition periods, adult longevity and the number of eggs laid/ female were recorded.

Statistical analysis: The F-value (one-way ANOVA at p<0.05) and Duncan tests using SPSS computing program version 20 were adopted for calculating biological aspects of *A. obtectus* using ANOVA as described by Snedecor and Cochran²⁷.

RESULTS

Morphological description: All the eggs were loosely onto the seeds. Freshly laid eggs were milky white color and elongate in shape (Fig. 1). Figure 1a shows the group of A. obtectus eggs and Fig. 1b shows the high magnification of a single egg. The larva has six instars (Fig. 2). Figure 2(a-f) show head capsules of the 1st, 2nd, 3rd, 4th, 5th and 6th instar larva of respectively. On the other hand, the А. obtectus, corresponding whole body were in Fig. 2(g-l). The head capsule and body measurements of the larval instars were provided in Table 1. There are significant differences between all larvae instars in the length of head capsule and body. However, the width of head capsule and body may record insignificant differences between two or more successive larval instars. The pre-pupa is white in color, while, the pupa is exerts type with white yellowish in color (Fig. 3). Figure 3a and b show the lateral and dorsal view of pre-pupae, respectively. Whereas, Fig. 3c and d show the ventral and lateral view of the pupa, respectively. The body

Table 1: Length and width measurements of head capsule and body of different larval instars of A. obtectu	IS
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Larval instar	Range (Mean±SE)			
	Head capsule		Body	
	Length(mm)	Width (mm)	Length (mm)	Width (mm)
1st	0.317±0.023ª	0.309±0.023ª	0.406±0.041ª	0.146±0.016ª
	(0.28-0.45)	(0.28-0.40)	(0.325-0.52)	(0.130-0.195)
2nd	0.458±0.005 ^b	0.367 ± 0.019^{ab}	0.659±0.026 ^b	0.509±0.017 ^b
	0.47-0.63	0.29-0.43	0.585-0.78	0.45-0.58
3rd	0.500±0.001°	0.367 ± 0.019^{ab}	0.864±0.031°	$0.650 \pm 0.00^{ m b}$
	0.50-0.90	0.33-0.73	0.78-0.975	0.65-0.65
4th	0.609 ± 0.015^{d}	0.434±0.042 ^b	1.641 ± 0.05^{d}	1.048±0.095°
	0.64-0.79	0.56-0.80	1.43-1.82	0.65-1.365
5th	0.725±0.005 ^e	0.634±0.015°	2.016±0.032 ^e	1.430±0.051d
	0.74-0.88	0.57-0.77	1.95-2.15	1.30-1.69
6th	0.807±0.015 ^f	0.663±0.017 ^d	2.23±0.06 ^f	1.644±0.044 ^d
	0.69-0.87	0.56-0.71	2.08-2.54	1.56-1.88
F-value	201,159	44,776	187.58	55.589
p-value	<0.001	<0.001	<0.001	<0.001



Fig. 1(a-d): Egg of Acanthoscelides obtectus (a) Group, (b) High magnification



Fig. 2(a-l): Acanthoscelides obtectus: head capsules (a) 1st, (b) 2nd, (c) 3rd, (d) 4th, (e) 5th and (f) 6th and their whole body of larval instars, (g) 1st, (h) 2nd, (i) 3rd, (j) 4th, (k) 5th and (l) 6th

Table 2: Body length and width of pre-pupa, pupa and adult of <i>A. obtectus</i>					
	Length (mm)		Width (mm)		
Stages	Range	Mean±SE	Range	Mean±SE	
Pre-pupa	3.8-3.9	3.87±0.03	1.54 -1.75	1.62±0.07	
Pupa	3.5-3.8	3.66±0.05	1.65-1.95	1.80±0.06	
Adult	2.9-3.2	3.00±0.05	1.45-1.65	1.50±0.05	

able 2. Body length and width of pre-pupal pupal and adult of A obtectus

length and width of pre-pupa, pupa and adult were provided in Table 2. The adult head is grayish brown and its mouth parts are blackish in color (Fig. 4a-c). Antennal segments 1-4 filiform and segments 5-10 broadened and more serrated and the segment 11 non-serrated and acute apically. The color of antennal segments 1-4 is grey, 5-10 is dark blackish, while the color of the segment 11 is yellowish brown (Fig. 4c). Elytra about twice as long as broad and covered with patterned brown and gray (Fig. 4d). Hind wings were hepatitis with spot black (Fig. 4e). Legs reddish brown, except mid venter femur of meso and meta legs, which are yellow in color. Inner ridge

of ventral margin of hind femur legs has three teeth-like and spine at the posterior end (Fig. 4f-h). Anterior tooth twice as long as the posterior teeth, with a slight posterior inclination. Posterior teeth equal in size and have similar posterior inclination (Fig. 4h). Ovipositor elongated and supported by 2 pairs of dorsoventrally sclerotized rods, which unite sub-terminally. The ovipositor orifice is supported by a single pair, along with numerous setae of variable size (Fig. 4i-j). Figure 5 shows the heavy infested kidney beans by A. obtectus which had one or more holes (Fig. 5a) and newly emerged adult (Fig. 5b).



Fig. 3(a-d): (a-b) Pre-pupa and (c-d) Pupa of Acanthoscelides obtectus



Fig. 4(a-j): Adult of *Acanthoscelides obtectus*, (a) Females, (b) Male, (c) Mouth part and its antennae, (d) Fore wings, (e) Hind wings, (f) Fore leg, (g) Middle leg, (h) Hind leg, (i) Genitalia in a female and (j) Genitalia in a male



Fig. 5(a-b): (a) Heavy infested kidney beans by *Acanthoscelides obtectus* and (b) Newly emerged adult of *A. obtectus*



Fig. 6: Phylogenetic tree of *Acanthoscelides obtectus* (■) and *Callosobruchus chinensis* (▲) for the COI gene, reconstructed by Bootstrap test-neighbor joining. The Lepidopteran insect *Sitotroga cerealella* is used as outgroup

Molecular characterization: This study is considered the first attempt to differentiate *A. obtectus* beetle from *C. chinensis* beetle those found in Egypt by sequencing the COI barcode region. The partial sequence of COI gene of *A. obtectus* revealed 99% similarity with *A. obtectus* that previously recorded in GenBank and located in a separate glade "cluster III" in the phylogenetic tree (Fig. 6). The bean weevil *A. obtectus* found in Egypt was closely related to the Serbian and Finland strains. However, *C. chinensis* found in Egypt locate in another clade "cluster IV" far away from *A. obtectus* (Fig. 6).

The biology: The mean number of eggs was 11.9 eggs/female. The mean of incubation period was 6.10 ± 0.28 days. The mean of hatchability was $96.00\pm2.00\%$. The duration mean was 7.37 ± 0.82 , 5.00 ± 0.00 , 3.00 ± 0.37 , 3.13 ± 0.44 , 3.53 ± 0.40 and 3.33 ± 0.23 days for the 1st, 2nd, 3rd, 4th, 5th and 6th instar larva, respectively. The mean duration of pre-pupa and pupa was 2.81 ± 0.28 and 4.17 ± 0.21 days, respectively. The longevity attained 7.2 and 8.9 days in male and female, respectively. The pre-oviposition, oviposition and post-oviposition periods of matted female attained 1.2, 5.6 and 2.1 days, respectively (Table 3).

Table 3: Some biological parameters of A. obtectus	reared on kidney beans under	laboratory conditions
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Biological parameters	Minimum	Maximum	Mean±SE (days)
Incubation period	5	7	6.10±0.28
Hatchability (%)	80	100	96.00±2.0
1st larval instar	3.00	10.00	7.37±0.82
2nd larval instar	5.00	5.00	5.00±0.00
3rd larval instar	2.00	4.00	3.00±0.37
4th larval instar	2.00	5.00	3.13±0.44
5th larval instar	2.00	7.00	3.53±0.40
6th larval instar	2.00	4.00	3.33±0.23
Total larval period	16.00	35.00	25.36±2.26
Per-pupa period	2.00	5.00	2.81±0.28
Pupal stage	3.00	5.00	4.17±0.21
Male longevity	6.00	12.00	7.20±0.69
Female longevity	3.00	14.00	8.90±1.19
Pre-oviposition period	1.00	2.00	1.20±0.13
Oviposition period	2.00	10.00	5.60±0.88
Post-oviposition period	0.00	3.00	2.10±0.35
No. of egg/female	2.00	35.00	11.90±3.27
Total life cycle	24.00	59.00	41.24±3.94

DISCUSSION

Current, it is believed that leguminous crops in Egypt are infested by *Callosobruchus* spp.^{28,29} because they seem to very close morphologically with A. obtectus which needs more qualified taxonomist to differential it from Callosobruchus spp. (e.g., C. chinensis). Therefore, the accurate identification of exotic and potentially invasive taxa is very important in IPM programs. Traditionally, identification has been based on morphological diagnoses provided by taxonomic studies. Only experts such as taxonomists and trained technicians can identify taxa accurately because it requires special skills acquired through extensive experience. The DNA barcoding has become increasingly common since it was proposed in 2003, this simple technique has attracted attention from taxonomists, ecologists, conservation biologists, agriculturists, plant guarantine officers and others and the number of studies using the DNA barcode has rapidly increased³⁰.

This is the first intensive study on the dried bean beetle, *A. obtectus* Say (Coleoptera: Chrysomelidae, Bruchinae) using both morphological description and molecular characterization. Hence, the combination of morphology and molecular results can provide reliable identifications. Bean weevil is a serious pest of kidney beans and food legumes that has strong adapt-ability to the environment. It has multiple hosts in some parts of the Mediterranean area^{9,10}. Similar morphological characters of *A. obtectus* have been reported by Say³¹, Johnson³², Kingsolver⁷, Alvarez *et al*,³³ and Thakur^{34,35}. Morphological characters of *A. obtectus* were resemble structure of genitalia (male and female) of the Indian population of *A. obtectus* are similar to the native population of America described by Johnson³⁶ and Kingsolver⁷. The results of sequence analyses and phylogenetic tree of the gene COI confirmed the morphological identification. The partial sequence of COI gene of *A. obtectus* revealed 99% similarity with *A. obtectus* that previously recorded in GenBank and located in a separate glade in the phylogenetic tree.

The results of the biology were similar to Thakur and Renuka¹² and Godfrey and Long³⁷ that freshly laid eggs were milky white and ellipsoidal in shape. Since most of the eggs were not glued onto the seeds it is essential for the freshly hatched first instar larva to find and select the host seeds for the remaining stages of development and food requirements. The data of Parsons and Credland³⁸, Paul *et al.*³ and Thakur³⁴, who studied with the eggs were not glued onto the seeds. The females lay eggs in clusters under or nearby a single seed. Similar observations on biology, ovipositor and larval-pupal development of bruchids have been observed by Southgate¹¹ and Thakur and Banyal³⁹. Oviposition lasted for 7-10 days and the incubation period was 8-10 days and larval development was completed in 14-20 days¹².

It is, however, very likely that in *A. obtectus* the males pass on nutrients to the males, which would extend female longevity under aphagous conditions. This may plain why mated females without beans lived longer than the virgins; the mated males get extra nutrients but do not spend it on eggs. This hypothesis is supported the report of Fox⁴⁰ who found in another aphagous bruchid beetle, *Callosobruchus maculutus*, that ejaculated-derived nutrients contributed to female somatic maintenance¹⁴. Similar results have already been reported for adults do not feed on the seeds. *A. obtectus*, as other bruchid beetles, is physiologically suited for adult aphagy and females emerge with adequate energy to develop and lay most of their potential eggs. Thus, adults need neither food nor water to reproduce¹⁴. The optimal generational development of *A. obtectus* occurred at 30°C, although the

insect completed its development within a range of 20-32 °C in 34-63 days⁴¹. In general, more molecular investigations are recommended in stored products insects for accurate identification. This will help better understanding the insect species in their products and environment and benefit for their controlling programs.

CONCLUSION

The insect *A. obtectus* adapted itself on the environmental conditions in Egypt and became a key pest on legume seeds beside other bruchid beetles. This insect could be discriminated from *C. chinensis* by its specific morphological characteristics and partial sequence of COI gene. It is recommended to take account consideration the presence of *A. obtectus* as a key pest in the control program of leguminous insects in Egypt.

SIGNIFICANCE STATEMENT

This study discovered the new key pest *A. obtectus* beside *C. chinensis* on leguminous seeds in Egypt that can be beneficial for integrated pest management programs for insect pests of leguminous seeds. This study will help the researchers to uncover the critical areas of distinguish *A. obtectus* from *C. chinensis* that many researchers were not able to explore. Thus a new theory on insect pests on leguminous in Egypt may be arrived at the consideration of an imported *A. obtectus* as a key insect pest on leguminous seeds in Egypt.

REFERENCES

- 1. Broughton, W.J., G. Hernandez, M. Blair, S. Beebe, P. Gepts and J. Vanderleyden, 2003. Beans (*Phaseolus* spp.)-model food legumes. Plant Soil, 252: 55-128.
- 2. Nchimbi-Msolla, S. and R.N. Misangu, 2002. Seasonal distribution of common bean (*Phaseolus vulgaris* L.) bruchid species in selected areas in Tanzania. Proceedings of the Bean Seed Workshop, January 12-14, 2002, Arusha, Tanzania.
- Paul, U.V., J.S. Lossini, P.J. Edwards and A. Hilbeck, 2009. Effectiveness of products from four locally grown plants for the management of *Acanthoscelides obtectus* (Say) and *Zabrotes subfasciatus* (Boheman) (both Coleoptera: Bruchidae) in stored beans under laboratory and farm conditions in Northern Tanzania. J. Stored Prod. Res., 45: 97-107.
- 4. Bailey, P.T., 2007. Pests of Field Crops and Pastures: Identification and Control. CSIRO Publishing, Collingwood, VIC 3066, Australia, ISBN-13: 9780643099425, pp: 456.

- Alvarez, N., M. Hossaert-McKey, J.Y. Rasplus, D. McKey and L. Mercier *et al.*, 2005. Sibling species of bean bruchids: A morphological and phylogenetic study of *Acanthoscelides obtectus* Say and *Acanthoscelides obvelatus* Bridwell. J. Zool. Syst. Evol. Res., 43: 29-37.
- Schmale, I., F.L. Wackers, C. Cardona and S. Dorn, 2002. Field infestation of *Phaseolus vulgaris* by *Acanthoscelides obtectus* (Coleoptera: Bruchidae), parasitoid abundance and consequences for storage pest control. Environ. Entomol., 31: 859-863.
- Kingsolver, J.M., 2004. Handbook of the bruchidae of the United States and Canada (Insecta, Coleoptera). Vols. 1 and 2, Technical Bulletin 1912, November 2004, U.S. Department of Agriculture, USA., pp: 1-524.
- 8. Romero-Napoles, J., 2010. A new species of *Acanthoscelides* Schilsky, 1905 (Coleoptera: Bruchidae) from Nuevo Leon, Mexico, with a key to the obtectus species-group. Coleopterists Bull., 64: 125-128.
- Regnault-Roger, C., M. Ribodeau, A. Hamraoui, I. Bareau, P. Blanchard, M.I. Gil-Munoz and F.T. Barberan, 2004. Polyphenolic compounds of Mediterranean Lamiaceae and investigation of orientational effects on *Acanthoscelides obtectus* (Say). J. Stored Prod. Res., 40: 395-408.
- Ayvaz, A., O. Sagdic, S. Karaborklu and I. Ozturk, 2010. Insecticidal activity of the essential oils from different plants against three stored-product insects. J. Insect Sci., Vol. 10. 10.1673/031.010.2101.
- 11. Southgate, P.J., 1979. Biology of the Bruchidae. Annu. Rev. Entomol., 24: 449-473.
- Thakur, D.R. and Renuka, 2014. Biology and biointensive management of *Acanthoscelides obtectus* (Say) (Coleoptera: Chrysomelidae)-a pest of kidney beans wordwide. Proceedings of the 11th International Working Conference on Stored Product Protection, November 24-28, 2014, Chiang Mai, Thailand, pp: 115-126.
- 13. Vera-Graziano, J. and S. Cruz-Izquierdo, 2016. Insect population parameters of *Acanthoscelides obtectus* (say.) in grains of five cultivars of common bean (*Phaseolus vulgaris* L.). Agrociencia, 50: 347-353.
- Savkovic, U., M. Dor Devic, D.S. Jovanovic, J. Lazarevic, N. Tucic and B. Stojkovic, 2016. Experimentally induced host-shift changes life history strategy in a seed beetle. J. Evol. Biol., 29: 837-847.
- Njoroge, A.W., H. Affognon, C. Mutungi, U. Richter, O. Hensel, B. Rohde and R.W. Mankin, 2017. Bioacoustics of *Acanthoscelides obtectus* (Coleoptera: Chrysomelidae: Bruchinae) on *Phaseolus vulgaris* (Fabaceae). Florida Entomol., 100: 109-115.
- Vuts, J., C.M. Woodcock, L. Konig, S.J. Powers, J.A. Pickett, A. Szentesi and M.A. Birkett, 2018. Host shift induces changes in mate choice of the seed predator *Acanthoscelides obtectus* via altered chemical signalling. PLoS ONE, Vol. 13. 10.1371/journal.pone.0206144.

- Tucic, N., I. Gliksman, D. Seslija, D. Milanovic, S. Mikuljanac and O. Stojkovic, 1996. Laboratory evolution of longevity in the bean weevil (*Acanthoscelides obtectus*). J. Evol. Biol., 9:485-503.
- Hagstrum, D.W. and B. Subramanyam, 2009. A review of stored-product entomology information sources. Am. Entomol., 55: 174-183.
- Mutungi, C., H.D. Affognon, A.W. Njoroge, J. Manono, D. Baributsa and L.L. Murdock, 2015. Triple-layer plastic bags protect dry common beans (*Phaseolus vulgaris*) against damage by *Acanthoscelides obtectus* (Coleoptera: Chrysomelidae) during storage. J. Econ. Entomol., 108: 2479-2488.
- Faroni, L.R.A. and A.H. Sousa, 2006. Aspectos Biologicos e Taxonomicos dos Principais Insetos-Praga de Produtos Armazenados. In: Tecnologia de Armazenagem em Sementes, Almeida, F.A.C., M.E.M. Duarte and M.E.R.M.C. Mata (Eds.). UFCG, Campina Grande, Brazil, pp: 371-402.
- 21. Labeyrie, V., 1962. Les Acanthoscelides. In: Entomologie Appliquee a l'Agriculture, Volume 1: Traite, Balachowsky, A.S. (Ed.). Masson, Paris, France, pp: 469-490.
- Pemonge, J., M.J. Pascual-Villalobos and C. Regnault-Roger, 1997. Effects of material and extracts of *Trigonella foenumgraecum* L. against the stored product pests *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) and *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae). J. Stored Prod. Res., 33: 209-217.
- 23. Hamraoui, A. and C. Regnault-Roger, 1995. Oviposition and larval growth of *Acanthoscelides obtectus* Say (Col., Bruchidae) in regard to host and non-host plants from leguminosae family. J. Applied Entomol., 119: 195-199.
- Sharkawi, S.G., 1964. The morphology, biology, ecology and control of *Vespa orientalis*, Fab. (Hymenoptera, Vespidae).
 M.Sc. Thesis, Faculty of Agriculture, Cairo University, Egypt.
- 25. Carpenter, A.C. and J.E. Board, 1997. Growth dynamic factors controlling soybean yield stability across plant populations. Crop Sci. Soci. Am., 37: 1520-1526.
- Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei and S. Kumar, 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance and maximum parsimony methods. Mol. Biol. Evol., 28: 2731-2739.
- 27. Snedecor, G.W. and W.G. Cochran, 1956. Statistical Methods Applied to Experiments in Agriculture and Biology. The Iowa State College, Ames, Iowa, USA., Pages: 534.
- Mourad, A.K. and O.A. Zaghloul, 1997. Effect of some environmental factors on *Callosobruchus chinensis* (L.) (Coleoptera: Bruchidae) infesting mungbean in Egypt. Proceedings of the 49th International Symposium on Crop Protection, May 6, 1997, Gent, Belgium, pp: 623-631.

- Mahgoub, S.M., M.S. Hamed, S.M. Ali and M.S.A. Gharib, 2005. Susceptibility of some Egyptian pulses to infestation by *Callosobruchus chinensis* (L.) and *Callosobruchus maculatus* (F.). Egypt. J. Agric. Res., 83: 1601-1612.
- 30. Jinbo, U., T. Kato and M. Ito, 2011. Current progress in DNA barcoding and future implications for entomology. Entomol. Sci., 14: 107-124.
- 31. Say, T., 1831. Descriptions of North American Curculionides and an arrangement of some of our known species agreeably to the method of Schoenherr. New Harmony, Indiana, pp: 1-30.
- 32. Johnson, C.D., 1990. Systematics of the seed beetle genus *Acanthoscelides* (Bruchidae) of Northern South America. Trans. Am. Entomol. Soc., 116: 297-618.
- Alvarez, N., D. Mckey, M. Hossaert-Mckey, C. Born, L. Mercier and B. Benrey, 2005. Ancient and recent evolutionary history of the bruchid beetle, *Acanthoscelides obtectus* Say, a cosmopolitan pest of beans. Mol. Ecol., 14: 1015-1024.
- Thakur, D.R., 2009. Acanthoscelides obtectus (Say) (Coleoptera: Bruchidae) a threat to kidney beans in India. Proceedings of the International Conference on Grain Legumes: Quality Improvement, Value Addition and Trade, February 14-16, 2009, Indian Institute of Pulses Research, Kanpur, India, pp: 262-263.
- Thakur, D.R., 2012. Taxonomy, distribution and pest status of Indian biotypes of *Acanthoscelides obtectus* (Coleoptera: Chrysomelidae: Bruchinae): A new record. Pak. J. Zool., 44: 189-195.
- Johnson, C.D., 1990. Six new species of *Acanthoscelides* from North and Central America (Coleoptera: Bruchidae). Coleopterists Bull., 44: 3-18.
- Godfrey, L.D. and R.F. Long, 2008. Dry bean: Weevils. UC IPM Pest Management Guidelines Publication 3446, University of California Agriculture and Natural Resources, USA., pp: 37. http://ipm.ucanr.edu/PMG/r52300411.html
- Parsons, D.M.J. and P.F. Credland, 2003. Determinants of oviposition in *Acanthoscelides obtectus*. A nonconformist bruchid. Physiol. Entomol., 28: 211-231.
- 39. Thakur, D.R. and H.S. Banyal, 2007. Developmental attributes and larval morphology of *Caryedon crineus* Arora (Bruchidae: Coleoptera). Indian J. Entomol., 69: 293-297.
- 40. Fox, C.W., 1993. Multiple mating, lifetime fecundity and female mortality of the bruchid beetle, *Callosobruchus maculatus* (Coleoptera: Bruchidae). Funct. Ecol., 7: 203-208.
- Soares, M.A., E.D. Quintela, G.M. Mascarin and S.P. Arthurs, 2015. Effect of temperature on the development and feeding behavior of *Acanthoscelides obtectus* (Chrysomelidae: Bruchinae) on dry bean (*Phaseolus vulgaris* L.). J. Stored Prod. Res., 61: 90-96.