Journal of Entomology and Nematology

Volume 7 Number 2, March, 2015
ISSN 2006-9855

Academic Journals

ABOUT JEN

The**Journal of Entomology and Nematology (JEN)** (ISSN: 2006-9855) is published monthly (one volume per year) by Academic Journals.

Journal of Entomology and Nematology (JEN) is an open access journal that provides rapid publication (monthly) of articles in all areas of the subject such as applications of entomology in solving crimes, taxonomy and control of insects and arachnids, changes in the spectrum of mosquito-borne diseases etc.

The Journal welcomes the submission of manuscripts that meet the general criteria of significance and scientific excellence. Papers will be published shortly after acceptance. All articles published in JEN are peer-reviewed.

Submission of Manuscript

Please read the **Instructions for Authors** before submitting your manuscript. The manuscript files should be given the last name of the first author

Click here to Submit manuscripts online

If you have any difficulty using the online submission system, kindly submit via this email jen@academicjournals.org.

With questions or concerns, please contact the Editorial Office at jen@academicjournals.org.

Editorial Board

InstructionsforAuthor

Electronic submission of manuscripts is strongly encouraged, provided that the text, tables, and figures are included in a single Microsoft Word file (preferably in Arial font).

The **cover letter** should include the corresponding author's full address and telephone/fax numbers and should be in an e-mail message sent to the Editor, with the file, whose name should begin with the first author's surname, as an attachment.

Article Types
Three types of manuscripts may be submitted:

Regular articles: These should describe new and carefully confirmed findings, and experimental procedures should be given in sufficient detail for others to verify the work. The length of a full paper should be the minimum required to describe and interpret the work clearly.
Short Communications: A Short Communication is suitable for recording the results of complete small investigations or giving details of new models or hypotheses, innovative methods, techniques or apparatus. The style of main sections need not conform to that of full-length papers. Short communications are 2 to 4 printed pages (about 6 to 12 manuscript pages) in length.

Reviews: Submissions of reviews and perspectives covering topics of current interest are welcome and encouraged. Reviews should be concise and no longer than 4-6 printed pages (about 12 to 18 manuscript pages). Reviews are also peer-reviewed.

Review Process

All manuscripts are reviewed by an editor and members of the Editorial Board or qualified outside reviewers. Authors cannot nominate reviewers. Only reviewers randomly selected from our database with specialization in the subject area will be contacted to evaluate the manuscripts. The process will be blind review.
Decisions will be made as rapidly as possible, and the journal strives to return reviewers' comments to authors as fast as possible. The editorial board will re-review manuscripts that are accepted pending revision. It is the goal of the AJFS to publish manuscripts within weeks after submission.

Regular articles

All portions of the manuscript must be typed double-spaced and all pages numbered starting from the title page.

The Title should be a brief phrase describing the contents of the paper. The Title Page should include the authors' full names and affiliations, the name of the corresponding author along with phone, fax and E-mail information. Present addresses of authors should appear as a footnote.

The Abstract should be informative and completely self-explanatory, briefly present the topic, state the scope of the experiments, indicate significant data, and point out major findings and conclusions. The Abstract should be 100 to 200 words in length.. Complete sentences, active verbs, and the third person should be used, and the abstract should be written in the past tense. Standard nomenclature should be used and abbreviations should be avoided. No literature should be cited.
Following the abstract, about 3 to 10 key words that will provide indexing references should be listed.

A list of non-standard **Abbreviations** should be added. In general, non-standard abbreviations should be used only when the full term is very long and used often. Each abbreviation should be spelled out and introduced in parentheses the first time it is used in the text. Only recommended SI units should be used. Authors should use the solidus presentation (mg/ml). Standard abbreviations (such as ATP and DNA) need not be defined.

The Introduction should provide a clear statement of the problem, the relevant literature on the subject, and the proposed approach or solution. It should be understandable to colleagues from a broad range of scientific disciplines.

Materials and methods should be complete enough to allow experiments to be reproduced. However, only truly new procedures should be described in detail; previously published procedures should be cited, and important modifications of published procedures should be mentioned briefly. Capitalize trade names and include the manufacturer's name and address. Subheadings should be used. Methods in general use need not be described in detail.

Results should be presented with clarity and precision. The results should be written in the past tense when describing findings in the authors' experiments. Previously published findings should be written in the present tense. Results should be explained, but largely without referring to the literature. Discussion, speculation and detailed interpretation of data should not be included in the Results but should be put into the Discussion section.

The Discussion should interpret the findings in view of the results obtained in this and in past studies on this topic. State the conclusions in a few sentences at the end of the paper. The Results and Discussion sections can include subheadings, and when appropriate, both sections can be combined.

The Acknowledgments of people, grants, funds, etc should be brief.

Tables should be kept to a minimum and be designed to be as simple as possible. Tables are to be typed double-spaced throughout, including headings and footnotes. Each table should be on a separate page, numbered consecutively in Arabic numerals and supplied with a heading and a legend. Tables should be self-explanatory without reference to the text. The details of the methods used in the experiments should preferably be described in the legend instead of in the text. The same data should not be presented in both table and graph form or repeated in the text.

Figure legends should be typed in numerical order on a separate sheet. Graphics should be prepared using applications capable of generating high resolution GIF, TIFF, JPEG or Powerpoint before pasting in the Microsoft Word manuscript file. Tables should be prepared in Microsoft Word. Use Arabic numerals to designate figures and upper case letters for their parts (Figure 1). Begin each legend with a title and include sufficient description so that the figure is understandable without reading the text of the manuscript. Information given in legends should not be repeated in the text.

References: In the text, a reference identified by means of an author‘s name should be followed by the date of the reference in parentheses. When there are more than two authors, only the first author‘s name should be mentioned, followed by ’et al‘. In the event that an author cited has had two or more works published during the same year, the reference, both in the text and in the reference list, should be identified by a lower case letter like ’a‘ and ’b‘ after the date to distinguish the works.

Examples:

Abayomi (2000), Agindotan et al. (2003), (Kelebeni, 1983), (Usman and Smith, 1992), (Chege, 1998; 1987a,b; Tijani, 1993,1995), (Kumasi et al., 2001) References should be listed at the end of the paper in alphabetical order. Articles in preparation or articles submitted for publication, unpublished observations, personal communications, etc. should not be included in the reference list but should only be mentioned in the article text (e.g., A. Kingori, University of Nairobi, Kenya, personal communication). Journal names are abbreviated according to Chemical Abstracts. Authors are fully responsible for the accuracy of the references.

Examples:

Chikere CB, Omoni VT and Chikere BO (2008). Distribution of potential nosocomial pathogens in a hospital environment. Afr. J. Biotechnol. 7: 3535-3539.

Moran GJ, Amii RN, Abrahamian FM, Talan DA (2005). Methicillinresistant Staphylococcus aureus in community-acquired skin infections. Emerg. Infect. Dis. 11: 928-930.

Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

Pelczar JR, Harley JP, Klein DA (1993). Microbiology: Concepts and Applications. McGraw-Hill Inc., New York, pp. 591-603.

Short Communications

Short Communications are limited to a maximum of two figures and one table. They should present a complete study that is more limited in scope than is found in full-length papers. The items of manuscript preparation listed above apply to Short Communications with the following differences: (1) Abstracts are limited to 100 words; (2) instead of a separate Materials and Methods section, experimental procedures may be incorporated into Figure Legends and Table footnotes; (3) Results and Discussion should be combined into a single section.

Proofs and Reprints: Electronic proofs will be sent (e-mail attachment) to the corresponding author as a PDF file. Page proofs are considered to be the final version of the manuscript. With the exception of typographical or minor clerical errors, no changes will be made in the manuscript at the proof stage.

Fees and Charges: Authors are required to pay a $550 handling fee. Publication of an article in the Journal of Entomology and Nematology is not contingent upon the author's ability to pay the charges. Neither is acceptance to pay the handling fee a guarantee that the paper will be accepted for publication. Authors may still request (in advance) that the editorial office waive some of the handling fee under special circumstances

Submission of a manuscript implies: that the work described has not been published before (except in the form of an abstract or as part of a published lecture, or thesis) that it is not under consideration for publication elsewhere; that if and when the manuscript is accepted for publication, the authors agree to automatic transfer of the copyright to the publisher.

Journal of Entomology and Nematology

Table of Contents: Volume 7 Number 2, March, 2015

ARTICLES

academicJournals

Vol. 7(2), pp. 5-10, March, 2015
DOI: 10.5897/JEN2015.0119
Article Number: 9B0BBFD51629
ISSN 2006-9855

http://www.academicjournals.org/JEN

Journal of Entomology and Nematology

Full Length Research Paper

Insect larvae associated with dropped pomegranate fruits in an organic orchard in Tunisia

Mohamed Braham

Laboratory of Entomology and Insect Ecology, University of Sousse, Regional Research Center of Horticulture and Organic Agriculture, 4042 Chott-Mariem Sousse, Tunisia.

Received 10 February, 2015; Accepted 17 March, 2015

In an attempt to find out the reasons of pomegranante fruit drop due to insect attacks, regular collection of all fallen fruits beneath trees of nine different varieties was done during the fruiting season from August 2013 to December 2013 in an organic pomegranate orchard in the Chott-Mariem region of Tunisia. Apparently heatlthy and cracked fruits were dissected in the laboratory to identify insect larvae found inside. Results indicate that fruits were attacked by three larvae insect species: the Mediterranean fruit fly, *Ceratitis capitata* Wiedemann (Diptera, Tephritidae), the carob moth, *Ectomyelois ceratoniae* Zeller (Lepidoptera, Pyralidae) and the pomegranate butterfly, *Virachola livia* Klug 1834 (Lepidoptera, Lycaenidae). Two different insect larvae can be found jointly inside fruits but no fruits were attacked by the three insect larvae together. All fruits varieties harbor the larvae of *C. capitata* whereas *E. ceratoniae* larvae were present in eight of nine varieties. Varieties numbered 1, 8 and 9 were free from *V. livia* larvae attacks and hence can be considered as resistant cultivars. With respect to Lepidoptera larvae attacks (*E. ceratoniae* and *V. livia*), we can assume Variety 1 as resistant. Nevertheless, more research was needed to corroborate these results.

Key words: Pomegranate, fruit drop, *Ectomyelois ceratoniae*, *Virachola livia*, *Ceratitis capitata.*

INTRODUCTION

Pomegranate, *Punica granatum* L. native to Persia is widely cultivated througout Iran, India, the drier parts of South-East Asia, Mediterranean region, Africa and dry hot areas of the United States and Latin America (Glozer and Ferguson, 2011; Mansour, 1995). The pomegranate is cultivated for its edible fruits and/or for decorative purposes. Its utilization consists of a large number of horticultural varieties mainly characterized by fruits traits such as fruit and seed color, taste and shape. The tree has been traditionally cultivated since ancient times under diverse climatic conditions (Evreinoff, 1957; Mars, 1995). Fruits are consumed fresh or processed for juice, syrup and other purposes. Different parts of the pomegranate tree (leaves, fruits and bark skin) have been used tradionally for the medicinal properties and for other purposes such as tanning (Mars, 2000). In Tunisia, pomegranate tree is well known in the coastal regions of the north, the center and the south and in many areas inside the country. It is also considered as a principal fruit tree in the oases. Local varieties are numerous and well

* E-mail: braham.mohamed@gmail.com.

Figure 1. Fallen pomegranate fruit

adapted to agroecological conditions (Mars, 1995). Having long been considered as a secondary and non commercial plantation, pomegranate cultivation has increased recently due maily to the greater awareness of its benefit to human health confirmed by recent scientific findings (Gil et al., 2000; Lansky and Newman, 2007). In Tunisia, the cultivated area extended from 5650 ha in 1980 to 11300 ha in 2008 given a production of 75000 tons in 2009 (Anonymous, 2014). Currently, the cultivation of pomegranate is experiencing several problems apart from pest attacks such as fruit cracking, fruit rot and fruit dropping. Insect pests of the fruit can cause major problems; one of these pests is the carob moth *Ectomyelois ceratoniae* Zeller 1839 (Lepidoptera, Pyralidae) (Gothilf, 1969), in which the female lays eggs in the calyx of developing fruits, and in a few days, the caterpillars enter the fruit by way of the calyx and cause arils rot. In Tunisia, Dhouibi (2000) reported the yearly damage rate caused by *E. ceratoniae* on Pomegranate fruits from 1996 to 1999 varied from 29 to 72% reaching 90% in 1983. Recently, increased damages of the pomegranate butterfly, *Virachola livia* Klug 1834 (Lepidoptera: Lycaenidae), was reported by Ksentini et al. (2011) causing fruit rot. These fruit borers may cause loss of an entire crop. More recently, we observed increased symptoms of damage similar to Mediterranean fruit fly *Ceratitis capitata* Wiedemann 1824 (Diptera, Tephritidae) attacks. *C. capitata* was reported as pomegranate pest in Turkey, Spain and other regions of the Mediterranean (Özturk et al., 2005; Özturk, and Ulusoy, 2009; Juan et al., 2000; Delrio and Cocco, 2012). Pomegranate cultivation and its potential yield are affected by many disorders like fruit cracking, fruit splitting and fruit drop. Beside the fall of fruits after fruit set occuring usually in May-June, the drop of mature fruits is common generally assigned to insect attacks mainly *E. ceratoniae*. Therefore, it is necessary to know the causes of fruit falling properly and determine which insect species are responsible to permit better control alternatives.

MATERIALS AND METHODS

Experimental site

The study was conducted in an experimental organic pomegranate orchard belonging to Research Center for Horticulture and Organic Agriculturein Chott-Mariem (Sousse, Tunisia 35.8°North ; 10.6° East). The area is located in the eastern coast of the Mediterranean at about 2.5 km far from the sea and characterized by a semi-arid climate with hot summers and mild winters. Monthly average temperatures in 2013 varied from 10.56°C in January to 28.62°C in August obtained from a meteological station situated at about 2 km from the study site belonging to the National Researches Institute of Rural Engeneering, Water and Forest (Tunisia). Annual rainfall recorded is about 300 mm concentrated mainly between October and April. The orchard, four years aged of about 0.25 ha was divided into two plots of the same area seperated by a path of 6 meters wide. The study plot is composed of 9 rows of 12 trees each for a total of 108 unsprayed trees. On the row, trees were seperated by 2.5 m and 3.5 m between the lines. The plot is surrounded in the North by a plot of fig tree (*Ficus carica*), in the south by a plot of olive tree in the west by a the second plot of pomegranate, of the same area having the same varieties and in the East by a bare land. Drip irrigation was used. Each row was planted with a different variety; thus, nine different local commercial cultivars were represented in which sour, sour-sweet and sweet varieties are represented (Dr. Mars, personal communication). Since the ongoing research of pomological characteristics of each variety conducted by Dr Mars's team, each cultivar was refered here as VAR1, VAR2 to VAR9.

Fruit collection and handling

The collection of fallen fruits was undertaken during the fruiting season (from 23 August 2013 to 6 December 2013). Fallen fruits (Figure 1) were collected usually at biweekly interval beneath each tree for each row (variety) and transported to the laboratory where they were dissected. Larvae found inside fruits were categorized as Dipteran or Lepidopteran larvae. For Lepidopteran species, only insect larvae were taken into account since we cannot differentiate species at eggs or pupae stages. Hence, larvae were categorized as *V.* larvae (Figure 2), *E.* larvae (Figure 3) or fruitflies larvae *C. capitata* (Figure 4). *E. ceratoniae* larvae were identified according to Solis (2006), *V. livia* according to Hanna (1939) and *C. capitata* according to Balachowsky et Misnil (1935). Extremely rotten fruits were eliminated and were not included as considered as attacked by *Drosophila melanogaster* Meigen (Diptera, Drosophilidae) larvae. *D. melanogaster* is specialized to feed as larvae on rotting vegetable matter that is undergoing fermentation due to yeast or bacterial contamination (EOL, 2014) or attacked by the fungus *Alternaria* spp. (Kahramanoglu et al., 2014).

Statistical analysis

The mean number of fallen fruits either attacked by insect larvae or un-attacked according to varieties were submitted to one way ANOVA using the software SPSS 17.0 (SPSS, 2008). Dates of collection were considered as repeated data. When significant difference was detected, a Duncan's multiple Range test was used to separate means at P≤0.05.

RESULTS AND DISCUSSION

Total number of fallen fruits

The total number of fallen fruits during the collection period

Figure 2. *V.livia* larva (September, 2013)

Figure 3. *E. ceratoniae* larva inside the pomegranate fruit (August, 2014)

(from August 23, 2013 to December, 6 2013, 25 collection dates) shows significant difference among varieties (One-way ANOVA; $F_{8,216}$= 5.133; P=0.001). The varieties numbered three and four suffer more fruit drop and variety number seven undergoes low fruit drops (Figure 5). Indeed VAR3 and VAR4 suffered significant fruit drops not caused by insect attacks (Table 1). The number of fallen fruits presumably caused by insect attacks, due to the presence of larvae inside fruits shows significant difference among varieties (Table 1). Indeed variety 1 harbors less insect larvae and variety 2 shows maximum attack even though the two varieties are located side by side. The less insect attacks in variety 1 may be attributed to the amount of acid content of this variety as reported by Melgarejo et al. (2000). This is confirmed by detailed insect larvae attack in which only *C. capitata* larvae were found in fallen fruits of variety 1 (Table 1) in only one date (on 31 October 2013).

Figure 4.*C.capitata* larva inside pomegranate fruit(September, 2013)

The details of insect damages show significant differences among cultivars regarding Lepidoptera larvae; *E. ceratoniae and V. livia*. Larvae attacks were assigned to *E. ceratoniae* more than *V. livia* (Table 2). All varieties were more attacked by *E. ceratoniae* larvae than *V. livia* larvae. Thus Moawad et al. (2011) reported that *V. livia* attacks healthy pomegranate fruits whereas *E. ceratoniae* attacks cracked fruits.

Insect infestation within varieties

The within variety insect larval densities (*E. ceratoniae,* larvae*, V. livia* larvae and *C*. *capitata* larvae showed no statistical difference for all varieties except variety 9 (one way ANOVAs; Variety 1:$F_{2,72}$=1; P= 0.373; Variety 2: $F_{2,72}$=0.60 P=0.50; Variety 3: $F_{2,72}$= 0.55 P=0.57; Variety 4 : $F_{2,72}$=0.37, P=0.72; Variety 5: $F_{2,72}$=1.9, P=0.14; Variety 6: $F_{2,72}$=3.10, P=0.052; Variety7: $F_{2,72}$=0.15, P=0.89; Variety 8: $F_{2,72}$=3.10, P=0.052 and Variety 9: $F_{2,72}$=3.9, P=0.02). *C. capitata* larvae were present in all varieties without real distinction between cultivars. This can be explained by (1) the insect has no choice among pomegranate varieties and thus pomegranate fruits can be considered as primary hosts. Indeed, if the *C. capitata* female has to choose among hosts present in the area, it would prefer several ripe fruits available. There were fig fruits, *Ficus carica* and two citrus species *Citrus sinensis* cv Thomson and *C. clementina* Hort. ex Tanaka. Indeed, in Morocco, Fahad et al. (2014), studying the biology and ecology of the Mediterranean fruit fly on Rosaceous tree in the Sefrou region, reported high *C. capitata* male captures in sexual traps installed in pomeganate orchard. However, no data were given regarding fruit infestation; and (2) the insect attacks already dropped fruits in which decaying process has begun and therefore, the insect

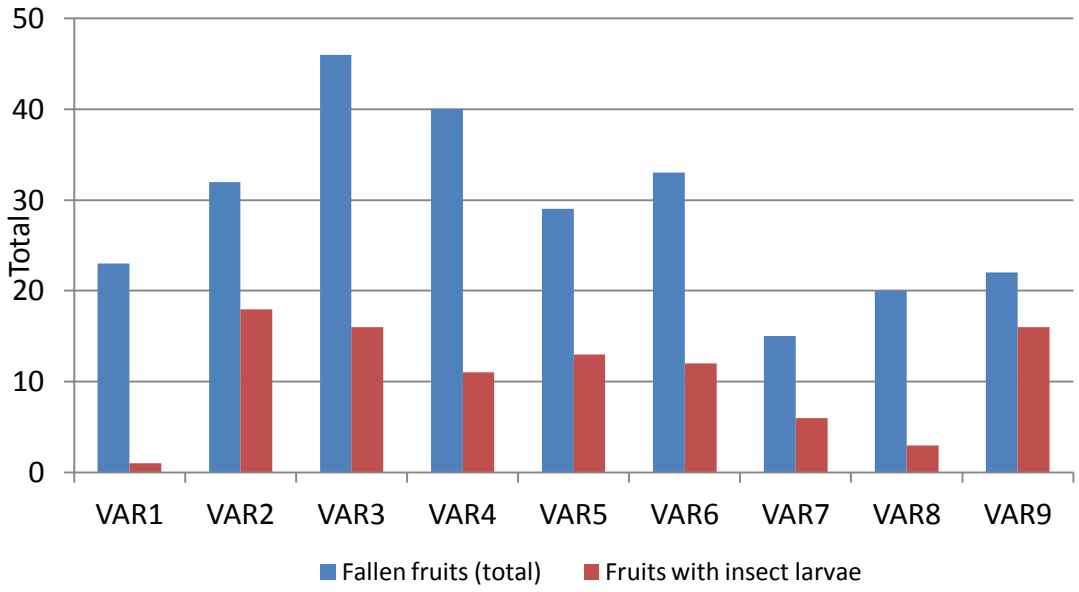

Figure 5. Number of fallen fruits and number of fruits attacked by insect larvae (*E. ceratoniae*, *V. livia*and or *C. capitata* (Chott-Mariem, 2013).

Table 1. Mean number of fallen pomegranate fruits (± standard deviation) with or without insect larvae (each variety was represented by 12 trees)

Varieties	Average fallen fruits with insect larvae ± SD[(1)]	Average fallen fruits without insects ± SD [(1)]	Average total fallen fruits ± SD [(1)]	N[(2)]
VAR1	0.04±0.2a	0.88±0.9bcd	0.92±0.95ab	25
VAR2	0.72±0.8d	0.56±0.71ab	1.28±0.8bc	25
VAR3	0.64±0.81cd	1.2±1.1d	1.84±1.06d	25
VAR4	0.44±0.71bcd	1.16±0.85cd	1.6±0.64cd	25
VAR5	0.52±0.58bcd	0.64±0.75abc	1.16±0.89bc	25
VAR6	0.48±0.58bcd	0.84±0.89bcd	1.32±0.9bc	25
VAR7	0.24±0.52abc	0.36±0.63ab	0.6±0.76a	25
VAR8	0.12±0.33ab	0.68±0.85abcd	0.8±0.91ab	25
VAR9	0.64±0.80cd	0.24±0.43a	0.88±0.92ab	25
Statistical analysis	$F_{8,216}$ =3.45; P= 0.001	$F_{8,216}$ 3.80 = P=0.001	$F_{8,216}$ = 5.133; P=0.001	

[(1)]Means in columns followed by different letters are significantly different (P≤0.05, Duncan's multiple Range test. [(2)]N : denotes collection dates (from 23 August to 6 December 2013).

has no real choice regarding the fruits characteristics such as color, thickness and shape. All varieties were attacked by *E. ceratoniae* larvae except variety 1 which is also free of *V. livia* larvae. Varieties 1, 8 and 9 were free from *V. livia* larvae which can be considered as resistant varieties. With respect to Lepidoptera larvae attacks, we can consider variety 1 as a resistant variety.

Presence versus absence of larvae species inside fruits

When we consider all varieties, for the presence-absence of the three insect larvae species, the larvae of *E. ceratoniae* were present solitary in the fruits at the percentage of 40%; exceeding *V. livia* and *C. capitata* larvae (Figure 6). Fruits can be attacked jointly by two different insect species (*C. capitata* plus *E. ceratoniae; C. capitata* plus *V. livia;* and *E. ceratoniae* plus *V. livia*) but no fruits were found attacked by the three insect species perhaps due to cannibalism between larvae (Figure 6).

To our knowledge, this is the first study to deal with the reasons of pomegranate fruit drop due to insect attacks in the centre-East region of Tunisia although Ksentini et al. (2011) when reporting the first occurrence of *V. livia* in Tunisia mentioned 5.2% of fruits were infested by *V. livia*

Table 2. Mean number of pomegranate fruits attacked by insect larvae (± standard deviation) according to varieties during the study period

Varieties	*E. ceratoniae* larvae ± SD [(1)]	*V. livia* larvae ±SD [(1)]	*C. capitata* larvae ±SD)	N[(2)]
VAR1	0a	0a	0.04±0.20	25
VAR2	0.24±0.52c	0.2±0.4c	0.36±0.56	25
VAR3	0.36±0.63c	0.2±0.4c	0.29±0.55	25
VAR4	0.20±0.64c	0.24±0.59c	0.12±0.33	25
VAR5	0.08±0.27b	0.20±0.40c	0.32±0.55	25
VAR6	0.08±0.27b	0.08±0.27b	0.32±0.55	25
VAR7	0.08±0.27b	0.08±0.27b	0.12±0.33	25
VAR8	0.08±0.27b	0a	0.04±0.2	25
VAR9	0.44±0.76c	0a	0.32±0.62	25
Statistical Analysis	$F_{8,214}$ =2.45; P= 0.01	$F_{8,214}$ = 2.18 = P=0.03	$F_{8,214}$ = 1.98; P=0.06	

([1])Means in columns followed by different letters are significantly different (P≤0.05, Duncan's multiple Range test) ([2])N : denotes collection dates.

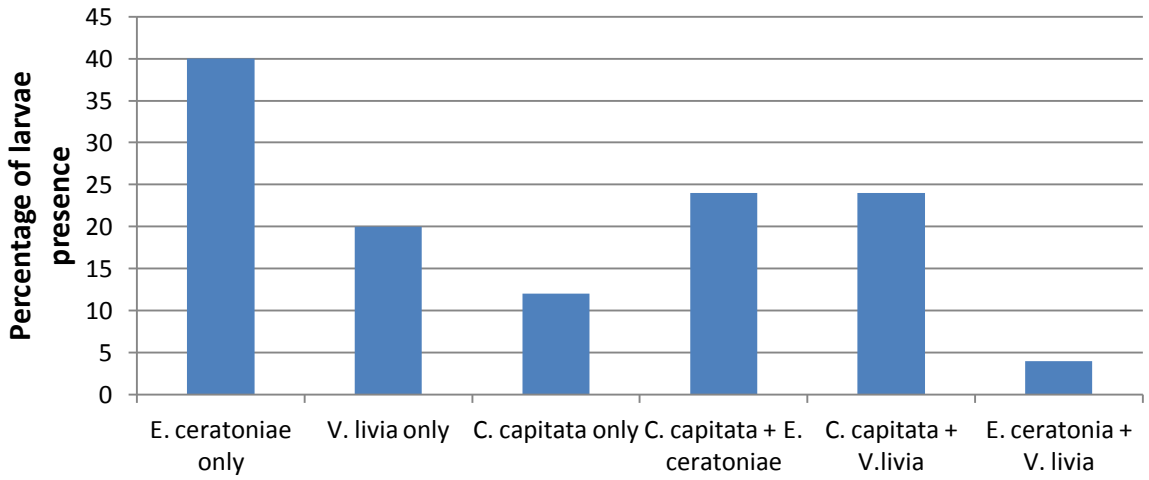

Figure 6. Percentage of species larvae present in pomegranate drop fruits.

and the insect was responsible for 52% of fruit rot in 2006 in Gabès governorate (South).

Better knowledge of the causes of fruit drop is important for these reasons (1) when establishing new orchard, the choice of varieties is of paramount importance. Determing which of pomegranate varieties are susceptibles, tolerants or resistants to insect attacks especially for the major Lepidoptera species (*E. ceratoniae* and *V. livia*) is crucial (Fesharaki et al., 2011) and (2) the insect pests responsible for fruit drop can be controlled efficiently. The rotten and decaying fruits showing no insect larvae may be infected by the fungus *Alternaria* spp. as reported by Kahramanoglu et al. (2014) in Cyprus. Indeed, Moawad et al. (2011) investigated the insect attacking five different pomegranate varieties in Saudi Arabia and reported three species: *V. livia, E. ceratoniae* and *Pseudococcus maitimus.* They stated the local variety (Al-taif) was resistant to *V. livia* larvae and the imported variety Wondeful was more resistant to *E. ceratoniae* larvae. In Turkey, Özturk et al. (2005) reported seven key insects attacking pomegranate in the Eastern Mediterranean Region of Turkey which are: *E. ceratoniae* (Zell.) (Lep.: Pyralidae), *C. capitata* Wied. (Dip.: Tephritidae), *Aphis punicae* Passerini (Hemiptera Aphididae), *Siphoninus phillyreae* (Haliday) (Hem.: Aleyrodidae), *Planococcus citri* (Risso) (Hemiptera:

Pseudococcidae), *Zeuzera pyrina* (L.) (Lep.: Cossidae) and *Carpophilus* spp. (Col.: Nitidulidae).

In our study beside the Med fly, two Lepidopteran species were present in fruits having more or less similar biologies. Shortly after hatching, larva needs to establish a feeding site, negotiating some or all of the following: leaf hairs, surface waxes, hard plant parts, laticifers, glands or tissues filled with allelochemicals, locally induced plant changes, variable microenvironments, predators,

pathogens, and parasitoids (Zalucki et al., 2002). The control of *C. capitata* is relatively easy using insecticide bait stations or cover sprayers. However, the control of Lepidoptera species is tricky and needs to monitor insect adult flight by the use of pheromones which are commercially lacking.

Conflict of interest

Author did not declare any conflict of interest.

ACKNOWLEDGEMENTS

The author wish to thank Dr Mars M. Director of the Regional Research Center for Horticulture and Organic Agriculture to permiting the use of pomegranate orchard. I thank Miss Bendhiefi A. and Mr Chtiwi L. for the technical assistance.

REFERENCES

Anonymous (2014). www.agriportail.tn. accessed 11 November 2014.
Balachowsky AL, Misnil L (1935). Les insectes nuisibles aux plantes cultivées: leurs mœurs, leur destruction. Traité d'entomologie agricole concernant la France, la Corse, l'Afrique du nord et les régions limitrophes, Volume 1. Les Presses des établissements Busson, sous la direction technique de L. Méry, (Eds). Paris. 242-253 (in French)
Delrio G, Cocco A (2012). Integrated Control of Citrus Pests in the Mediterranean Region. Chapter 15.Tephritidae.Vincenzo Vacante and Uri Gerson (Eds).Bentham Sciences Publishers. pp. 206-222.
Dhouibi MH (2000). Lutte intégrée pour la protection du palmier dattier en Tunisie. Centre de publication universitaire. Tunis (Tunisia), 140 p (in French).
EOL (2014). Encyclopedia of life. Eol.org. accessed 18 November 2014.
Evreinoff VA (1957). Contribution à l'étude du grenadier. J. d'Agriculture tropicale et de botanique appliquée. 4. N°3-4(124-138). http://dx.doi.org/10.3406/jatba.1957.2380
Fahad K, Gmira N, Benziane T, Sekkat A (2014). Etude de la bio-écologie de la moucheméditerranéenne des fruits Ceratitis capitata Wiedemann (1824) sur Rosacées dans la région de Sefrou (Maroc). Entomologie Faunistique – Faunistic Entomol. 67: 85-95.
Fesharaki SH, Karimizadeh J, Jalalzand AR,Besharatnejad MH, Modaresi M (2011). Studying on Damage of Carob Moth in Three Pomegranate cultivars of Isfahan (Iran). Procedia Environmental Sciences 8: 257–261. http://dx.doi.org/10.1016/j.proenv.2011.10.041
Gil MI, Tomas-Barberan FA, Hess-Pierce B, HolcroftDM, Kader AA (2000). Antioxidant Activity of Pomegranate Juice and its Relationship with phenolic composition and processing. J. Agric. Food Chem. 48: 4581−4589. http://dx.doi.org/10.1021/jf000404a
Glozer K, Ferguson L (2011). Pomegranate production in Afghanistan. UC Davis. College of Agricultural and Environmental Sciences. pp. 1-32.
Gothilf S (1969). The biology of the carob moth Ectomyelois ceratoniae (Zell.) in Israel. Effect of food, temperature and humidity on development. Israel J. Entomol. IV: 107-116.
Hanna AD (1939). The pomegranate fruit butterfly Virachola livia Klug : Morphology, life history and control. Entomological Section. Bulletin Number 186. Government Press (Bulâq). 54 p.
Juan P, Martinez JJ, Oltra MA, Ferrandez M (2000). Current situation of pomegranate growing (Punica granatum) in southern Alicante. Chemical control of pests and diseases and financial cost. Options Méditerranéennes Séries A. Séminaires Méditerranéenns. 42:157-161.
Ksentini I, Jardak T, Zeghal N (2011). First report on Virachola livia Klug. (Lepidoptera: Lycaenidae) and its effects on different pomegranate varieties in Tunisia. Bulletin OEPP/EPPO Bulletin. 41:178–182.
Lansky EP, Newman RA (2007). Punica granatum (pomegranate) and its potential for prevention and treatment of inflammation and cancer. J. Ethnopharmacol. 109(2): 177-206. http://dx.doi.org/10.1016/j.jep.2006.09.006
Mansour KM (1995). Underutilized fruit crops in Egypt. Cahiers. Options Méditerranéennes. 13-19.
Mars M (1995). La culture du grenadier (Punica granatum L.) et du figuier (Ficus carica L.) en Tunisie. Cahiers Options Méditerranéennes 13:85-95.
Mars M (2000). Pomegranante plant material: genetic resources and breeding, a review. Options méditerranéennes. Série A. Séminaires Méditérranéens 42: 55-62.
Melgarejo P, Salazar DM, Artes F (2000). Organic acids and sugars composition of harvestedpomegranate fruits, Eur. Food Res. Technol. 211(3):185–190. http://dx.doi.org/10.1007/s002170050021
Moawad SS, Hassan SA, Al-Barty AM (2011). Enumeration and estimation of insect attack fruits of some cultivars of Punica granatum. Afr. J. Biotechnol. 10(19):3880-3887.
Kahramanoglu I, Uzanmaz S, Nizam I (2014). Incidence of heart rot at pomegranate fruits caused by Alternaria spp. in Cyprus. Afr. J. Agric. Res. 9(10): 905-907.
Özturk N, UlusoyMR, Bayhane E (2005). Pest and natural enemy species determined in pomegranate orchards in the Eastern Mediterranean Region, Turkey. Türk. Entomol. Derg. 29 (3): 225-23.
Özturk N, Ulusoy MR (2009). Pests and natural enemies determined in pomegranate orchards in Turkey. Acta Hort. (ISHS) 818:277284.
Solis MA (2006). Key to selected Pyraloidea (Lepidoptera) larvae intercepted at U.S. ports of entry. Revision of Pyraloidea in Keys to some frequently intercepted Lepidopterous larvae by Wiseman (1986) USDA Systematic Entomology Laboratory. Paper 1.
SPSS (2008). Spss Statistics for windows 17.0. Chicago.Il.USA.
Zalucki MP, Clarke AR, Malcolm SB (2002). Ecology and behavior of first instar larval lepidoptera. Annu. Rev. Entomol. 47:361–393. http://dx.doi.org/10.1146/annurev.ento.47.091201.145220

academicJournals

Vol. 7(2), pp. 11-17, March, 2015
DOI: 10.5897/JEN2015.0116
Article Number: 79BA3C651630
ISSN 2006-9855

http://www.academicjournals.org/JEN

Journal of Entomology and Nematology

Full Length Research Paper

Isolation and identification of fungal growth on *Tribolium castaneum* in stored wheat flour

Bosly, H. A. and El-Banna O. M.

Department of Biology, Faculty of Science, Jazan University, Jazan, Saudi Arabia.

Received 31 January, 2015; Accepted 2 March, 2015

Stored wheat flour samples purchased from Jazan local markets in Saudi Arabia were checked for the presence of pests, insect and fungal species. The insects existing in the flour were separated and identified as red flour beetle *Tribolium castaneum* and from 60 individuals (dead bodies) inspected, 17 individuals exhibited abnormal deformation, decomposition and or superficial fungal growths. Incubation of the dead insects' bodies resulted in emergence of fungal growth on the surface of these bodies. Nine fungal species that belonged to eight fungal genera were isolated. Results indicate that *Beauveria bassiana* and *Verticillium lecanii* were the most dominant fungi among those isolated from *T. castaneum* growing in flour followed by *Sporothrix* sp., *Hirsutella versicolor*, *Granulomanus* sp., modern *Rhizoctonia solani*, *Moelleriella* sp., *Aspergillus fumigatus* and *A. flavus*.

Key words: Stored wheat flour, *Tribolium castaneum*, fungi, Jazan, Saudi Arabia.

INTRODUCTION

Saudi Arabia is a sub-tropical country, with a warm climate that favors the multiplication of microorganisms and destructive pests of stored products (Tirado et al., 2010). *Tribolium castaneum* is one of the most destructive beetle pests of stored products and a major pest of cereals worldwide especially in tropical and sub-tropical regions (Islam and Talukder, 2005). Damage to agricultural crops both in the field and during harvest and storage due to fungi is also very considerable as it reaches billions of dollars around the world (Kendrick, 1992). Insects, nematodes, fungi, and microorganisms constantly compete with humans for these commodities. In a closer look, interactions of these organisms might be more complicated. The stored wheat flour is subjected to invasion with the red flour beetle, *Tribolium castaneum* (Wakil et al., 2003).

Some insect species, including many pests, are particularly susceptible to infection by naturally occurring, insect-pathogenic fungi. These fungi are very specific to insects, often to particular species, and do not infect animals or plants. They are generally safe for the environment and thus considered to be among the most promising alternatives to chemical–based insect control (Sahayaraj and Tomson, 2010). Fungal growth favored by moist conditions but fungi also have resistant stages that maintain infection potential under dry conditions. Fungi have considerable epizootic potential and can spread quickly through an insect population and cause its collapse (Hoffmann and Frodsham 1993; Maria et al., 2006; Sahayaraj and Tomson, 2010). Primary hosts of

*Corresponding author. E mail: dr_2009_bosly@hotmail.com.

the majority of these fungi are aphids, whiteflies, leaf-hoppers, flies, beetles, caterpillars, thrips, mites and some beetle larvae.

The most recorded entomopathogenic fungi species were, *Beauvaria bassiana, Asperigillus, Penicillium, Metarrhizium, Tolypocladium, Isaria, Lecanicillium Aschersoniabadia, Aachersonia marginata, Aschersonia samoensis, A. oxystoma, A. placenta, A. confluens, Hypocrellasia mensis, Moelleriella raciborskii, Hypocrella calendulina, Paecilomyces cinnamomeus, Conoideocrella tenuis* and *Verticillium* spp. (Lord, 2001; Shah and Pell, 2003). Lawrence and Milner (1996) and Shah and Pell (2003) indicated that *Ophiocordyceps unilateralis, O. myrmecophila, Torrubiella* sp., *Isaria* sp., *Paecilomyces lilacinus, Isariatenuipes, Gibellula pulchra, Nomuraeaa typicola, Hirsutella formicarum, Metarhizium* sp., *Akanthomyces pistillariiformis* and *Hirsutella citriformis* are entomopathogenic fungi applied as biocontrol agents. In addition to the previously mentioned fungi, Akanthomyces, Aschersonia, Beauveria, Conoideocrella, Cordyceps, Gibellula, Hirsutella, Hymenostilbe, Hypocrella, Isaria, Metarhizium, Moelleriella, Nomuraea, Ophiocordyceps, Paecilomyces, Torrubiella and Verticillium are entomopathogenic fungi and associated with many insects.

The goal of this work was to isolate and identify fungi naturally associated with *T. castaneum* and to study the effect of these fungi on their insect hosts and to keep them as stock for further research concerning biological control application.

MATERIALS AND METHODS

Source of wheat Flour samples

Twenty-four wheat flour samples in paper packets of 1 Kg each purchased from Jazan local markets in Saudi Arabia were stored at room temperature (22 - 35°C) and 65- 68% relative humidity for two years. The mean temperature and relative humidity were as follows; January daytime highest temperature was 88°F (31°C) and lowest was 71°F (22°C), while July had average daytime highest temperature of 104°F (40°C) and lowest of 86°F (30°C) (Bosly and Kawanna, 2014). At the end of the storage period, the flour was characterized with yellowish color and undesirable odors. The flour were checked for the presence of pests, insects and fungal species associated with or affecting them.

Separation and identification of the affected insects

The insects existing in the flour were separated and identified as Red flour beetle *T. castaneum* according to the key given by Hinton (1945) and Bousquet (1990).

Isolation of fungi associated with the separated insects

Isolation of fungi associated with the separated *T. castaneum* was done in three successive stages as follows:

Separation of the affected insects

The dead bodies of *T. castaneum* (60 individuals) separated from the flour using a silk sieve, were cleaned by the aid of camel hairbrush to remove the flour. The insect's dead bodies were inspected by the binuclear for any deformations or decomposition. The individuals exhibited abnormal deformation, decomposition and or superficial fungal growths were photographed by a (zoom in) digital camera.

Incubation of the candidate insects

The individuals of *T. castaneum* that showed decomposition or fungal growths were incubated to allow the emergence of fungal growth from both outside and inside the affected insects. For incubation, plastic Petri dishes (9 cm) each containing sterilized filter paper (Whatman No1) were used. Sterilized glass slide was fixed above the filter paper. Using sterilized fine tip forceps, the individuals were transferred and put above the slide. Two individuals were put on each slide. The filter papers were kept wet by dispensing 1 ml of sterilized distilled water according to Dhingra and Sinclair (1985).The dishes were covered and sealed using para film and incubated at 25±2°C for 7 days. After elapse of the incubation period, insects were checked for emergence of fungal growth by the binuclear and the individuals that the fungi observed on their surfaces were labeled and used in the isolation experiment.

Isolation and identification of the emerged fungi

For isolation of the emerged fungi associated with the insects, Petri dishes (9 cm) containing PDA medium were used. Aseptically, small part of the fungal growth was taken from the surface of the insect body using a sterilized needle and transferred onto the surface of PDA medium in Petri dish. The dishes were labeled, sealed with parafilm and incubated at 25 ±2°C. The dishes were checked for fungal growth after 3 and 7 days. The emerged fungi were picked-up and transferred to new PDA medium plates, purified and identified following keys given by Ainsworth (1971), Ellis (1971 and 1976), Barnett and Hunter (1972), Domsch et al. (1993)and Gadd et al. (2006). Frequency of each identified fungus was calculated according to the following equation:

$$Frequency\ percentage = \frac{\text{Number of fungal colonies for each fungus X } 100}{\text{Total number of fungal colonies for the isolated fungi}}$$

Light microscope examination

To identify the emerged fungi, slides from cultures of any of the previously mentioned fungi grown on the PDA medium for 7 days were prepared, fixed, stained with lacto phenol-cotton blue and examined by light microscope (ModelZeizz AX10) and photographs were captured using AxioCamICc1 using the program (Axiovission4.8.2-06-2010). Stock cultures on PDA under paraffin oil were kept in a refrigerator at 5-10°C and sub-cultured onto fresh medium every 2-3 weeks. These will be the potential biological materials for further research and application.

RESULTS

Separation and identification of the affected insects

The apparently healthy red flour beetle *T. castaneum* is

Figure 1. Photograph shows dorsal (left) and ventral (right) sides of the isolated non-infected *T. castaneum.*

Figure 2. Photograph shows ventral side of T. *castaneum* at the beginning of infection by *Verticilliumlecanii*.

Figure 3. Different degrees of infection and colonization with *Beauveriabassiana*.

Figure 4. Photograph shows that the body of T.*castaneum*is already occupied by *Beauveriabassiana*.

represented in Figure 1. About 60 insect dead bodies of the *T. castaneum* checked for the different degrees of degeneration were separated from the stored wheat flour, inspected and photographed. The insects exhibited various morphological deformations as showed in Figures 2, 3, 4 and 5. The most characteristic deformation was the separation of the insect head (Figure 6), decom-position of their wings (Figure 7), legs (Figure 8) and the abdomen (Figure 9). Some parts of the insects dead bodies were covered with colored fungal growths that ranged from olive green, white, grey, brown to black. The fungal infection ranged from distributed colonies on the insect body (Figure 2) or parts of it covered with the fungal growth (Figures 4, 5 and 7).

Isolation and identification of the fungi associated with the separated insects

Isolation from the candidate insect samples showed emergence of fungal growths by incubation which resulted in isolation of nine fungal species belonging to eight fungal genera (Table 1). The fungi isolated were purified and identified to the generic and/or the species level according to their morphological characteristics.

Figure 5. Abdomen completely colonized by *Hirsutellaversicolor*.

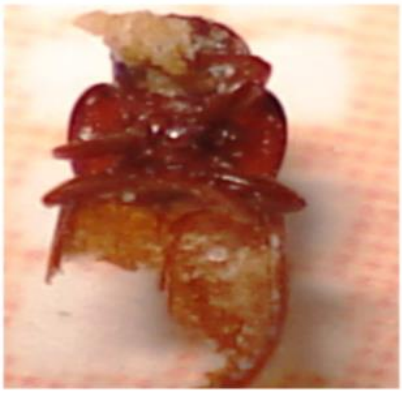

Figure 8. Photograph showing the decomposition of the lower part of the abdomen and the fungal growth of *Sporothrix* spp. that covers the frontal mouth parts.

Figure 6. Photograph shows that the half of the dorsal *T. castaneum* decomposed and the head removed as a result of infection by *Aspergillus flavus*

Figure 9. Decomposition of the abdomen and the *T. castaneum* dead body colonized by the fungal growth.

Figure 7. *T. castaneum* infected with *Rhizoctonia solani*.

Results indicate that *B. bassiana* and *V. lecanii* were the most dominant fungi among those isolated from red flour beetle of wheat flour samples collected from Jazan showing 28.1 and 26.6%, respectively, followed by *Sporothrix* spp. (14.1%), *Hirsutella versicolor* (9.4%), *Granulomanus* sp. (6.2%), modern *Rhizoctonia solani* and *Moelleriella* sp. (4.7%), *Aspergillus fumigatus* and *A. flavus* (3.1%).

Light microscopy

The morphological features of the isolated fungi were studied through examination of slides prepared from the fungal colonies obtained on PDA medium. The preparations were stained by lactophenol-cotton blue and examined by light microscope at magnification 40x. The photographs are represented in Figure 10.

Table 1. Fungi isolated from *T.castaneum* samples by culturing on PDA medium and their frequencies.

Isolated fungi	Number of fungal colonies	(%) Frequency *
Aspergillus flavus	2	3.1
Aspergillus fumigates	2	3.1
Beauveria bassiana	18	28.1
Hirsutella versicolor	6	9.4
Modern Rhizoctonia solani	3	4.7
Granulomanus sp	4	6.2
Nomuraeacy lindrospora	3	4.7
Sporothrix spp	9	14.1
Verticillium lecanii	17	26.6
Total	64	100

$$* \, Frequency\ percentage = \frac{\text{Number of fungal colonies for each fungus X } 100}{\text{Total number of fungal colonies for the isolated fungi}}$$

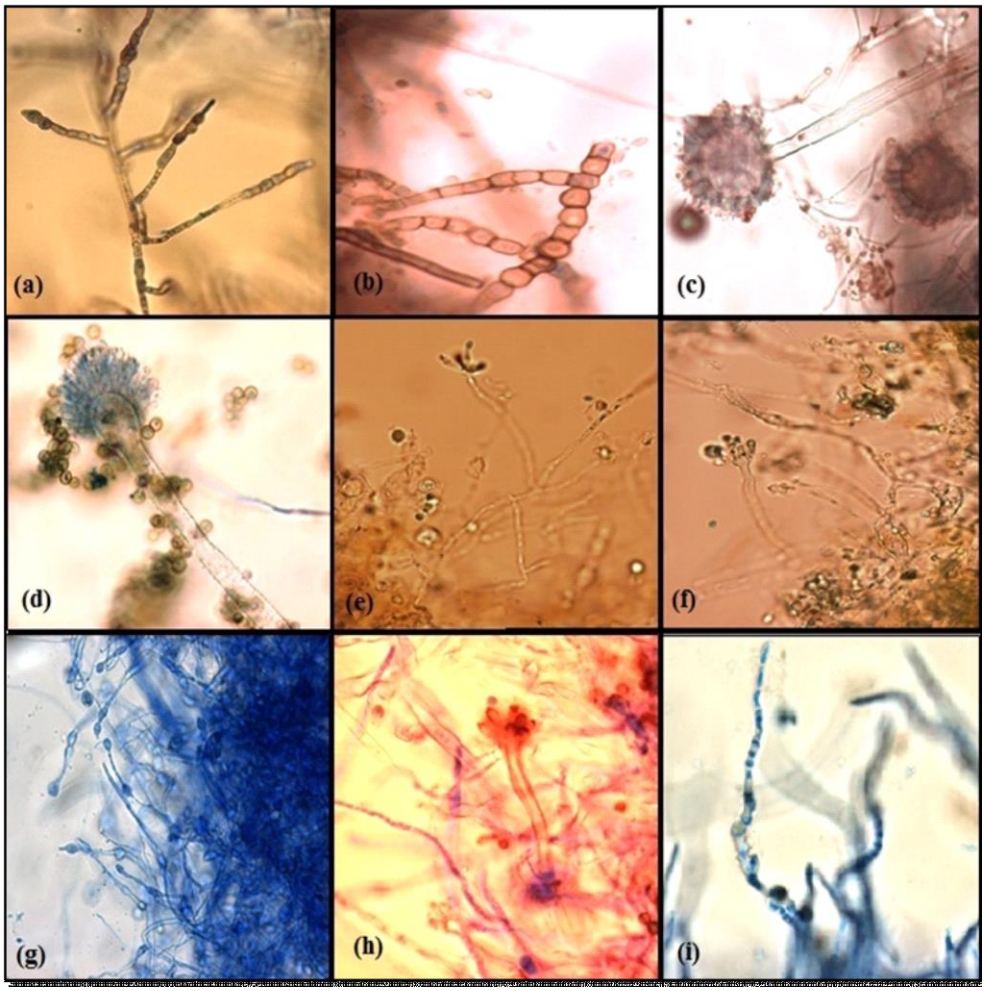

Figure 10. Light photographs (40X) showing the morphology of the fungi isolated from the candidate samples of *Tribolium castaneum* stained with lacto phenol-cotton blue: *Verticillium lecanii* (a); modern *Rhizoctonia solani* (b); *Aspergillus flavus* (c); *Aspergillus fumigatus* (d); *Beauveria bassiana* (e); *Nomuraeacy lindrospora* (f); *Hirsutella versicolor* (g); *Sporothrix* sp. (h) and *Granulomanus* sp. (i)

DISCUSSION

Several studies confirmed the development and survival of *T*. *castaneum* in wheat flour (Khattak and Shafique, 1986; Shafique et al., 2006; Bosly and Kawanna, 2014). Infestation by insects encourages growth of fungi including those that produce mycotoxins, and results in contamination of commodities with insect bodies and waste products etc. The diversity and distribution of entomopathogenic fungal species depends on environmental conditions, diversity of insect species and their hosts. Dispersal of the fungus probably played an important role in the co-evolution with its insect host and the co-evolution of the insect with its plant host (Chaverri et al., 2005). The high moisture and diversified and plentiful insects in tropical countries, especially those like our country (KSA) provide ideal conditions for the entomopathogenic fungi to develop as stated in Gadd et al. (2006). Previously, Shah and Pell (2003) indicated that the dead insect's body infected with an entomopathogenic fungi may be firm and "cheese-like" or an empty shell, often but not always with cream, green, red, or brown fungal growth, either enveloping the body or emerging from joints and body segments. Incubation of the candidate insects for 7 days at 25±2°C resulted in emergence of fungal growths on the surface of some incubated insects dead bodies. On the other hand, the rest of the incubated insects still clean even after prolonged incubation period to 14 days. Gabartya et al. (2014) described that *B*. *bassiana* and *M*. *anisopliae* are entomopathogenic and their infection begins when conidia (asexual spores, the seeds of a fungus) attach to insect's cuticle, the spores germinate and penetrate the insect's skin and enter the host. Once the fungus penetrates the host it produces toxins that overcome the insect immune system and the hyphae penetrate through the cuticle to the outside and cause white (*B*. *bassiana*) or green (*M*. *anisopliae*) sporulation on the insect's body. The obtained results about those fungi indicate that invading insects occurred by penetrating their cuticle or "skin." Once inside the insect, the fungus rapidly multiplies throughout the body. Death is caused by tissue destruction and, occasionally, by toxins produced by the fungus. The fungus frequently emerges from the insect's body to produce spores that, when spread by contact with other insects, can spread infection (Moino et al., 2002). Another explanation is the fungi secondary metabolites that increase the chance of insect death (Fan et al., 2013). The previously mentioned fungi have the ability to decompose cuticle layer to be able to penetrate the insect body and colonize it leading to death of these insects. The fungi act through the insect integument in several ways. Long chain hydrocarbons help in or play an important role in initial attachment of the entomopathogenic fungal spores to cuticle given that such dry phialocondia attach in nonspecific manner through hydrophobic interaction (Lord, 2001 and 2009; Shah and Pell, 2003). The chemical, biological or physical treatments for controlling the insects during storage may contribute towards reducing spread of fungal spores within the storage system (Huang et al., 1997; Athanassiou et al., 2008; Brijwani et al., 2010; Mohale et al., 2010; Beckett, 2011; Villers, 2014).

Conclusion

The natural death of the insects separated from the stored wheat flour gave us the idea of this investigation and the further studies of biological control of the red wheat flour *T*. *castaneum*. The fungi were isolated from insects naturally exhibiting different degrees of deformation, decomposition and superficial fungal growth. The more destructive fungal genera that is *B. bassiana*, *H. versicolor*, *Sporothrix* sp. and *V. lecanii* preserved for further study which will concern biological control of *T. castaneum*

It can be also be concluded that, storage of wheat flour under Jazan conditions which is characterized by high temperature and humidity resulted in the presence of *T*. *castaneum*. Moreover abnormal deformation, decomposetion and or superficial fungal growths flour should be stored in conditions to prevent the flour from attracting insects and fungi to avoid the loss of flour and the health damage resulting from the usage.

ACKNOWLEDGMENT

The authors would like to thank the Fungi Research Department, Plant Pathology Institute, Agriculture Research Center (ARC), Giza, Egypt for the identification of the isolated fungi.

Conflict of interest

The authors declared no conflict of interest.

REFERENCES

Ainsworth GC (1971). Ainsworth and Bisby's Dictionary of the Fungi. Sixth Edition Farnham Royal, Commonwealth Agricultural Bureaux [for the] Commonwealth Mycological Institute. ISBN-0851980759.

Athanassiou CG, Kavallieratos NG, Yiatilis AE, Vayias BJ, Mavrotas CS, Tomanović Z (2008). Influence of temperature and humidity on the efficacy of spinosad against four stored-grain beetle species. J. Insect Sci. 8(60): 1-9. http://dx.doi.org/10.1673/031.008.6001

Barnett HL, Hunter BB (1972). Illustrated Genera of Imperfect fungi. 3rd edition Burgess Publishing Co. USA.

Beckett SJ (2011). Insect and mite control by manipulating temperature and moisture before and during chemical-free storage J. Stored Prod. Res. 47: 284-292. http://dx.doi.org/10.1016/j.jspr.2011.08.002

Bosly HA, Kawanna MA (2014).Fungi species and red flour beetle in stored wheat flour under jazan region conditions. Toxicol. Health 30(4): 304–310. http://dx.doi.org/10.1177/0748233712457449

Bousquet Y (1990). Beetles Associated with Stored Products in Canada: An Identification Guide, 240p. Agriculture Canada

Publication 1837, Canada.
Brijwani M, Subramanyam B, Flinn PW, Langemeier MR (2010) Structural heat treatments against Triboliumcastaneum (Herbst) (Coleoptera: Tenebrionidae): effect of flour depth, life stage and floor. Paper presented at: Proceedings of the 10th International Working Conference on Stored Products Protection June 27 - July 2, Estoril, Portugal. pp. 622-627.
Chaverri P, Bischoff JF, Evans HC, Hodge KT (2005). Regiocrella, a new entomopathogenic genus with a pycnidialanamorph and its phylogenetic placement in the Clavicipitaceae. Mycologia 97(6): 1225-1237. http://dx.doi.org/10.3852/mycologia.97.6.1225
Dhingra OD, Sinclair JB (1985). Basic Plant Pathology Methods. CRC Press, Inc Boca Raton. Florida. USA.
Domsch KH, Gams W, Anderson TH (1993). Compendium of soil fungi. IHW-Verlag, Eching, Germany.
Ellis MB (1971). DematiaceousHyphomycetes. Commonwealth Mycological Institute. Kew, Surrey, England.
Ellis MB (1976). More DematiaceousHyphomycetes. Commonwealth Mycological Institute. Kew, Surrey, England.
Fan J, Xie Y, Xue J, Liud R (2013). The Effect of Beauveriabrongniartii and its Secondary Metabolites on the Detoxification Enzymes of the Pine Caterpillar, Dendrolimustabulaeformis. J. Insect Sci. 13:44. http://dx.doi.org/10.1673/031.013.4401
Gabartya A, Salema HM, Foudab MA, Abasb AA, Ibrahimc AA (2014). Pathogencity induced by the entomopathogenic fungi Beauveriabassiana and Metarhiziumanisopliae in Agrotisipsilon (Hufn.). J. Radiation Res. Appl. Sci. 7(1):95-100. http://dx.doi.org/10.1016/j.jrras.2013.12.004
Gadd GM, van West P, Robson GD (2006). Exploitation of Fungi. Cambridge: Cambridge University Press.
Hinton HE (1945). A monograph of the beetles associated with stored products- Volume 1. British Museum (Natural History), London. 443p
Hoffmann MP, Frodsham AC (1993). Natural Enemies of Vegetable Insect Pests. Cooperative Extension, Cornell University, Ithaca, NY. 63 p.
Huang Y, Tan JMW, Kini RM, Ho SH (1997) Toxic and antifeedant action of nutmeg oil against Triboliumcastaneum (Herbst) and SitophiluszeamaisMotsch. J. Stored Prod. Res. 33(4): 289-298. http://dx.doi.org/10.1016/S0022-474X(97)00009-X
Islam S, Talukder FA (2005). Toxic and residual effects of Azadirachtaindica, Tageteserecta and Cynodondactylon seed extracts and leaf powders towards Triboliumcastaneum. J. Plant Dis. Protect.112 (6):594-601.
Kendrick B (1992). The Fifth Kingdom, Second Edition 1992. Focus Texts, Focus Information Group, Inc., PO Box 369, Newburyport, MA 01950. ISBN 0941051285.
Khattak SUK, Shafique M (1986). Varietal susceptibility of ten wheat cultivars flour to red flour beetle, Tribolium castaneum (Herbst) (Coleoptera:Tenebrionidae). Pak. J. Zool. 18:257-261.
Lawrence JF, Milner RJ (1996). Associations between arthropods and fungi. Fungi Aust. 1B:137-202.
Lord JC (2001). Desiccant dusts synergize the effect of Beauveriabassiana (Hyphomycetes: Moniliales) on stored-grain beetles. J. Econ. Entomol. 94:367-372. http://dx.doi.org/10.1603/0022-0493-94.2.367
Maria PM, Christos GA, Nickolas GK, Yacoub AB, George NB (2006). Effectiveness of Metarhiziumanisopliae (Metschinkoff) Sorokin applied alone or in combination with diatomaceous earth against Tribolium confusum Du Val larvae: Influence of temperature, relative humidity and type of commodity. Crop Prot. 25:418-425. http://dx.doi.org/10.1016/j.cropro.2005.07.003
Mohale S, Allotey J, Saime BA (2010). Control of Tribolium confusum J Du Val. by diatomaceous earth (Protect-It[TM]) on stored groundnut (Arachishypogaea) and Aspergillus flavus linkspore dispersal. AJFAND. 10(6):2678-2694.
Moino JrA, Alves SB, Lopes RB, Oliveria PM, Neves J, Pereria RM, Vieira SA (2002). External development of the entomopathogenic fungi Beauveriabassiana and Metarhiziumanisopliaein the subterranean termite Heterotermestenuis. Sci. agric. (Piracicaba, Braz.). 59(2):267-273.
Sahayaraj K, Tomson M (2010). Impact of two pathogenic fungal crude metabolites on mortality, biology and enzymes of Dysdercusc ingulatus (Fab.) (Hemiptera: Pyrrhocoridae). J. Biopestic. 3:163-167.
Shafique M, Ahmad M, Chaudry MA (2006). Feeding Preference and Development of Tribolium castaneum (Herbst.) in Wheat Products. Pak. J. Zool. 38(1):27-31.
Shah PA, Pell JK (2003). Entomopathogenic fungi as biocontrol agents. Appl. Microbiol. Biotechnol. 61(5-6):413-423. http://dx.doi.org/10.1007/s00253-003-1240-8
Tirado MC, Clarke R, Jaykus LA, Mc Quatters-Gollop A, Frank JM (2010). Climate change and food safety: A review. Food Res. Int. 43(7):1745-1765. http://dx.doi.org/10.1016/j.foodres.2010.07.003
Villers P (2014). Aflatoxins and safe storage. Front Microbiol. 5:158. http://dx.doi.org/10.3389/fmicb.2014.00158
Wakil W, Hassan M, Javed A, Anwar S (2003). Comparison of nutritional losses of insect infested wheat in laboratory and public storages. Pak. J. Arid Agric. 6:1-6.

Journal of Entomology and Nematology

Related Journals Published by Academic Journals

- *Biotechnology and Molecular Biology Reviews*
- *African Journal of Microbiology Research*
- *African Journal of Biochemistry Research*
- *African Journal of Environmental Science and Technology*
- *African Journal of Food Science*
- *African Journal of Plant Science*
- *Journal of Bioinformatics and Sequence Analysis*
- *International Journal of Biodiversity and Conservation*

academicJournals