Volume 15, Issue 2, July 2022
ISSN 1791-3691 (Print)
ISSN 2732-656X (OnLine)

Hellenic Plant Protection Journal

A semiannual scientific publication of the
BENAKI PHYTOPATHOLOGICAL INSTITUTE

Editorial Policy

The *Hellenic Plant Protection Journal (*HPPJ) (ISSN 1791-3691) is the scientific publication of the Benaki Phytopathological Institute (BPI) replacing the *Annals of the Benaki Phytopathological Institute* (ISSN 1790-1480) which had been published since 1935. Starting from January 2008, the *Hellenic Plant Protection Journal* is published semiannually, in January and July each year.

HPPJ publishes scientific work on all aspects of plant health and plant protection referring to plant pathogens, pests, weeds, pesticides and relevant environmental and safety issues. In addition, the topics of the journal extend to aspects related to pests of public health in agricultural and urban areas.

Papers submitted for publication can be either in the form of a complete research article or in the form of a sufficiently documented short communication (including new records). Only original articles which have not been published or submitted for publication elsewhere are considered for publication in the journal. Review articles in related topics, either submitted or invited by the Editorial Board, are also published, normally one article per issue. Upon publication all articles are copyrighted by the BPI.

Manuscripts should be prepared according to instructions available to authors and submitted in electronic form on line at http://www.hppj.gr. All submitted manuscripts are considered and published after successful completion of a review procedure by two competent referees.

The content of the articles published in HPPJ reflects the view and the official position of the authors. The information and opinions contained herein have not been adopted or approved by the HPPJ Editorial Board. The HPPJ Editorial Board neither guarantees the accuracy of the information included in the published articles nor may be held responsible for the use to which information contained herein may be put.

For all parties involved in the act of publishing (the author(s), the journal editor(s), the peer reviewers, and the publisher) it is necessary to agree upon standards of expected ethical behavior. HPPJ follows the ethics statements of De Gruyter journals, which are based on the Committee on Publication Ethics (COPE) Code of Conduct guidelines available at www.publicationethics.org.

For back issues, exchange agreements and other publications of the Institute contact the Library, Benaki Phytopathological Institute, 8 St. Delta Str., GR-145 61 Kifissia, Attica, Greece, e-mail: m.kitsiou@bpi.gr.

This Journal is indexed by: *AGRICOLA, CAB Abstracts*-Plant Protection Database, *INIST* (Institute for Scientific and Technical Information) and *SCOPUS*.

The olive tree of Plato in Athens is the emblem of the Benaki Phytopathological Institute

sciendo

Hellenic Plant Protection Journal **15:** 49-56, 2022
DOI 10.2478/hppj-2022-0006

SHORT COMMUNICATION

Differentiation between *Botryosphaeria dothidea* and *Neofusicoccum* spp. based on a single nucleotide polymorphism in the ITS region

S. Palavouzis, A. Triantafyllopoulou, A.K. Tzima and E.J. Paplomatas*

Summary Fungi belonging to the Botryosphaeriaceae family are widespread pathogens of many angiosperms, causing disease on various high value crops. The most important members of the family for the Greek region and other Mediterranean countries are *Botryosphaeria dothidea*, *Neofusicoccum hellenicum*, *Neofusicoccum mediterraneum* and *Neofusicoccum parvum*. The frequently concurrent isolation of Botryosphaeriaceae species from the same host, as well as the extensive host range of *B. dothidea*, necessitate the development of rapid and reliable detection methods. This study presents a new and robust molecular diagnostic tool, in the form of a PCR method based on primers designed on an SNP (single nucleotide polymorphism) located in the ITS region (Internal Transcribed Region) of *B. dothidea* and *Neofusicoccum* species. SNP primers constructed with or without added mismatch nucleotides were combined with the same upstream universal primer to generate distinct amplicons. When evaluated in PCR assays, mismatched primers were found to have the highest differentiation capability. The potential for further development of SNP assays in order to differentiate between species is being evaluated.

Additional keywords: diagnosis, internal transcribed spacer region, molecular marker, *Neofusicoccum*, SNPs

Botryosphaeriaceae is a family of economically important phytopathogens, with worldwide distribution (Slippers *et al*., 2007). Members of the family are known to infect a wide range of woody plants, affecting buds, panicles, petioles, rachises, fruit, the mid rib of leaflets, shoots and branches. The pathogens cause fruit rot, dieback, cankers, panicle and shoot blight (Chen *et al*., 2014).

Pistachio (*Pistacia vera* L.) is one of the most important hosts for the species in the Botryosphaeriaceae family. In Greece and other Mediterranean countries, where pistachio cultivation is widespread, the main species responsible for high yield loses are *Botryosphaeria dothidea*, *Diplodia seriata, Neofusicoccum hellenicum*, *Neofusicoccum mediterraneum* and *Neofusicoccum parvum* (Chen *et al*., 2015; Lazzizera *et al*., 2008).

To date, species differentiation within the Botryosphaeriaceae family was based on RFLPs (Slippers *et al*., 2007) or phylogenetic analysis of fingerprinting data (Abdollahzadeh and Zolfaghari, 2014) and gene sequences (Pavlic *et al*., 2009**;** Lopes *et al*., 2017). Only at the genus level, primers have been developed for detection of Botryosphaeriaceous species (Ridgway *et al*., 2011). The ITS region has been a target of choice for the design of species specific primers due to the high sensitivity achieved in single and nested PCR (Flowers *et al*., 2003).

With so many plant pathogenic species causing disease, the ability to rapidly and correctly delimit species and/ or genera is of vital importance for the successful management of these pathogens. At the same time, since some Botryosphaeriaceous species are homothallic, an impeding danger arises regarding genetic recombination that could

Laboratory of Phytopathology, Department of Crop Science, School of Plant Sciences, Agricultural University of Athens, Iera Odos 75, GR-118 55 Athens, Greece.
* Corresponding author: epaplom@aua.gr

lead to host jumps (Batista *et al*., 2021) or the rapid development of resistance of these fungi to various fungicides. Along these lines, our study presents a robust and quick PCR method for the differentiation between *B. dothidea* and *Neofusicoccum* spp. targeting the ITS region.

Initially, sequences of *N. hellenicum*, *N. mediterraneum*, *N. parvum* and *B. dothidea* were collected from the NCBI database ("National Center for Biotechnology Information (NCBI)[Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information," 1988). Additionally, sequences for each species, derived from isolates of our own collection, were deposited on NCBI and utilized in the study. ITS sequences of *B. dothidea* and *N. hellenicum*, *N. mediterraneum*, *N. parvum* were aligned using Clustal Omega (Madeira *et al*., 2019). Differences between sequences of the species under study were detected using the MEGA software (Kumar *et al*., 2018). Based on sequence similarities, designing of SNP primers for *B. dothidea* and *Neofusicoccum* spp. with the SNP base located at the 3′ end and combined with a common upstream primer, was selected as the preferred strategy.

Downstream SNP primers were designed with a common upstream ITS5 primer. SNP base was detected on the 3′ end (A on *B. dothidea* sequence and T on *Neofusicoccum* sequence) and matching primers were developed (T on BotdoITS_384R primer and A on NeofusITS_384R primer, respectively). However, reproducible and consistent differentiation was proved difficult using these primers. Thus, new primers were designed based on the initial SNP primers. This time ITS3, which is positioned at the 5.8s rRNA, was used as the upstream primer (Table 1).

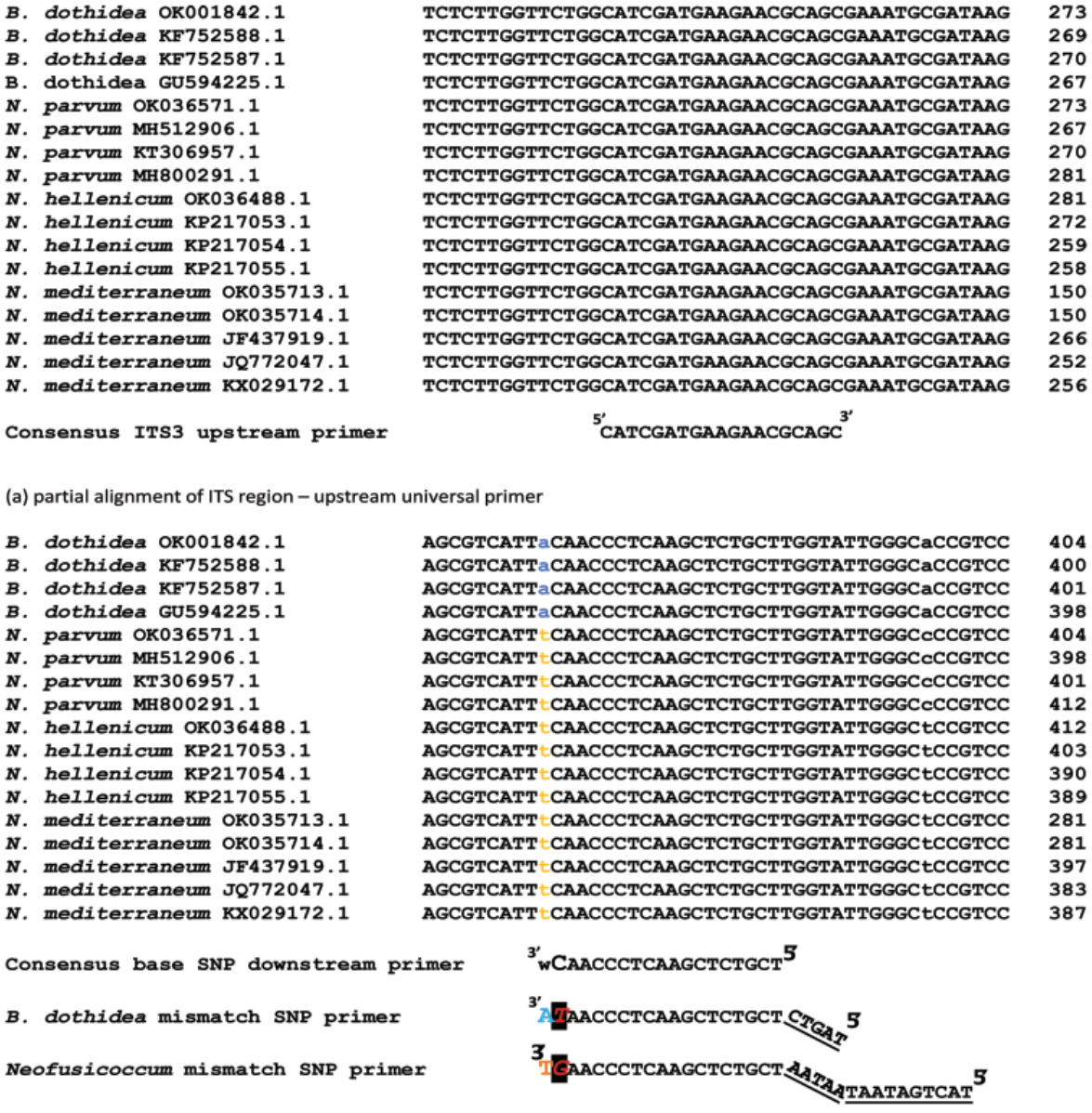
Based on the data from Gaudet *et al*. (2009), the newly designed downstream SNP primers included a destabilizing mismatch within the 3′ end of the primers. The mismatch was different for each of the downstream SNP primers. In addition, a tail of different length between the two primers was added on the 5′ end, taking care to keep the melting temperature of the primers similar (Fig. 1). Primers were checked for their specificity and characteristics using the software Primer 3 (Untergasser *et al*., 2012) and IDT DNA oligo analyzer (Owczarzy *et al*., 2008).

The SNP on the downstream primers selected between the species, was T for *B.*

Table 1. Primer pair sequences utilized in the present study for discrimination of *Botryosphaeria dothidea* and *Neofusicoccum* spp. For primers designed in the present study in the ITS region, the 3′ SNP base is indicated in **bold**, the mismatch base is shown in *italics & underlined* and the 5′ tail sequence in *italics*.

Primer name	Sequence (5′ > 3′)	Description	Reference
ITS4	TCCTCCGCTTATTGATATGC	Universal Downstream primer	White *et al.* (1990)
ITS5	GGAGTAAAAGTCGTAACAAGG	Universal Upstream primer	White *et al.* (1990)
BotdoITS_384R	CAGAGCTTGAGGGTTG**T**	SNP Downstream *B. dothidea* primer	This study
NeofusITS_384R	CAGAGCTTGAGGGTTG**A**	SNP Downstream *Neofusicoccum* primer	This study
ITS3	GCATCGATGAAGAACGCAGC	Universal Upstream primer	White *et al.* (1990)
TL-MS-BotdoITS_386R	*ATCAG*AGCAGAGCTTGAGGGTT*A***T**	SNP + mismatch Downstream *B. dothidea* primer	This study
TL-MS-NeofusITS_386R	*ATGACTATTATTATT*AGCAGAGCTTGAGGGTT*C***A**	SNP + mismatch Downstream *Neofusicoccum* primer	This study

dothidea primer and A for *Neofusicoccum* spp. primer. In order to improve the specificity of the PCR, a mismatch was added next to the 3′ end. That was A for the *B. dothidea* primer and C for the *Neofusicoccum* primer. The bases used for mismatch were chosen to ensure differentiation efficiency and secondary structure of the primers. To facilitate visual gel separation, a 5 bp tail was added to the 5′ end of *B. dothidea* primer and a 15 tail on the 5′ end of the *Neofusicoccum* spp. primer (Fig. 1). Although the prim-



(a) partial alignment of ITS region – upstream universal primer

Figure 1. Illustrating the design of downstream primers TL-MS-BotdoITS_386R and TL-MS-NeofusITS_386R for selective amplification of *Botryosphaeria dothidea* and *Neofusicoccum* spp., respectively when paired with universal primer ITS3. Isolates from our own collection are indicated with an asterisk. Primer position is indicated on partial alignment of 46 bp of the ITS region of selected *B. dothidea* and *Neofusicoccum* spp. sequences to demonstrate sequence homology and SNPs between *B. dothidea, N. parvum, N. hellenicum* and *N. mediterraneum.* Next to each sequence, the name and genbank accession number is indicated. For downstream primers TL-MS-BotdoITS_386R and TL-MS-NeofusITS_386R, the SNP is indicated in bold letters, the mismatch with red letter in black background, while the 5′ tail added to each primer is shown with underlined letters.

er pairs in the SNP primer tool (ITS3 / TL-MS-BotdoITS_386R / TL-MS-NeofusITS_386R) have low delta-G primer-dimer values, making their combination in a single reaction theoretically possible, the small difference in the length of generated PCR products limits their use as a differentiating tool in a multiplex reaction.

As a first step, the quality of all genomic DNA samples was assessed using universal primers ITS4 and ITS5. The conditions for the final amplification were as follows: initial denaturation at 95°C for 3 minutes, followed by 35 cycles of 30 s at 95°C, 30 s at 55°C, 60 s at 72°C, and a final extension period of 10 min at 72°C. The 20 μL PCR mixture contained 1x KAPA PCR buffer with 1.5 mM $MgCl_2$ (Sigma-Aldrich), 200 μM of each dNTP, 500 μM of each primer, 1 U of KAPA Taq polymerase and 25 ng DNA as template.

For the primer pairs ITS5 (5'-GGAGTAAAAGTCGTAACAAGG-3')/BotdoITS_384R (5'-CAGAGCTTGAGGGTTGT-3') and ITS5 / NeofusITS_384R (5'-CAGAGCTTGAGGGTTGA -3') the conditions for the final amplification were as follows: initial denaturation at 95°C for 3 minutes, followed by 35 cycles of 30 s at 95°C, 30 s at 58°C, 40 s at 72°C, and a final extension period of 10 min at 72°C. The 20 μL PCR mixture contained 1x KAPA PCR buffer with 1.5 mM $MgCl_2$ (Sigma-Aldrich), 200 μM of each dNTP, 500 μM of each primer, 1 U of KAPA Taq polymerase and 25 ng DNA as template.

For the primer pairs ITS3 (5'- GCATCGATGAAGAACGCAGC-3')/TL-MS-BotdoITS_386R (5'-ATCAGAGCAGAGCTTGAGGGTTAT-3') and ITS3 / TL-MS-NeofusITS_386R (5'-ATGACTATTATTATTAGCAGAGCTTGAGGGTTCA - 3') the conditions for the final amplification were as follows: initial denaturation at 95°C for 3 minutes, followed by 35 cycles of 30 s at 95°C, 30 s at 64°C, 20 s at 72°C, and a final extension period of 10 min at 72°C. Lower annealing temperatures 61, 62, 63°C were also tested, but 64°C was selected due to higher stringency. The 20 μL PCR mixture contained 1x KAPA PCR buffer with 1.5 mM $MgCl_2$ Sigma-Aldrich, 200 μM of each dNTP, 500 μM of each primer, 1 U of KAPA Taq polymerase (Sigma-Aldrich) and 25 ng DNA as template.

For the SNP primers assays, DNA from the following species was used: *B. dothidea*, *N. parvum, N. mediterraneum*, *N. hellenicum,* while *Diplodia seriata* was included as an outgroup. DNA from *Colletotrichum acutatum* and *Verticillium dahliae* were utilized as negative controls.

All reactions were performed in a Veriti (Applied Biosystems) PCR machine cycler. After amplification 10 μL aliquots from each PCR product were separated by gel electrophoresis in 1% agarose for ITS5/ BotdoITS_384R and ITS5 / NeofusITS_384R PCR amplicons and in 2.5% agarose for ITS3 / TL-MS-BotdoITS_386R and ITS3/ TL-MS-NeofusITS_386R PCR amplicons in 1x TAE buffer (40 mM Tris base, 20 mM acetic acid, 1 mM EDTA, pH 8) at 5 V / cm for 100 min. Gels were stained with Ethidium Bromide (1 μg / ml) for 20 min and visualized on a gel documentation system (Lumibis, DNR bio imaging systems).

For the ITS5 / BotdoITS_384R and ITS5 / NeofusITS_384R primer pairs, which have only one 3' SNP base as discriminating factor, annealing temperatures 58-61°C were chosen. However, at 58°C there was cross-species amplification and above 58°C only primer dimers or lack of product. Also, the results from each PCR were not easily reproducible. PCR with primers *B. dothidea* ITS5/ BotdoITS_384R amplified the expected fragment in *Botryosphaeria. dothidea* but also in *Neofusicoccum* spp. and *Diplodia. seriata* (Fig. 2a). In addition, PCR with *Neofusicoccum* spp. primers ITS5/ NeofusITS_384R amplified the expected fragment in all species, including non – target fungi, *C. acutatum*, *V. dahliae* (Fig. 2b). In conclusion, the single base 3' SNP primers did not differentiate efficiently and repeatedly *B. dothidea* from *Neofusicoccum* species.

For the ITS3/ TL-MS-BotdoITS_386R and ITS3/ TL-MS-NeofusITS_386R primer pairs, annealing temperatures 61 - 64°C were chosen. In all temperatures that were tested, the primers successfully differentiated between *B. dothidea* and *Neofusicoccum* spp., with *B. dothidea* SNP primers amplifying only *B.*

dothidea DNA (Fig. 3a) and *Neofusicoccum* spp. SNP primers amplifying *Neofusicoccum* species only (Fig. 3b). An exception was DNA from *D. seriata,* which showed amplification using primers for *B. dothidea*. That can be explained by *B. dothidea* and *D. seriata* being homologous on the base sequences of the SNP primer. However, this confusion can easily be resolved, based on morphological differences of *D. seriata* conidia size, shape and color from *B. dothidea*. Therefore, the PCR differentiation will be needed only if *B. dothidea* is involved in the analysis. Based on the different length of the 5′ tail in SNP mismatch primers, the product for primer pair ITS3/ TL-MS-BotdoITS_386R was 150 bp while for primer pair ITS3/ TL-MS_NeofusITS 160 bp (Fig. 3).

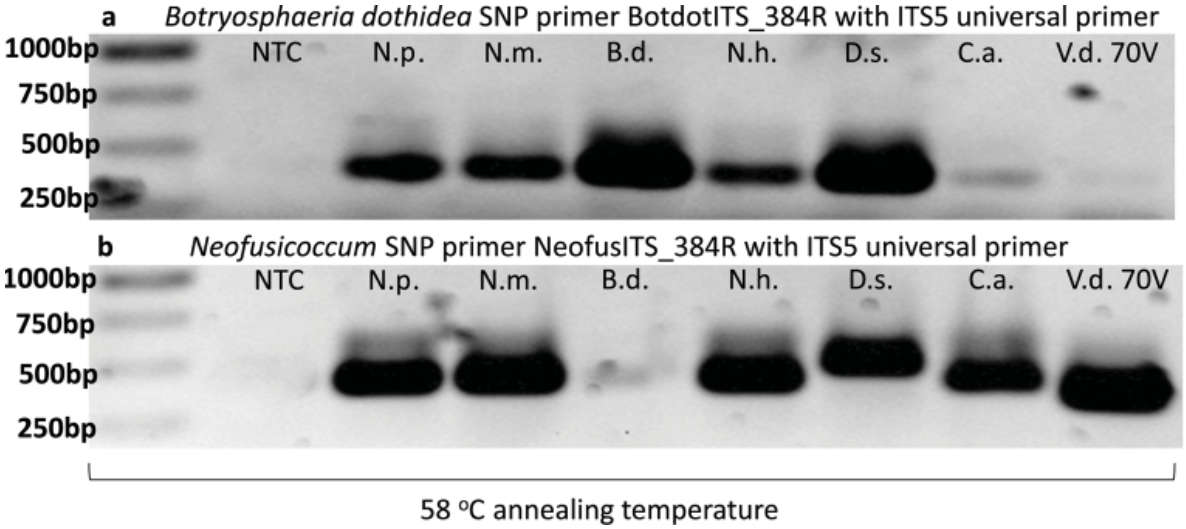


Figure 2. PCR with downstream primers bearing a single 3′ SNP paired with the ITS5 primer and annealing temperature 58°C did not discriminate between *Botryosphaeria dothidea* and *Neofusicoccum* spp. a) PCR with primers ITS5/ BotdoITS_384R amplified the expected fragment in *B. dothidea* (B.d), but also in *Neofusicoccum* spp. (N.p, N.m. N.h.) and D. seriata (D.s.). b) PCR with primers ITS5/ NeofusITS_384R amplified the expected fragment in all species, including non-target fungi. Abbreviations: N.p.=*Neofusicoccum parvum*, N.m.=*N. mediterraneum*, B.d.=*Botryosphaeria dothidea*, N.h.=*N. hellenicum*, D.s.=*Diplodia seriata*, C.a.=*Colletotrichum acutatum*, V.d. 70V=*Verticillium dahliae* strain 70V. Electrophoresis of PCR products was performed in 1% agarose gel.

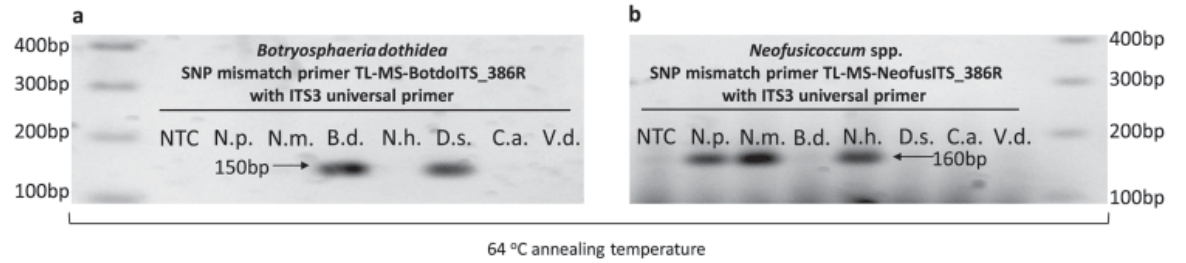


Figure 3. Successful discrimination between *B. dothidea* and *Neofusicoccum* spp. using downstream primers bearing a 3′ SNP plus mismatch and with optimization of the annealing temperature at 64°C. a) PCR with primer pair ITS3/ TL-MS-BotdoITS_386R showed amplification of the expected fragment only for *B. dothidea* (B.d) and *D. seriata* (D.s.), b) PCR with primer pair ITS3/ TL-MS_NeofusITS_386R amplified the expected fragment specifically in *Neofusicoccum* spp. (N.p., N.m., N.h.). Abbreviations: N.p.=*Neofusicoccum parvum*, N.m.=*N. mediterraneum*, B.d.=*Botryosphaeria dothidea*, N.h.=*N. hellenicum*, D.s.=*Diplodia seriata*, C.a.=*Colletotrichum acutatum*, V.d. =*Verticillium dahliae* strain 70V. Electrophoresis was performed on 2.5% agarose gel.

Based on the plethora of species in the Botryosphaeriaceae family contributing to the same disease complex, molecular tools that enable rapid and accurate detection and differentiation, are deemed critically important. In this study, a novel, robust diagnostic tool was developed, to detect and differentiate between species of *B. dothidea* and three species of the genus *Neofusicoccum* (*N. mediterraneum*, *N. hellenicum*, *N. parvum*). These pathogens were selected as the focal point of our research because they prevail among species isolated from pistachio and other hosts (olive, pomegranate, white willow etc.) in Greece (personal communication with Dr. Tsopelas; E.J. Paplomatas, data not shown.). Furthermore, *B. dothidea* by itself is deemed critically important, since it emerges as the most widespread pathogen of the Botryosphaeriaceae family, with a documented presence in more than 24 genera of host plants, many of them of economic significance, such as pistachio and olive in the Mediterranean basin (Batista *et al*., 2021; Marsberg *et al*., 2016). As such, the ability to rapidly and accurately delimit this species, while bypassing time-consuming traditional taxonomic tools, is of major importance.

As the initial results showed, primers designed with only a 3'SNP base as discriminating factor were not successful in classifying our isolates. On the other hand, SNPs with mismatch next to the 3' end primers were efficient at differentiating these important species. In accordance with previous studies, SNP primers with mismatch base have been found to be more efficient in differentiating species or alleles compared to 3' SNP base only. Their mode of action involves inhibiting or significantly delaying, amplification of the targeted sequence (Carvalho *et al*., 2021). In the present study, the incorporation of the mismatch in the SNP primer, improved specificity and enabled the targeting of the ITS region, which is known to display high sensitivity due to the high copy numbers in genome. The developed one step PCR based protocol for differentiation of Botryosphaeriaceae species is superior to other methods, as it requires no additional steps such as RLFP- PCR (Slippers *et al*., 2007) or polyacrylamide electrophoresis for SSCP analysis (Ridgway *et al*., 2011). Furthermore, its practical application will be of outmost importance, especially in the case of perennial plant crops, which constitute a long-term investment on the producers' side.

Based on the methods evaluated, PCR with SNP primers including a mismatch is a robust and fast track protocol to differentiate between important species in the Botryosphaeriaceae. Further research, into the usage of SNP polymorphisms in the ITS region, or/and other genetic loci, for the differentiation of species in the Botryosphaeriaceae family is required.

Declarations

Funding

This Research Project is co-financed by Greece and the European Union (European Social Fund) through the Operational Program «Human Resources Development, Education and Lifelong Learning 2014-2020» and the Program encoded EDBM103, titled "Support for researchers with an emphasis on young researchers-cycle B'", in the context of the project "Risk evaluation of development of a resistant form of disease via transfer of mating type genes from the Botryosphaeria dothidea species to species of the genus Neofusicoccum" (MIS 5048471).

Conflicts of interest/Competing interests
The authors declare no conflict of interest.

Availability of data and material
Sequence data that support part of the findings of this study are available at the GenBank database under the accession numbers: OK001842, OK035713, OK035714, OK036488, OK036571

Code availability
Not applicable

Literature Cited

Abdollahzadeh, J. and Zolfaghari S. 2014. Efficiency of rep-PCR fingerprinting as a useful technique for molecular typing of plant pathogenic fungal species: Botryosphaeriaceae species as a case study. *FEMS Microbiology Letters*, 361: 144–157.

Batista, E., Lopes, A. and Alves, A. 2021. What do we know about botryosphaeriaceae? An overview of a worldwide cured dataset. *Forests,* 12.

Carvalho, J., Yadav, S., Garrido-Maestu, A., Azinheiro, S., Trujillo, I., Barros-Velázquez, J. and Prado, M. 2021. Evaluation of simple sequence repeats (SSR) and single nucleotide polymorphism (SNP)-based methods in olive varieties from the Northwest of Spain and potential for miniaturization. *Food Chemistry: Molecular Sciences,* 3: 1–10.

Chen, S., Li, G., Liu, F. and Michailides, T.J. 2015. Novel species of Botryosphaeriaceae associated with shoot blight of pistachio. *Mycologia* 107: 780–792.

Chen, S.F., Morgan, D.P. and Michailides, T.J. 2014. Botryosphaeriaceae and Diaporthaceae associated with panicle and shoot blight of pistachio in California, USA. *Fungal Diversity,* 67: 157–179.

Flowers, J., Hartman, J. and Vaillancourt, L. 2003. Detection of Latent Sphaeropsis sapinea Infections in Austrian Pine Tissues Using Nested-Polymerase Chain Reaction. *Phytopathology,* 93: 1471–1477.

Gaudet, M., Fara, A.-G., Beritognolo, I. and Sabatti, M. 2009. Allele-Specific PCR in SNP Genotyping. In: *Single Nucleotide Polymorphisms: Methods and Protocols* (A.A. Komar, ed.), 415–424.

Kumar, S., Stecher, G., Li, M., Knyaz, C. and Tamura, K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Molecular Biology and Evolution,* 35: 1547–1549.

Lazzizera, C., Frisullo, S., Alves, A. and Phillips, A.J.L. 2008. Morphology, phylogeny and pathogenicity of Botryosphaeria and Neofusicoccum species associated with drupe rot of olives in southern Italy. *Plant Pathology,* 57: 948–956.

Lopes, A., Phillips, A.J. and Alves, A. 2017. Mating type genes in the genus Neofusicoccum: Mating strategies and usefulness in species delimitation. *Fungal Biology,* 121: 394–404.

Madeira, F., Park, Y. Mi, Lee, J., Buso N., Gur T., Madhusoodanan, N., Basutkar, P., Tivey, A.R.N., Potter., S.P., Finn., R.D. and Lopez, R. 2019. The EMBL-EBI search and sequence analysis tools APIs in 2019. *Nucleic Acids Research,* 47: W636–W641.

Marsberg , A., Kemler, M., Jami, F., Nagel, J.H., Postma-Smidt, A., Naidoo, S., Wingfield, M.J., Crous, P.W., Spatafora, J.W., Hesse, C.N., Robbertse, B. and Slippers B. 2017. *Botryosphaeria dothidea*: a latent pathogen of global importance to woody plant health. *Molecular Plant Pathology*, 18: 477–488.

National Center for Biotechnology Information (NCBI)[Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information, 1988. Available at: https://www.ncbi.nlm.nih.gov. Accessed May 11, 2021.

Owczarzy, R., Tataurov, A.V, Wu, Y., Manthey, J.A., McQuisten, K.A., Almabrazi, H.G., Pedersen, K.F., Lin, Y., Garretson, J., McEntaggart, N.O., Sailor, C.A., Dawson, R.B. and Peek, A.S. 2008. IDT SciTools: a suite for analysis and design of nucleic acid oligomers. *Nucleic Acids Research,* 36: W163-9.

Pavlic, D., Slippers, B., Coutinho, T.A. and Wingfield, M.J. 2009. Multiple gene genealogies and phenotypic data reveal cryptic species of the Botryosphaeriaceae: A case study on the Neofusicoccum parvum/N. ribis complex. *Molecular Phylogenetics and Evolution,* 51: 259–268.

Ridgway, H.J., Amponsah, N.T., Brown, D.S., Baskarathevan, J., Jones, E.E. and Jaspers, M.V. 2011. Detection of botryosphaeriaceous species in environmental samples using a multi-species primer pair. *Plant Pathology,* 60: 1118–1127.

Slippers, B., Smit, W.A., Crous, P.W., Coutinho, T.A., Wingfield, B.D. and Wingfield, M.J. 2007. Taxonomy, phylogeny and identification of Botryosphaeriaceae associated with pome and stone fruit trees in South Africa and other regions of the world. *Plant Pathology,* 56: 128–139.

Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B.C., Remm, M. and Rozen, S.G. 2012. Primer3--new capabilities and interfaces. *Nucleic Acids Research*, 40: 1–12.

Received: 1 November 2021; Accepted: 17 November 2021

ΣΥΝΤΟΜΗ ΑΝΑΚΟΙΝΩΣΗ

Διαφοροποίηση μεταξύ *Botryosphaeria dothidea* και ειδών του γένους *Neofusicoccum* με βάση πολυμορφισμό ενός νουκλεοτιδίου στην περιοχή ITS

Σ. Παλαβούζης, Α. Τριανταφυλλοπούλου, Α.Κ. Τζίμα και Ε.Ι. Παπλωματάς

Περίληψη Οι μύκητες που ανήκουν στην οικογένεια Botryosphaeriaceae είναι ευρέως διαδεδομένα παθογόνα πολλών αγγειόσπερμων, προκαλώντας ασθένειες σε διάφορες οικονομικά σημαντικές καλλιέργειες. Τα σημαντικότερα μέλη της οικογένειας για την Ελλάδα και άλλες μεσογειακές χώρες είναι τα *Botryosphaeria dothidea, Neofusicoccum hellenicum, Neofusicoccum mediterraneum* και *Neofusicoccum parvum*. Η μεγάλη συχνότητα ταυτόχρονης απομόνωσης διαφόρων ειδών της οικογένειας Botryosphaeriaceae από τον ίδιο ξενιστή, καθώς και το εκτεταμένο εύρος ξενιστών του *B. dothidea*, καθιστούν αναγκαία την ανάπτυξη ταχέων και αξιόπιστων μεθόδων ανίχνευσης. Η παρούσα μελέτη παρουσιάζει ένα νέο και ισχυρό μοριακό διαγνωστικό εργαλείο που βασίζεται στη μέθοδο της PCR με τη χρήση εκκινητών σχεδιασμένων σε σημεία με πολυμορφισμό απλού νουκλεοτιδίου (SNP) στην περιοχή ITS (εσωτερική μεταγραφόμενη περιοχή) των ειδών *B. dothidea* και *Neofusicoccum* spp. Οι εκκινητές SNP που κατασκευάστηκαν με ή χωρίς πρόσθετα νουκλεοτίδια χωρίς ομολογία συνδυάστηκαν με τον ίδιο ανοδικό γενικό εκκινητή για να ενισχύσουν διακριτά τμήματα. Όταν αξιολογήθηκαν σε αναλύσεις PCR, οι εκκινητές με πρόσθετα νουκλεοτίδια χωρίς ομολογία βρέθηκαν να έχουν την υψηλότερη ικανότητα διαφοροποίησης. Αξιολογείται περαιτέρω η δυνατότητα ανάπτυξης δοκιμών SNP προκειμένου να γίνει διαφοροποίηση μεταξύ των ειδών Neofusicoccum.

Hellenic Plant Protection Journal **15:** 49-56, 2022

sciendo

Hellenic Plant Protection Journal **15:** 57-71, 2022
DOI 10.2478/hppj-2022-0007

Efficacy of fungicide alternatives against late wilt disease of maize and their influence on plant morphogenesis and yield characters

M.M. Abdel-Kader*, M.S.A. Khalil and N.S. El-Mougy

Summary Efficiency of various organic acids, organic salts, essential oils, algae (an extract of *Chlorella vulgaris* and a commercial product), and bioagents against *Magnaporthiopsis maydis*, causing maize late wilt disease, was evaluated in laboratory and field conditions. For the *in vitro* tests, isolated *M. maydis* field strains from Egypt were used. Additionally, in field experiments different application methods were tested for their efficacy throughout two successive growing seasons. Results showed maximum growth inhibition of *M. maydis* at different concentrations of salicylic acid, ascorbic acid, benzoic acid and humic acid as well as sodium benzoate, potassium sorbate, di-potassim phosphate and calcium choloride, in descending order. The essential oils of carnation, lemongrass and black seed followed a similar trend. The minimum pathogenic fungal growth was achieved when the pathogen was exposed to the antagonistic *Trichoderma viride* followed by *Trichoderma harzianum, Bacillus subtilis* and *Pseudomonas fluorescens* treatments. Under field conditions, the highest disease reduction was recorded after treatment with salicylic acid (all application methods), humic acid (all application methods), sodium benzoate (seed dressing), carnation oil (seed dressing or seed dressing +foliar spray) and the bioagents *B. subtilis* and *P. fluorescens* (soil drench). Overall, all treatments and all application methods led to significant lower disease incidence compared to the untreated control in both growing seasons. Additionally, all treatments achieved an enhancement of plant morphogenesis and yield characters. The most effective combinations of compounds/bioagents and application methods can be considered for future use in IPM management of late wilt disease of maize.

Additional keywords: application method, biological pesticides, essential oils, organic acids, organic salts, salicylic acid

Introduction

Maize is the most-produced cereal worldwide (Ranum *et al*., 2014). In Africa alone, more than 300 million people depend on maize as their main food crop (Ranum *et al*., 2014). In addition, maize is also very important as feed for farm animals. Currently, maize is grown in more than 170 countries on approximately 180 million hectares of land. Yellow maize represents 90% of the world's production, while in Africa, 90% of the total maize production is white maize (Ranum *et al*., 2014). In Egypt, maize is the second most important cereal (750,000 feddan = 315,000 ha/5.9 million tons annually) after wheat crop while another 4.5 million tons of corn was imported in 2016 (Anonymous, 2017).

Late wilt disease in maize is caused by *Magnaporthiopsis maydis*. It is one of the main economically important diseases of maize in Egypt (Samra *et al*., 1963; Johal *et al*., 2004). It is considered endemic throughout Egypt (Sabet *et al*., 1970), while about 100% infection occurs in some fields and the yield losses approach 40-70% in non-resistant cultivars (Johal *et al*., 2004).

Disease symptoms include a rapid wilting of the lower leaves after flowering, development of hollow and shrunken stalks turning to dark yellow-to-brown stained pith (El-Shafey and Claflin, 1999). *Magnaporthiopsis maydis* is a soil-borne fungus that enters the tissue of the root and colonizes

Plant pathology Department, National research Centre, Giza 12622, Egypt
* Corresponding author: mokh_nrc@yahoo.com

the xylem (Sabet *et al*., 1970). Less commonly, this pathogen can be seed-borne (E1-Shafey *et al*., 1976) and may irregularly cause pre-emergence damping-off under heavy soil infestation (Sabet *et al*., 1970).

In India, Singh and Siradhana (1987) stated that the highest disease incidence occurs when rainfall is above average or at frequent irrigation. Nevertheless, it has been shown that in Egypt, the frequent irrigation (irrigation intervals of 9-10 days) decreased infection and excessive soil moisture reduced the wilt disease severity (Satyanarayana and Begum, 1996). Also, early sowing (El-Shafey *et al*., 1988) and frequent watering or saturated soils causing moisture stress (Samra *et al*., 1966) are considered the physical control measures of maize late wilt.

Application of systemic fungicides for the control of *M. maydis* should be repeated several times during the growing season because of their limited persistence in corn roots, only for 90 days (Singh and Siradhana, 1989). Moreover, it has been recorded that grain treatments with captan, carbendazim, carboxin and thiram lead to significant decrease in late wilt disease as well as yield increase approximately by 11-91% (Begum *et al*., 1989; Satyanarayana and Begum, 1996). Seed treatments with azoxystrobin have failed against maize late wilt due to several factors, i.e., *M. maydis* isolates, their virulence or chemical sensitivity and environmental conditions (Degani *et al*., 2019).

Nowadays a great attention has been focused on agents of natural origin as a positive alternative to chemical pesticides, which additionally are safe for the humans, animals and the environment (Whipps, 2001). Within this context, this study tested the fungicidal activity of various organic acids, organic salts, essential oils and bioagents against *M. maydis in vitro*. In a following step, we examined further their efficacy *in situ* in two cultivation seasons of maize using different application methods. Additionally, the enhancement of plant growth and yield characters of maize were also evaluated.

Materials and methods

Chemicals and reagents used

The organic acids salicylic acid, humic acid, ascorbic acid, and benzoic acid and the organic salts sodium benzoate, potassium sorbate, di-potassium phosphate and calcium chloride were purchased from Al-Gamhoria Company Ltd. for Chemicals and Medicinal Instruments (Cairo, Egypt).The essential oils of carnation (*Dianthus caryophyllus* L.), lemongrass (*Cymbopogon schoenanthus* L.) and black seed (*Nigella sativa* L.) were obtained from CID Company, Giza-Egypt. The essential oils were stored in dark bottles at 4°C until use.

In field trials, the extract of alga *Chlorella vulgaris* was kindly obtained from Algal Biotechnology Unit, National Research Centre, Giza, Egypt. The plant growth promoter/fertilizer Biactive is a commercial alga (*Ascophyllum nodosum*) biostimulant enriched with cytokinins, auxins, gibberellins, betains, manitol, alginic acid, oligosaccharids, amino acids and natural microelements (http://adleragro.com/en/productos/familia-bioestimulantes/biactive/).

Biological material

The antagonistic fungal isolates *Trichoderma harzianum*, *T. viride* as well as the antagonistic bacterial isolates *Bacillus subtilis* and *Pseudomonas fluorescens* were kindly obtained from the Culture Collection Unit, Plant Pathology Department, National Research Centre (NRC), Egypt. Regarding *T. harzianum,* a spore suspension (sporesXwater) was made from a seven days old culture and the final concentration was adjusted to10^8 conidia per 1 mL water using a hemocytometer (Brite-line Sigma-Aldresh). Concerning the *in situ* trials, the maize grains seeds *cv.* M84 were obtained from the Field Crop Research Department, Agricultural Research Centre, Giza, Egypt.

Isolation and identification of *M. maydis*

Maize samples showing late wilt disease symptoms were collected from different fields and transferred to the laboratory.

The root samples were thoroughly washed with tap water and dried between two sterilized filter papers, then cut into small pieces (0.5cm) and their surface was sterilized by dipping them in 1% sodium hypochlorite solution for 2 min. Afterwards, samples were washed three times using sterilized distilled water and placed onto filter paper to air dry. Five pieces of the cleaned and dried samples were plated into Petri dishes (9 cm) containing PDA medium supplemented with streptomycin sulphate at 100 ppm to avoid bacterial contamination. The plates were incubated at 25±2°C for 7 days under light/dark conditions. In the final step the purification of the isolated fungi was performed by using the hyphal tip method. The purified isolates were subjected to microscopic examination and the isolated fungi were identified according to Gilman (1957) and Barnet and Hunter (1972).

In vitro evaluation of the effect of the tested materials against *M. maydis*

The evaluation *in vitro* of the effect of the organic acids, organic salts, antagonistic fungi and bacteria against maize late wilt was based on the reduction of the linear growth of *M. maydis*. The tested concentrations were 0.125, 0.25, 0.5, 1.0, 2.0, 4.0, 6.0 and 8% (w:v or v:v) for all materials. The essential oils were diluted in sterilized distilled water in order to acquire emulsified stocks of high concentrations. Moreover a few drops of the emulsifier Tween 20 (Sigma Co.) were added to the essential oil volumes to obtain an emulsion feature. Different weights of each organic acid or organic salt as well as different volumes of each feature of essential oil emulsion were added to conical flasks containing 100 mL sterilized PDA medium before its solidification, to obtain the wanted concentrations. Approximately 20 ml of each supplemented medium were poured into Petri-dishes (9 cm). The control treatment consisted of PDA medium free of the tested materials. A mycelial disk (5 mm-diameter) of *M. maydis* from a seven days old culture was placed on the center of the Petri dishes.

The efficacy of the antagonistic bioagents, was evaluated *in vitro* using the dual culture technique (Ferreira *et al*., 1991). Treatments of the bioagents, *T. harzianum*, *T. viride* (a mycelial disk of 5mm diameter), *B. subtilis*, *P. fluorescens* (growth streaking) were placed onto a PDA plate 10 mm at the edge of the Petri dish. A 5-mm mycelial disk of the pathogen *M. maydis* was placed on the opposite side of the dish at the same distance. The control treatment was inoculated with a well of the bioagents treatment or a mycelial disk of the pathogen alone.

In total ten replicates were conducted for each treatment. They were incubated at 25±2°C until the fungus reached full growth in the control treatment (10 days); then the linear growth was measured (mm).

In situ efficacy evaluation of the tested materials against *M. maydis*

Two field experiments were conducted during the successive summer growing seasons 2017 and 2018 at a field located at Kafr-Eldawar, Alexandria governorate. This field was known for the high natural infection of *M. maydis*. The tested materials which showed a high toxicity effect against *M. maydis in vitro* were examined further under field conditions. Additionally, an extract of alga *C. vulgaris* as well as the commercial biostimulat (Biactive) were also tested in these experiments. The treatments applied in the two successive seasons were the following:

A. Untreated control
B. Soil drench
1. *T. harzianum*
2. *T. viride*
3. *B. subtilis*
4. *P. fluorescens*

C. Seed dressing (1.0g or 1ml/100g seeds)
5. Salicylic acid
6. Humic acid
7. Potassium sorbate
8. Carnation oil
9. Lemongrass oil
10. *Chlorella vulgaris* extract
11. Biactive (commercial alga stimulant)

D. Foliar spray (1g or 1ml /1L)
12. Salicylic acid
13. Humic acid
14. Potassium sorbate
15. Carnation oil
16. Lemongrass oil

E. Seed dressing + foliar spray (1.0g or 1ml/100g seeds +1g or 1ml /1L)
17. Salicylic acid + Salicylic acid
18. Humic acid + Humic acid
19. Potassium sorbate + Potassium sorbate
20. Carnation oil + Carnation oil
21. Lemongrass oil + Lemongrass oil

The field experiment consisted of 105 plots, [3.5m x 3m (10.5 m^2) each]. Each individual plot comprised of 6 rows. Five plots were used as replicates for each test material and five plots were used as the untreated control. Maize grains M84 *cv.* were sown in all plots at the rate of 3grains/hole, 20 holes/row. The randomized complete block design was used. All plots received the traditional agricultural practices, irrigation, fertilization, etc.

Soil drench was applied at the rate of 250ml (10^8 cfu/mL) per one m^2. Bioagents were incorporated individually in the top 20 cm of the soil surface at planting row sites considering relevant treatments (Abdel-Kader, 1997). Meanwhile, for seed dressing, the inoculation of the bioagents was used at the rate of 10^8 cfu/mL.

Specific quantity of the used organic acids, organic salts and essential oils was added to water (20L) to obtain the intended concentration applied for each treatment for foliar spray. All foliar spray treatments were applied three times with 15-day intervals starting at the beginning of the flowering stage.

Efficacy assessment of the different treatments and application methods was based on the disease incidence. The disease incidence was determined using the formula of (DI %) = (No of infected plants/ total No. of examined plants in each plot) x 100. Reduction in disease incidence was calculated as percentage of disease incidence in certain treatment in relation to the percentage of disease incidence in the control treatment. Monitoring and scouting for disease incidence in all cultivated plots were preformed 15 days after the third spray treatment. The field experiments were carried out at the same field for the two successive growing seasons (2017 and 2018). Plant growth and yield characters of maize were also evaluated.

Statistical analysis

The obtained data were subjected to IBM SPSS software version 14.0. Analysis of variance was determined, and the mean values were compared by Duncan's multiple range test at $P < 0.05$.

Results

The efficacy of various organic acids, organic salts, essential oils and bioagents against *M. maydis* in maize was evaluated *in vitro* and *in situ* at the summer growing seasons of 2017 and 2018.

In vitro effect of the tested materials against *M. maydis*

The inhibitory effect of the organic acids, salicylic acid, humic acid, ascorbic acid and benzoic acid, the organic salts sodium benzoate, potassium sorbate, di-potassium phosphate, calcium chloride, the essential oils carnation, lemongrass and black seeds and bioagents *T. hazianum*, *T. viride*, *B. subtilis* and *P. fluorescens* against the linear growth of *M. maydis* is presented in Table 1.

The linear fungal mycelium growth decreased significantly corresponding to the increase in concentrations of all tested materials. The minimum effective concentration varied among the different tested materials. Organic acids achieved the highest inhibition against the fungal growth followed by the essential oils and the organic salts. Salicylic acid of the organic acids decreased the linear fungal growth from 90 mm to 6.0 mm at the concentration 0.125% (w/v) and led to the maximum growth inhibition (100%) at 0.25% (w/v). Ascorbic acid

Table 1. Linear growth (mm) of *Magnaporthiopsis maydis*, causing late wilt disease of maize, after the *in vitro* application of various organic acids, organic salts, essential oils and bioagents.

Treatment		Linear (diameter) fungal growth (mm)							
		Concentrations (%)							
		0.125	0.25	0.5	1.0	2.0	4.0	6.0	8.0
		Mean ± s.d.	Mean ± s.d.	Mean ± s.d.	Mean ± s.d.	Mean ± s.d.	Mean ± s.d.	Mean ± s.d.	Mean ± s.d.
Organic acids	Salicylic acid	6.0 ± 0.1h	0.0 ± 0.0i	0.0 ± 0.0i	0.0 ± 0.0i	0.0 ± 0.0i	0.0 ± 0.0i	0.0 ± 0.0i	0.0 ± 0.0i
	Humic acid	67.7 ± 1.4c	25.0 ± 0.8e	0.0 ± 0.0i	0.0 ± 0.0i	0.0 ± 0.0i	0.0 ± 0.0i	0.0 ± 0.0i	0.0 ± 0.0i
	Ascorbic acid	26.7 ± 0.7e	0.0 ± 0.0i	0.0 ± 0.0i	0.0 ± 0.0i	0.0 ± 0.0i	0.0 ± 0.0i	0.0 ± 0.0i	0.0 ± 0.0i
	Benzoic acid	37.3 ± 0.6d	0.0 ± 0.0i	0.0 ± 0.0i	0.0 ± 0.0i	0.0 ± 0.0i	0.0 ± 0.0i	0.0 ± 0.0i	0.0 ± 0.0i
Organic salts	Sodium benzoate	66.7 ± 0.9c	26.7 ± 0.7e	21.3 ± 0.7e	0.0 ± 0.0i	0.0 ± 0.0i	0.0 ± 0.0i	0.0 ± 0.0i	0.0 ± 0.0i
	Potassium sorbate	45.0 ± 1.0d	21.3 ± 0.9e	36.0 ± 1.1d	0.0 ± 0.0i	0.0 ± 0.0i	0.0 ± 0.0i	0.0 ± 0.0i	0.0 ± 0.0i
	Di-potassim phosphate	90.0 ± 0.0a	90.0 ± 0.0a	76.0 ± 5.1b	73.0 ± 5.5b	29.7 ± 1.2e	25.0 ± 2.3e	0.0 ± 0.0i	0.0 ± 0.0i
	Calcium choloride	90.0 ± 0.0a	90.0 ± 0.0a	71.3 ± 3.7b	66.7 ± 1.3c	45.0 ± 1.0d	30.7 ± 3.8e	10.7 ± 0.5g	0.0 ± 0.0i
Essential oil	Carnation	21.3 ± 1.7e	13.3 ± 0.9f	5.3 ± 0.6h	0.0 ± 0.0i	0.0 ± 0.0i	0.0 ± 0.0i	0.0 ± 0.0i	0.0 ± 0.0i
	Lemongrass	37.3 ± 0.8d	21.3 ± 1.7e	13.3 ± 0.6f	0.0 ± 0.0i	0.0 ± 0.0i	0.0 ± 0.0i	0.0 ± 0.0i	0.0 ± 0.0i
	Black seed	90.0 ± 0.0a	90.0 ± 0.0a	90.0 ± 0.0a	76.0 ± 1.4b	63.3 ± 2.3c	38.0 ± 1.3d	0.0 ± 0.0i	0.0 ± 0.0i
Antagonists									
Bioagent	*T. viride*	7.6 ± 1.0h							
	T. harzianum	14.6 ± 1.2f							
	B. subtilis	19.0 ± 1.3e							
	P. fluorescens	41.0 ± 0.6d							
Control		90.0 ± 0.0a							

Means ± standard deviations within a column followed by the same letter are not significantly different (Duncan multiple range test at $P < 0.05$).

and benzoic acids followed the same trend causing complete fungal growth inhibition also at the concentration of 0.25% (w/v). Humic acid showed a lower effect, achieving complete fungal growth inhibition at the concentration of 0.5% (v/v). The results on essential oils revealed that the fungal growth was completely inhibited (100%) at the concentration of 1% of carnation and lemongrass essential oils, while the same effect was recorded for black seeds oil at concentration of 6.0%. As regards the organic salts, sodium benzoate and potassium sorbate could inhibit the fungal growth completely (100%) at 1.0%. The lowest inhibitory effect was observed with di-potassium phosphate and calcium chloride for which the maximum fungal growth inhibition was recorded at 4.0% and 6.0%, respectively (Table 1).

Concerning the effect of antagonistic agents, the fungi showed higher inhibition than the bacteria. *Trichoderma viride* decreased the pathogen fungal growth from 90 mm to 7.67 mm followed by a decrease to 14.67 mm at the *T. harzianum* treatment. Likewise, the pathogen fungal growth decreased to 19.0 mm and 41.0 mm when it was exposed to the bacterial bioagents *B. subtilis* and *P. fluorescens*, respectively (Table 1).

Efficacy of the tested materials against *M. maydis in situ*

Efficacy of the tested materials in the field trials (most effective ones *in vitro*), based on percentages of disease incidence, is presented in Table 2 and Figure 1 for the two growing seasons. All treatments/application method combinations showed higher control of disease incidence compared to the untreated control in both growing seasons. As the efficacy profile of the treatments was similar in both seasons (comparison between treatments within each growing season), the data of the two seasons for all recorded parameters of plant morphogenesis and yield were pooled and analyzed together.

Soil drench with the antagonistic bacteria, *B. subtilis* and *P. fluorescens* achieved 71.1% and 67.7% lower disease incidence than the untreated control, respectively. The corresponding disease incidence was 18.3% at the *B. subtilis* treatment and 20.5% at the *P. fluorescens* treatment. The antagonistic fungi *T. viride* and *T. harzianum* reduced the disease incidence by 52.2% and 48.0%, respectively, compared to the control treatment (Fig. 1).

Regarding the seed dressing treatments, salicylic acid and sodium benzoate showed higher efficacy against late wilt incidence followed by humic acid, carnation oil and potassium sorbate. The recorded disease incidence at these treatments was 13.5%, 15.0%, 16.6%, 21.7% and 24.6%, corresponding to efficacy of 78.7%, 76.3%, 73.7%, 65.8% and 61.2%, in respective order. Lower control of disease incidence was observed with Biactive alga extract and lemongrass oil (disease incidence 30.0%, 33.3% and 35.5% and efficacy 51.6%, 47.5%, respectively) although they differed significantly to the untreated control.

For the combinations of [seed dressing + foliar spray with the same treatment], the highest efficacy (> 60%) compared to untreated control was recorded at the treatments of salicylic acid, lemongrass oil and humic acid, followed by the treatments of sodium benzoate, potassium sorbate and carnation oil (> 55%) (Table 2, Fig. 1).

A similar trend was also observed for the foliar spray application of the tested materials. Humic acid followed by salicylic acid spray treatments resulted in 16.5% and 18.3% disease incidence leading to 74.0% and 71.1% lower disease incidence than untreated control. Moderate efficacy was achieved by the treatments of lemongrass oil and sodium benzoate 49.0% and 44.0%, respectively (Fig. 1). Meanwhile, the lowest efficacy for foliar spray was recorded for carnation oil and potassium sorbate treatments; they controlled the disease incidence by 38.5%, 35.9%, respectively (Fig. 1).

Efficacy of the tested materials on plant morphogenesis and yield characters of maize

All treatments exhibited a significant

Table 2. Late wilt disease incidence of *Magnaporthiopsis maydis* in maize after application of various organic acids, organic salts, essential oils, bioagents and algae in field conditions in two growing seasons (2017, 2018).

Treatment		Late wilt disease incidence (%)		
		Season 2017 Mean ± s.d.	Season 2018 Mean ± s.d.	Average Mean ± s.d.
Soil drench	*T. viride*	29.2 ± 1.4d	31.4 ± 1.2c	30.3 ± 1.9cd
	T. harzianum	32.6 ± 1.3bc	33.4 ± 1.4c	33.0 ± 2.0c
	B. subtilis	17.3 ± 0.8f	19.3 ± 0.7e	18.3 ± 1.9fg
	P. fluoroscens	19.8 ± 1.2de	21.2 ± 1.3de	20.5 ± 1.3f
Seed dressing (1g or ±l/100g seeds)	Salicylic acid	14.8 ± 1.1g	12.2 ± 1.4g	13.5 ± 1.7h
	Humic acid	17.4 ± 1.3f	15.8 ± 1.6fg	16.6 ± 1.5g
	Sodium benzoate	14.3 ± 1.7g	15.7 ± 1.5f	15.0 ± 1.7g
	Potassium sorbate	23.3 ± 1.4de	25.9 ± 1.2d	24.6 ± 2.7e
	Carnation oil	20.8 ± 1.3de	22.6 ± 1.4de	21.7 ± 1.2f
	Lemongrass oil	34.6 ± 1.5bc	36.4 ± 1.6bc	35.5 ± 1.4c
	Algae extract	32.1 ± 1.1bc	34.5 ± 1.2d	33.3 ± 1.2c
	Biactive stimulant	29.8 ± 1.6e	31.6 ± 1.5cd	30.7 ± 1.3cd
Seed dressing + foliar spray (1g or l/100g seeds +1g or 1ml /1L)	Salicylic acid + Salicylic acid	11.2 ± 0.7g	9.8 ± 0.6g	10.50 ± 1.0h
	Humic acid + Humic acid	20.3 ± 0.6de	22.9 ± 0.8de	21.6 ± 1.0f
	Sodium benzoate + Sodium benzoate	26.4 ± 0.4d	24.6 ± 0.4de	25.5 ± 2.3de
	Potassium sorbate + Potassium sorbate	26.4 ± 0.8d	27.6 ± 0.7d	27.0 ± 0.1d
	Carnation oil + Carnation oil	19.2 ± 1.2de	22.0 ± 1.1de	20.6 ± 0.5f
	Lemongrass oil + Lemongrass oil	28.9 ± 1.3d	27.9 ± 1.2d	28.4 ± 2.0d
Foliar spray (1g or 1ml L)	Salicylic acid	17.8 ±0.7f	18.8 ± 0.9ef	18.3 ± 0.9fg
	Humic acid	15.7 ± 0.9fg	17.3 ± 0.6ef	16.5 ± 0.8g
	Sodium benzoate	36.6 ± 1.2bc	34.4 ± 1.1c	35.5 ± 2.2c
	Potassium sorbate	41.3 ± 1.2b	39.9 ± 1.3b	40.6 ± 1.5b
	Carnation oil	38.7 ± 1.3bc	39.3 ± 1.4b	39.0 ± 1.4bc
	Lemongrass oil	31.8 ± 1.4c	32.8 ± 1.6c	32.3 ± 0.7c
Control		62.8 ± 0.8a	64.2 ± 0.7a	63.5 ± 0.6a

Disease incidence (%) for each season was calculated as follows: Disease incidence = No. of infected plants/No. of total plants X 100.
Means ± standard deviations within a column followed by the same letter are not significantly different (Duncan multiple range test at $P < 0.05$).

positive effect on plant morphogenesis and yield characters compared to the untreated control (Table 3). At the control treatment, the recorded figures of plant height, No. of leaves/plant, No. of cobs/plant, No. of rows/cob and weight of 100 grain were 242.0, 12.3, 1.0, 12.0 and 31.6g, in respective order. The most effective application method proved to be the [seed dressing + foliar spray] followed by seed dressing, foliar spray, and oil drench with bioagents applications, in descending order.

The highest plant height (297.3 cm) was recorded at the [seed dressing + foliar spray] application method of [salicylic acid + salicylic acid] followed by treatments of [Humic acid + Humic acid], [Potassium sorbate + Potassium sorbate], [Sodium benzoate + Sodium benzoate], [Lemongrass oil + Lemongrass oil] and [Carnation oil + Carnation oil], leading to plant height of 293.0 cm, 286.3 cm, 280.6 cm, 280.0 cm and 273.0 cm, respectively compared with 242.0 cm in control treatment. The number of leaves

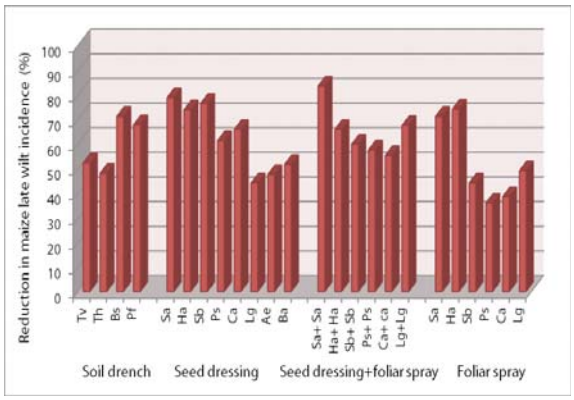


Figure 1. Reduction (%) in maize late wilt incidence in response to in situ soil drench, seed dressing seed dressing + foliar spray or foliar applications of several fungicide alternatives (average for growing seasons 2017, 2018). Reduction in disease incidence was calculated as percentage of disease incidence in certain treatment in relation to the percentage of disease incidence in the control treatment.
Tv= *Trichoderma viride*, Th= *Trichoderma harzianum*, Bs= *Bacilus subtilis*, Pf= *Pseudomonas fluorescens*, Sa= Salicylic acid, Ha= Humic acid, Sb= Sodium benzoate, Ps= Potassium sorbate, Ca-Carnation oil, Lg= Lemongrass oil, Ae= Algae extract and Ba= Bioactive stimulant.

per plant followed a similar trend as stated above. At the [seed dressing + foliar spray] application method the No. of leaves ranged between 16.0 - 14.3 leaves/plant whereas at the seed dressing application method it ranged between 15.3 - 12.6 leaves/plant; at foliar spray treatments this number was 13.6 – 15.0 leaves/plant and at untreated control 12.3 leaves/plant.

Furthermore, results reveal that the highest yield characters were recorded at [seed dressing + foliar spray] application method followed by seed dressing, foliar spray and soil drench with bioagents (Table 3, Fig. 2).

The highest measured yield characters were recorded at the [Salicylic acid + Salicylic acid] treatment; the recorded No. of cobs/plant, No. of rows/cob and weight of 100 grain were 2.0, 13.3 and 39.1g, respectively. Moderate results were acquired at the [Humic acid + Humic acid], [Sodium benzoate + Sodium benzoate], [Potassium sorbate + Potassium sorbate], [Lemongrass oil + Lemongrass oil] and [Carnation oil + Carnation oil] treatments. The No. of cobs/plant, No. of rows/cob and weight of 100 grain were 2.0, 15.6, 36.5g; 2.0, 15.6, 36.2g; 2.0, 14.0, 35.8g; 2.0, 13.3, 35.7g and 1.6, 15.3, 35.6g, for each treatment/yield character, respectively (Table 3).

A similar trend was also observed at the seed dressing treatments. The recorded No. of cobs/plant ranged between 1.0-2.0, the No. of rows/cob between 11.3-15.0 and the weight of 100 grain between 31.9g-35.5g. At foliar spray treatments the recorded ranges were 1.3-1.6 for the No. of cobs/plant, 12.6-14.6 for the No. of rows/cob, and 33.1-34.7g for the weight of 100 grain, respectively (Table 3).

Regarding the soil drench with bioagents, the highest plant morphogenesis and yield characters were recorded at *T. viride* treatment followed by *B. subtilis*, *T. harzianum* and *P. fluorescens*. The plant height ranged from 231.3 to 261.6 cm and the No. of leaves/plant from 14.0 to 14.3. The recorded ranges of No. of cobs/plant, No. of rows/cob and weight of 100 grain were 1.0-2.0, 14.0-15.6, and 35.4-36.7g, respectively (Table 3).

Discussion

Control of the late wilt of maize disease is very important due to its widespread in maize cultivation in Egypt but also in new reclaimed soil. In the present study, several alternative fungicides were studied in this respect: salicylic acid, humic acid (organic acids), sodium benzoate, potassium sorbate (organic salts), carnation, lemongrass (essential oils), algae products, and biocontrol agents, *T. harzianum*, *T. viride*, *B. subtilis* and *P. fluorescens*.

The obtained results revealed that salicylic acid, ascorbic acid and benzoic acid followed by humic acid achieved maximum growth inhibition (100%) of *M. maydis* at concentrations of 0.25% and 0.5% (humic acid). Meanwhile, the same effect was recorded at 0.5% of sodium benzoate, potassium sorbate and at 4.0, 6.0% of di-potassim phosphate, calcium choloride, respectively. Ismail *et al*. (1988) reported that germ tube

Table 3. Effect of organic acids, organic salts, essential oils, bioagents and algae on plant morphogenesis and yield characters of maize grown in field conditions (average for growing seasons 2017 and 2018).

Treatment	Plant morphogenesis and yield characters				
	Plant Height (cm)	No. of leaves/ plant	No. of cobs/ plant	No. of rows/ cob	Weight of 100 Grains/g
	Mean ± s.d.	Mean ± s.d.	Mean ± s.d.	Mean ± s.d.	Mean ± s.d.
Soil drench					
T. viride	231.3 ± 40.0a (b)	14.0 ± 1.0b	2.0 ± 0.0d	15.6 ± 2.0d	36.1 ± 1.0f
T. harzianum	242.3 ± 24.9b	14.0 ± 1.0b	1.6 ± 0.5bc	14.3 ± 2.0bc	35.4 ± 0.7e
B. subtilis	261.6 ± 10.4d	14.3 ± 1.5b	1.6 ± 0.5bc	14.0 ± 0.0bc	36.7 ± 0.4f
P. fluoroscens	258.3 ± 25.5c	14.0 ± 0.0b	1.0 ± 0.0a	15.3 ± 1.5d	35.6 ± 0.1e
Seed dressing (1.0g or 1ml/100g seeds)					
Salicylic acid	285.6 ± 11.0d	15.3 ± 0.5c	2.0 ± 0.5d	12.6 ± 2.3a	35.2 ± 1.5e
Humic acid	276.6 ± 23.0e	14.6 ± 1.1b	1.6 ± 0.0bc	13.3 ± 1.1b	35.5 ± 1.6e
Sodium benzoate	277.3 ± 11.2e	14.0 ± 0.0b	1.3 ± 0.5b	15.0 ± 1.0d	35.1 ± 1.3e
Potassium sorbate	277.6 ± 20.4e	15.0 ± 1.0c	1.3 ± 0.5b	12.6 ± 2.3a	34.7 ± 0.9d
Carnation oil	275.0 ± 13.2e	15.3 ± 0.5c	1.3 ± 0.5b	11.6 ± 1.5a	32.6 ± 1.4bc
Lemongrass oil	271.0 ± 27.6e	15.0 ± 2.0c	1.3 ± 0.5b	13.3 ± 1.5b	31.9 ± 3.0a
Algae extract	282.0 ± 17.3f	14.0 ± 0.0b	1.0 ± 0.0a	13.3 ± 1.1b	31.9 ± 0.7a
Commercial algae biofertilizer	253.0 ± 14.4c	12.6 ± 0.5a	1.3 ± 0.0b	11.3 ± 2.3a	32.4 ± 1.2bc
Seed dressing + foliar spray (1.0g or 1ml/100g 15.3seeds 1.0+1g or 1ml /1L)					
Salicylic acid + Salicylic acid	297.3 ± 4.0g	16.0 ± 1.0d	2.0 ± 0.0d	13.3 ± 1.1b	39.1 ± 1.1g
Humic acid + Humic acid	293.0 ± 5.2g	15.3 ± 0.5c	2.0 ± 0.0d	15.6 ± 0.5d	36.5 ± 2.1f
Sodium benzoate + Sodium benzoate	280.6 ± 5.5f	14.6 ± 2.0b	2.0 ± 0.0d	15.6 ± 1.5d	36.2 ± 1.8f
Potassium sorbate + Potassium sorbate	286.3 ± 9.2f	15.0 ± 0.0c	2.0 ± 0.0d	14.0 ± 0.0bc	35.8 ± 3.8e
Carnation oil + Carnation oil	273.6 ± 8.0e	14.3 ± 0.5b	1.6 ± 0.5bc	15.3 ± 1.1d	35.6 ± 1.1e
Lemongrass oil + Lemongrass oil	280.0 ± 5.5f	15.3 ± 0.5c	2.0 ± 0.0d	13.3 ± 1.1b	35.7 ± 1.8e
Foliar spray (1g or 1ml /1L)					
Salicylic acid	261.1 ± 6.5d	14.3 ± 1.1b	1.6 ± 0.5bc	13.3 ± 1.1b	35.1 ± 2.3e
Humic acid	256.3 ± 4.1c	14.3 ± 0.5b	1.3 ± 0.5b	14.0 ± 0.0bc	34.7 ± 0.2d
Sodium benzoate	247.3 ± 6.0b	13.6 ± 0.5ab	1.3 ± 0.5b	12.6 ± 3.0a	34.1 ± 0.4d
Potassium sorbate	256.6 ± 11.5c	14.6 ± 0.5b	1.6 ± 0.5bc	14.0 ± 2.0bc	34.1 ± 0.4d
Carnation oil	250.6 ± 11.8c	14.6 ± 0.5b	1.6 ± 0.5bc	14.6 ± 2.0bc	33.9 ± 0.9c
Lemongrass oil	260.6 ± 2.5d	15.0 ± 0.0c	1.3 ± 0.5b	13.3 ± 1.1b	33.1 ± 0.2c
Control	242.0 ± 9.0b	12.3 ± 0.5a	1.0 ± 0.0a	12.0 ± 0.5a	31.6 ± 1.5a

Means ± standard deviations within a column followed by the same letter are not significantly different by (Duncan multiple range test at $P < 0.05$).

length and spores germination of *Fusarium oxysporum* f. sp. *lycopersici* and *Aspergillus fumigates* were inhibited by salicylic acid. Also, Shashi-Chauhan (1989) reported that salicylic acid proved its high toxicity against the mycelial growth of *Trichophyton mentagrophytes, T. tonsuran, T. violaceum, T. rubrum, Microsporum gypseum* and *Epidermophyton floccosum*. Induction of disease resistance by applying salicylic acid against

several soil-borne plant pathogens, i.e. fungal root rot and wilt pathogens (Chen-Chunquan *et al.,* 1999; Mandavia *et al*., 2000), as well as fungal foliar diseases (Sathiyabama and Balasubramanian, 1999; Cameron, 2000) have been reported. In addition, foliar appli-

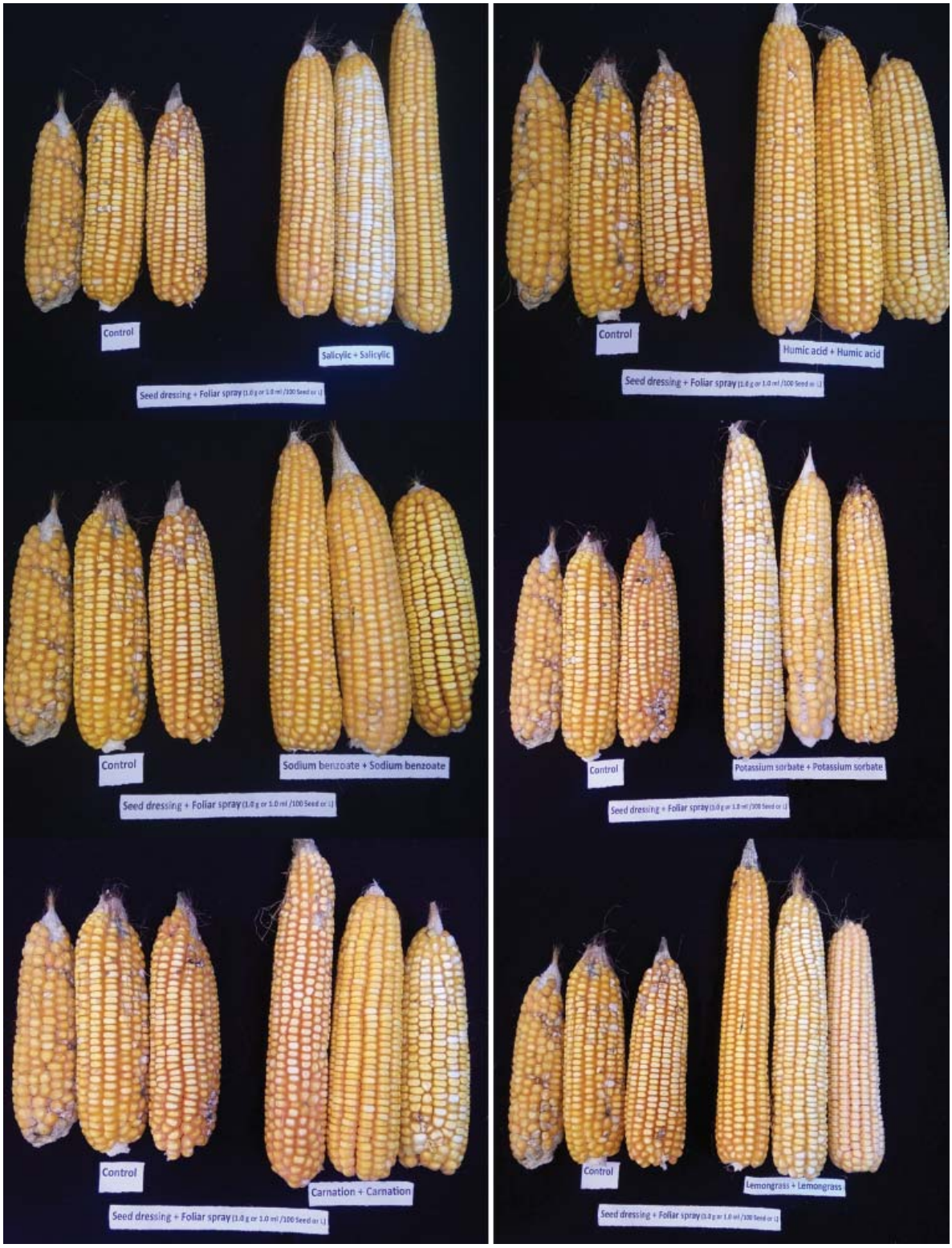


Figure 2. Corn cobs in maize plants treated with organic acids (salicylic acid, humid acid), organic salts (sodium benzoate, potassium sorbate) or essential oils (carnation oil, lemongrass oil) by [seed dressing + foliar spray] application, compared to untreated control plants, in field trials.

cation of humic acid proved to be effective in reducing early blight disease incidence in tomato plants (Farouk *et al*., 2012). Likewise, Saad *et al*. (2014) reported the inhibition activity of some antioxidants (ascorbic, benzoic, citric, salicylic acids and Bion 50 WG®) on the growth of the economically important pathogenic fungi *Alternaria solani* and *Fusarium solani in vitro,* while linear growth was decreased by increasing their concentrations.

El-Mougy *et al*. (2008) observed that organic acids, i.e., ascorbic, benzoic, citric and sorbic as well as organic salts, i.e., potassium sorbate and sodium benzoate at concentrations of 4% and 2%, respectively, completely inhibited the growth of causal agents of sour rot, green and blue molds of lemon fruits *in vitro* and the disease incidence under *in vivo* conditions. Sofos *et al*. (1986) and Sofos (1992) found that sorbic acid and its salts could inhibit various microorganisms (bacteria including sporeformers) as a result of a change in cell-transport function, the inhibition of enzymes involved in the glycolytic pathway or tricarboxylic acid cycle, inhibition of RNA, DNA, and protein synthesis, or the uncoupling of the oxidative phosphorylation in mitochondria. Cheng and Piper (1994) reported the depletion of ATP in conidia of *Saccharomyces cerevisiae* after exposure to sorbic acid.

The essential oils of carnation, lemongrass and black seed showed growth inhibition of *M. maydis in vitro* and *in situ* when applied as seed dressing, [seed dressing + foliar spray] or foliar spray. Essential oils have been generally reported as the main or adjuvant antimicrobial compounds in plant disease control (Kaur and Arora, 1999). Such compounds differ to conventional antimicrobials in structure and mode of action (Nascimento *et al*., 2000). Berhanu *et al.* (2020) stated that the phytochemicals occur naturally in the plant leaves, fruits, stems, bark, seeds, and roots, and defend and protect the plant against various diseases. For example, the antimicrobial activities of *Carissa spinarum* revealed an antibacterial activity against *Staphylococcus aureus*, *Staphylococcus aureus* ATCC 25923, Methicillin-resistant *Staphylococcus aureus* (MRSA), *Bacillus subtilis*, *Salmonella typhi*, *Escherichia coli*, *Escherichia coli* ATCC25922, *Escherichia coli* DSM 1103, *Pseudomonas aeruginosa* ATCC 35032, *Pseudomonas fluorescence*, *Proteus mirabilis*, *Mycoplasma mycoides* and *Streptococcus* species. Several compounds that have antimicrobial effects have been detected in the tested essential oils i.e., citral, geraniol, borneol and citronellol in lemongrass oil (Ganjewala and Gupta, 2016); eugenol, α-pinene, myrcene in carnation oil (Kirillov *et al*., 2017); 9-eicosyne, linoleic acid, palmitic acid and antioxidants in black seed oil (Dinagran *et al*., 2016) (www.holisticonline.com/Herbal-Med/_Herbs/h280.htm).

The treatments with the antagonistic fungi *T. viride, T. harzianum, B. subtilis* and P. *fluorescens* decreased the pathogenic fungal growth down to 7.6, 14.6, 19.0 and 41.0 mm, respectively, compared with 90mm linear growth in untreated control. Paulitz and Bélanger (2001) reported that during the past ten years, over 80 bio-control products have been marketed worldwide, based mostly on *Trichoderma* sp., *Ampelomyces quisqualis, Bacillus* sp., *Ulocladium* sp. and *Pseudomonas* sp. Abdel-Kader *et al.* (2012) have showed that reduction of powdery mildew and downy mildew diseases of cucumber, cantaloupe and pepper as well as early and late blights of tomato and pepper was achieved by spraying with the bioagents, *T. harzianum, T. viride, B. subtilis, P. fluorescens* and *S. cerevisiae*. Regarding their mode of action, it has been shown that *Bacillus* sp. synthesizes different metabolites that act as antifungal agents (Ahimou *et al*., 2000; Moyne *et a*l., 2001).

Algae are one of the chief biological agents that have been studied for the control of plant pathogenic fungi, particularly for soil-borne diseases (Hewedy *et al*., 2000). Cyanobacteria (blue-green algae) and eukaryotic algae had been reported to produce biologically active compounds, that have antifungal activity (Kulik, 1995; Schlegel *et al*., 1998), antibiotic and toxic activity (Bonjouklian *et al*., 1991; Kiviranta *et al*.,

1993). Moreover, *Anabaena* spp., *Scytonema* spp., and *Nostoc* spp. have been reported to be efficient for controlling soilborne fungi causing damping-off disease as well as the growth of soil fungus *Cunninghamella blakesleana* (Frankmolle *et al*., 1992; Chetsumon *et al*., 1993). Also, Kulik (1995) reported that culture filtrates or cell extracts from cyanobacteria and algae applied to seeds protect them from damping-off fungi such as *Fusarium* sp., *Pythium* sp. and *Rhizoctonia solani*. Furthermore, El-Mougy and Abdel-Kader (2013) reported that commercial blue-green algae extracts, Weed-Max at 2g/l (extracts in powder phase), and Oligo-X at 2 ml/l (extracts in liquid phase) could suppress soil-borne fungi causing root rot of cucumber, cantaloupe, tomato, and pepper plants under plastic greenhouse conditions and enhance the antagonistic ability of bioagents, fungi, bacteria and yeast.

It should be highlighted that the application method on the efficacy of the treatments was also important since the [seed dressing + foliar spray] application achieved significantly higher efficacy against disease incidence followed by seed dressing, foliar spray and soil drench application methods. Nevertheless, all tested application methods led to significantly lower disease incidence, and better plant morphogenesis and yield characters.

All treatments had a positive significant effect on maize plant morphogenesis and yield characters compared to the control. A possible effect of the tested compounds on plant nutrition cannot be ignored, i.e., SA as foliar application had a positive effect on increasing rocket yield and N, P, K, Fe, Mn, and Zn concentrations in leaves (Ahmed *et al*., 2002). Kalarani *et al.* (2002) reported that increasing nitrate reductase activity and chlorophyll content of tomato plants after SA spray as a distinct role. Likewise, humic acid is a heterogeneous mixture of many compounds which improve soil fertility and increase nutrients availability, enhance plant growth, yield, and decrease the harmful effect of stresses through various mechanisms inside plants and soil (Unlu *et al*., 2011; Moraditochaee, 2012). El-Beltagi *et al*. (2017) reported significant increase in plant growth and yield characters as well as changes in the leaf chemical composition of cotton plants by foliar application of potassium citrate (PC) and salicylic acid (SA). In addition, microalgae *Chlorella vulgaris* is used as biofertilizer and soil conditioner in agriculture systems (Song *et al*., 2005) and part of micronutrient foliar fertilizers (Shabaan, 2010). The tested commercial product Bioactive is also known to bring additional benefits to the plant at different growth stages such as development of the root, increasing vigor and photosynthetic efficiency, or a larger number and quality of the produced fruits (http://adleragro.com/en/productos/familia-bioestimulantes/biactive/).

Conclusion

In the present work, seed and/or by foliar application of the tested organic acids and salts, essential oils, algae, and soil drench application of the tested bioagents could achieve significant reduction of maize late wilt disease incidence *in vitro* and in field conditions. Overall, these compounds could be considered for potential use in IPM programs for the control of *M. maydis*, promoting sustainability in maize cultivation.

This research was financially supported in part by In-House Project No. 11030132 of National Research Centre, Egypt entitled "Integrated Management of Diseases Affecting Maize Crop during Vegetative Growth and Storage Periods".

Literature Cited

Abdel-Kader, M.M. 1997. Field application of *Trichoderma harzianum* as biocide for control of bean root rot disease. *Egyptian Journal of Phytopathology*, 25: 19-25.

Abdel-Kader, M.M., El-Mougy, N.S., Aly, M.D.E. and Lashin, S.M. 2012. Integration of Biological and Fungicidal Alternatives for Controlling Foliar Diseases of Vegetables Under Greenhouse Con-

ditions. *International Journal of Agricultural Forest*, 2: 38-48.

Ahimou, F., Jacques, P. and Deleu, M. 2000. Surfactin and iturin A effects on *Bacillus subtilis* surface hydrophobicity. *Enzyme Microbial Technology*, 27: 749-754.

Ahmed, H.A.H., Khalil, M.K. and Farrag, A.M. 2002. Nitrate accumulation, growth, yield and chemical composition of rocket (*Eruca vesicaria* subsp. *sativa*) plant as affected by NPK fertilization, kinetin and salicylic acid. *Annals of Agricultural Science*, Ain Shams Univ., 47 (1): 1- 26.

Anonymous 2017. Ministry of agriculture and Land Reclamation. Year Book of Department of Agricultural Economic Statistical, Egypt, p 215.

Barnett, H.L. and Hunter, B.B. 1972. Illustrated genera of imperfect fungi. 3rd edition, Burgess Publishing Co. 273 pp.

Begum, H., Mohammad, S., Rao, G.K. and Raj, R.B. 1989. Influence of seed dressing fungicides on the incidence of post flowering stalk rot (late wilt and charcoal rot), yield and profitability of maize. *Crop Research Hisar*, 2:142-146.

Bindu, S. and Kumar, P. 2009. *In vitro* antifungal potency of some plant extracts against *Fusarium oxysporum*. *International Journal of Green Pharmacy*, 3:63-65.

Bonjouklian, R., Smitka, T.A., Doolin, L.E., Molloy, R.M., Debono, M. and Shaffer, S.A. 1991. "Tjipanazoles, new antifungal agents from the blue-green alga *Tolypothrix tjipanasensis*," *Tetrahedron*, 47(37): 7739–7750.

Cameron, R.K. 2000. Salicylic acid and its role in plant defense responses: what do we really know. *Physiological* and *Molecular Plant Pathology*, 56: 91-93.

Chen-Chunquan, S., Belanger, R.R., Benhamou, N., Paulitz, T.C. and Chen, C.Q. 1999. Role of Salicylic acid in systemic resistance induced by Pseudomonas spp. against *Pythium aphanidermatum* in cucumber roots. *European Journal of Plant Pathology*, 105: 477-486.

Cheng, L. and Piper, P.W. 1994. Weak acid preservation block the heat shock response and heat shock-element-directed lacZ expression pf low pH *Saccharomyces cerevisiae* cultures an inhibitory action partially relieved by respiratory deficiency. *Microbiology*, 140: 1085-1096.

Chetsumon, A., Fujieda, K., Hirata, K., Yagi, K. and Miura, Y. 1993. "Optimization of antibiotic production by the cyanobacterium *Scytonema* sp. TISTR 8208 immobilized on polyurethane foam". *Journal of Applied Phycology*, 5 (6): 615–622.

Dinagaran, S., Sridhar, S. and Eganathan, P. 2016. Chemical composition and antioxidant activities of black seed oil (*Nigella sativa* L.). *International Journal of Pharmaceutical Sciences and Research*. Vol. 7(11): 4473-4479.

Degani, O., Movshowitz, D., Dor, S., Meerson, A., Goldbalt, Y. and Raninovitz, O. 2019. *Plant Disease*, 103(2): 238- 248. https://doi.org/10.1094/PDIS-05-18-0759-RE

El-Beltagi, H.S., Ahmed, S.H., Namich, A.A.M. and Abdel-Sattar, R.R. 2017 Effect of salicylic acid and potassium citrate on cotton plant under salt stress. *Fresenius Environmental Bulletin*, 26 (1): 1091-1100.

El-Mougy, N.S. and Abdel-Kader, M.M. 2013. "Effect of commercial cyanobacteria products on the growth and antagonistic ability of some bioagents under laboratory conditions," *Journal of Pathogens*, vol. 2013, Article ID 838329, 11 pages.

El-Mougy, N.S., El-Gamal, N.G. and Abd-El-Kareem, F. 2008. Use of organic acids and salts to control postharvest diseases of lemon fruits in Egypt. *Archives of Phytopathology and Plant Protection,* 41(7): 467- 476.

El-Shafey, H.A. and Claflin, L.E. 1999. Late wilt. In: White DG (ed) Compendium of corn diseases, 3rd ed. The American Phytopathological Society, St. Paul, pp 43–44.

El-Shafey, H.A., Abd-el-Rahim, M.F., Abd-el-Azim, O.Z. and Abd-eI-Hamid, M.S. 1976. Carryover of maize stalk-rot fungi in seed. *Agricultural Research Review*, 54: 29–42.

El-Shafey, H.A., El-Shorbagy, F.A., Khalil, I.I. and El-Assiuty, E.M. 1988. Additional sources of resistance to the late-wilt disease of maize caused by *Cephalosporium maydis*. Egypt. *Agricultural Research Review*, 66: 221-230.

Farouk, S., Youssef, S.A. and Ali, A.A. 2012. Exploitation of biostimulatants and vitamins as an alternative strategy to control early blight of tomato plants. *Asian Journal of Plant Science,* 11 (1): 36-43.

Ferreira, J.H.S., Mathee, F.N. and Thomas, A.C. 1991. Biological control of Eutypalota on grapevine by an antagonistic strain of *Bacillus subtilis*. *Phytopathology*, 81: 283–287.

Frankmolle, W.P., Larsen, L.K., Caplan, F.R., Patterson, G.M., Knübel, G., Levine, I.A. and Moore, R.E. 1992. "Antifungal cyclic peptides from the terrestrial blue-green alga *Anabaena laxa*. I. Isolation and biological properties". *Journal of Antibiotics*, 45(9): 1451–1457.

Ganjewala, D. and Gupta, A.K. 2016. Lemongrass (*Cymbopogon flexuosus* Steud.) Wats Essential Oil: Overview and Biological Activities. *Recent Progress in Medicinal Plants* (RPMP) Vol. 37: Essential Oils–II. 233-274.

Gilman, J.C. 1957. A manual of soil fungi. Iowa State College Press, Ames. 2nd Edition, pp.450.

Hewedy, M.A., Rahhal, M.M.H. and Ismail, I.A. 2000. "Pathological studies on soybean damping-off disease". *Egyptian Journal of Applied Science*, 15: 88–102.

Ismail, I.M.K., Salama, A.M., Ali, M.I.A. and Ouf, S.A. 1988. Effect of some phenolic compounds on

spore germination and germ-tube length of *Aspergillus fumigatus* and *Fusarium oxysporum* f. sp. *lycopersici*. *Egyptian Journal of Microbiology*, 23: 29-41.

Johal, L., Huber, D.M. and Martyn, R. 2004. Late wilt of corn (maize) pathway analysis: intentional introduction of *Cephalosporium maydis*. In: Pathways Analysis for the Introduction to the U.S. of Plant Pathogens of Economic Importance. USDA-APHIS Technical Report No. 503025.

Kalarani, M.K., Thangaraj, M., Sivakumar, R. and Mallika, V. 2002. Effects of salicylic acid on tomato (*Lycopersicon esculentum* Mill) productivity. *Crop Research*, 23 (3): 486-492.

Kaur, J. and Arora, D. 1999. Antimicrobial activities of species. *International Journal of Antimicrobial Agents*, 12: 257–262.

Kirillov, V., Stikhareva, T., Suleimen, Y., Serafimovich, M., Kabanova, S. and Mukanov, B. 2017. Chemical composition of the essential oil from carnation coniferous (*Dianthus acicularis* Fisch. ex Ledeb) growing wild in Northern Kazakhstan, Natural Product Research, 31(1): 117-123. DOI: 10.1080/14786419.2016.1214832

Kiviranta, J., Abdel-Hameed, A., Sivonen, K., Niemela, S.I. and Carlberg, G. 1993. "Toxicity of cyanobacteria to mosquito larvae screening of active compounds," *Environmental Toxicology* and *Water Quality,* 8 (1): 63–71.

Kulik, M.M. 1995. "Thepotential for using cyanobacteria (blue-green algae) and algae in the biological control of plant pathogenic bacteria and fungi," *European Journal of Plant Pathology*, 101 (6): 585–599.

Li, S., Xue, L., Xu, S., Feng, H. and An, L. 2009. Mediators, genes and signaling in adventitious rooting. *Botanical Review*, 75: 230-247.

Mandavia, M.K., Khan, N.A., Gajera, H.P., Andaria, J.H. and Parameswaram, M. 2000. Inhibitory effects of phenolic compounds on fungal metabolism in host-pathogen interactions in Fusarium wilt of Cumin. *Allelopathy Journal*, 7: 85-92.

Moraditochaee, M. 2012. Effects of humic acid foliar spraying and nitrogen fertilizer management on yield of peanut (*Arachis hypogaea* L.) in Iran. *ARPN Journal of Agricultural and Biological Science*, 7: 289–293.

Moyne, A.L., Shelby, R., Cleveland, T.E. and Tuzun, S. 2001. Bacillomycin D: an iturin with antifungal activity against *Aspergillus flavus*. *Journal of Applied Microbiology,* 90: 622-629.

Naqui, S.H.A., Khan, M.S.Y. and Vohora, S.B. 1994. Antibacterial, antifungal and anthelmintic investigation on Indian medicinal plants. *Fitoterapia*, 62: 221–228.

Nascimento, G.G., Locatelli, J. and Freitas, P.C. 2000. Antibacterial activity of plants extracts and phytochemical on antibiotic resistant bacteria. *Brazilian Journal of Microbiology*, 31: 247–256.

Nishimoto, R. 2019. Global trends in the crop protection industry. *Journal of Pesticides Sciences* 44(3): 141-147. https://www.jstage.jst.go.jp/article/jpestics/44/3/44_D19-101/_article

Paulitz, T.C. and Bélanger, R.R. 2001. Biological control in greenhouse systems. *Annual Review of Phytopathology*, 39: 103-133.

Ranum, P., Pena-Rosas, J.P. and Garcia-Casal, M.N. 2014. Global maize production, utilization, and consumption. *Annals of the New York Academy of Sciences,* 1312: 105-12. https://doi.org/10.1111/nyas.12396

Saad, A.S.A., Kadous, E.A., Tayeb, E.H., Massoud, M.A., Ahmed, S.M. and Abou El-Ela, A.S.A. 2014. The inhibitory effect of some antioxidants and fungicides on the growth of *Alternaria solani* and *Fusarium solani in vitro*. *Middle East Journal of Agricultural Research*, 3(2): 123-134.

Sabet, K.A., Zaher, A.M., Samra, A.S. and Mansour, I.M. 1970. Pathogenic behavior of *Cephalosporium maydis* and *C. acremonium*. *Annals of Applied Biology*, 66:257-263.

Samra, A.S., Sabet, K.A. and Abdel-Rahim, M.F. 1966. Effect of soil conditions and cultural practices on infection with stalk rots. pp 117-164. In: Samra, A.S. and Sabet, K.A. (eds) Investigations on Stalk-rot Disease of Maize in U.A.R. Ministry of Agric. Government Printing Offices, Cairo, Egypt.

Samra, A.S., Sabet, K.A. and Hingorani, M.K. 1963. Late wilt disease of maize caused by *Cephalosporium maydis*. *Phytopathology*, 53: 402–406.

Sathiyabama M, Balasubramanian R (1999) Treatment of groundnut leaves with Salicylic acid controls the development of rust disease caused by Puccinia arachidis Speg. *Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz*, 106: 166-173.

Satyanarayana, E. and Begum, H. 1996. Relative efficacy of fungicides (seed dressers) and irrigation schedule for the control of late wilt of maize. *Current Research University of Agricultural Science Bangalore*, 25: 59-60.

Schlegel, I., Doan, N.T., de Chazal, N. and Smith, G.D. 1998. "Antibiotic activity of new cyanobacterial isolates from Australia and Asia against green algae and cyanobacteria," *Journal of Applied Phycology*, 10 (5): 471–479.

Shabaan, M.M. 2010. Green microalgae water extracts as foliar feeding to wheat plants. *Pakistan Journal of Biological Science*, 4: 628–632.

Sharma, P., Jha, A.B., Dubey, R.S. and Pessarakli, M. 2012. Reactive Oxygen Species, Oxidative Damage, and Antioxidative Defense Mechanism in Plants under Stressful Conditions. *Journal of Botany*, Volume *2012*, Article ID 217037, *26* pages.

Shashi-Chauhan, S.M. and Chauhan, S. 1989. *In vitro* efficacy of certain antifungal lotions on the growth of deratophytic fungi. *National Acade-*

my Science Letters, 12: 11-13.

Singh, S.D. and Siradhana, B.S. 1987. Influence of some environmental conditions on the development of late wilt of maize induced by *Cephalosporium maydis*. *Indian Journal of Mycology and Plant Pathology,* 17: 1-5.

Singh, S.D. and Siradhana, B.S. 1989. Chemical control of late wilt of maize induced by *Cephalosporium maydis*. *Indian Journal of Mycological Plant Pathology,* 19: 121-122.

Sofos JN (1992) Sorbic acid, mode of action. In: Ledeberg J, editor. Encyclopedia of microbiology. San Diego, CA: Academic Press. pp 43 – 52.

Sofos, J.N., Pierson, M.D., Blocher, J.C. and Busta, F.F. 1986. Mode of action of sorbic acid on bacterial cells and spores. *International Journal of Food Microbiology*, 3:1-17.

Song, T., Martensson, L., Eriksson, T., Zheng, W. and Rasmussen, U. 2005. Biodiversity and seasonal variation of the cyanobacterial assemblage in a rice paddy field in Fujian, China. *The Federation of European Materials Societies (FEMS) Microbiology Ecology*, 54: 131–140.

Unlu, H.O., Unlu, H., Karakurt, Y. and Padem, H. 2011. Changes in fruit yield and quality in response to foliar and soil humic acid application in cucumber. *Scientific Research Essays*, 6: 2800–2803.

Whipps, J.M. 2001. Microbial interactions and biocontrol in the rhizosphere. *Journal of Experimental Botany*, 52: 487-511.

Received: 3 January 2020; Accepted: 4 February 2022

Αποτελεσματικότητα εναλλακτικών μυκητοκτόνων κατά του φυτοπαθογόνου μύκητα *Magnaporthiopsis maydis* στον αραβόσιτο και επίδραση στη μορφογένεση των φυτών και σε χαρακτηριστικά της απόδοσης

M.M. Abdel-Kader, M.S.A. Khalil και N.S. El-Mougy

Περίληψη Μελετήθηκε η αποτελεσματικότητα διαφόρων οργανικών οξέων, οργανικών αλάτων, αιθέριων ελαίων, φυκών (εκχύλισμα *Chlorella vulgaris* και εμπορικού προϊόντος) και βιολογικών παραγόντων κατά του *Magnaporthiopsis maydis*, που προκαλεί τον όψιμο μαρασμό του αραβοσίτου, σε συνθήκες εργαστηρίου και αγρού. Για τις *in vitro* δοκιμές χρησιμοποιήθηκαν στελέχη του *M. maydis* που απομονώθηκαν από το πεδίο στην Αίγυπτο. Επιπλέον, σε πειράματα αγρού σε δύο διαδοχικές καλλιεργητικές περιόδους δοκιμάστηκε η αποτελεσματικότητα διαφορετικών μεθόδων εφαρμογής των παραπάνω παραγόντων. Τα αποτελέσματα των *in vitro* δοκιμών έδειξαν μέγιστη ανάσχεση της ανάπτυξης του *M. maydis* σε διαφορετικές συγκεντρώσεις σαλικυλικού οξέος, ασκορβικού οξέος, βενζοϊκού οξέος και χουμικού οξέος καθώς και βενζοϊκού νατρίου, σορβικού καλίου, μονόξινου φωσφορικού καλίου και χλωριούχου ασβεστίου, με φθίνουσα σειρά. Παρόμοια τάση έδειξαν και τα αιθέρια έλαια γαρύφαλλου (*Dianthus caryophyllus* L.), λεμονόχορτου (*Cymbopogon schoenanthus* L.) και μελάνθιου (*Nigella sativa* L.). Η ελάχιστη ανάπτυξη του παθογόνου επιτεύχθηκε με την έκθεση στον ανταγωνιστή μύκητα *Trichoderma viride* και ακολούθως στους μικροοργανισμούς *Trichoderma harzianum*, *Bacillus subtilis* και *Pseudomonas fluorescens*. Υπό συνθήκες αγρού, η μεγαλύτερη μείωση της ασθένειας καταγράφηκε στις εφαρμογές με σαλικυλικό οξύ (όλες οι μέθοδοι εφαρμογής), χουμικό οξύ (όλες οι μέθοδοι εφαρμογής), βενζοϊκό νάτριο (επικάλυψη σπόρων), γαρυφαλέλαιο (επικάλυψη σπόρων ή επικάλυψη σπόρων σε συνδυασμό με ψεκασμό φυλλώματος) και τους βιολογικούς παράγοντες *B. subtilis* και *P. fluorescens* (ριζοπότισμα). Συνολικά, όλες οι επεμβάσεις και όλες οι μέθοδοι εφαρμογής οδήγησαν σε σημαντικά μικρότερη συχνότητα της ασθένειας σε σχέση με το μάρτυρα και στις δύο καλλιεργητικές περιόδους. Επιπλέον, με όλες τις επεμβάσεις επιτεύχθηκε ενίσχυση της μορφογένεσης των φυτών και των χαρακτηριστικών της απόδοσης. Οι πιο αποτελεσματικοί συνδυασμοί ουσιών/βιολογικών παραγόντων και μεθόδων εφαρμογής μπορεί να ληφθούν υπόψη για μελλοντική χρήση στην ολοκληρωμένη διαχείριση της ασθένειας στον αραβόσιτο.

Hellenic Plant Protection Journal **15:** 57-71, 2022

sciendo

Hellenic Plant Protection Journal **15:** 72-75, 2022
DOI 10.2478/hppj-2022-0008

SHORT COMMUNICATION

First record of *Sphenophorus placidus vestitus* (Coleoptera: Curculionidae: Dryophthorinae) in Cyprus

E. Kakouris[1], C. Makris[2] and J. Demetriou[3,4,5*]

Summary The Nearctic weevil *Sphenophorus placidus vestitus* Chittenden, 1904, an alien pest which is known to infest turfgrasses is reported for the first time on the island of Cyprus. A single specimen was collected from Famagusta (Ammochostos), Protaras in 2012. The species distribution and possible economic impacts are shortly discussed. A checklist for the alien Dryophthorinae of Cyprus is presented.

Additional keywords: alien species, Eastern Mediterranean, island invasions, Rhynchophorini, Sphenophorina

The subfamily Dryophthorinae consists of 1200 species in 152 genera which are widespread throughout the globe and divided in five tribes: Cryptodermatini, Dryophthorini, Orthognathini, Rhynchophorini and Stromboscerini (Oberprieler *et al*., 2014). The tribe Rhynchophorini includes some of the most notorious alien to Europe beetles, attacking woody monocotyledons, such as *Rhynchophorus ferrugineus* (Olivier, 1790) and *Diocalandra frumenti* (Fabricius, 1801) infesting palm-trees, *Scyphophorus acupunctatus* Gyllenhal, 1838 mainly associated with agaves, and representatives of the genus *Sitophilus* Schoenherr, 1838 being common stored product pests (Sauvard *et al*., 2010).

Only six species of the genus *Sphenophorus* Schönherr, 1838 are distributed in the Western Palearctic, namely, *S. abbreviatus* (Fabricius, 1787), *S. meridionalis* (Gyllenhal, 1838), *S. parumpunctatus* (Gyllenhal, 1838), *S. piceus* (Pallas, 1776), *S. striatopunctatus* (Goeze, 1777) and the alien *S. placidus vestitus* (Chittenden, 1904) [=syn. *S. venatus vestitus* (Say, 1832)] (Alonso-Zarazaga *et al*., 2017; Prena, 2018). Up to date, out of these species only three have been found to inhabit Cyprus (*S. abbreviatus*, *S. piceus* and *S. striatopunctatus*) (Alonso-Zarazaga *et al*., 2017).

Native to the Nearctic zoogeographic realm, *S. placidus vestitus* has been accidentally transported outside its native range to the Palearctic, reaching Eastern Palearctic countries such as Iraq, Japan, Korea and Qatar (Aoyagi *et al*., 1990; Yang et al., 2009; Pelletier, 2005; Aletby *et al*., 2015). In the Western Palearctic, the species has been reported in France (including Corsica) (Orousset *et al*., 2008; INPN 2021), Greece (Korotyaev and Apt, 2018), Morocco (Pelletier, 2005; Alonso-Zarazaga and Sánchez-Ruiz, 2009), and Spain (including Canary Islands) (Alonso-Zarazaga and Sánchez-Ruiz, 2009). This is the first record for *S. placidus vestitus* in Cyprus.

A single specimen was collected alive in Cyprus, Famagusta (Ammochostos), Paralimni, Protaras (35.0205 °N, 34.0516 °E), 15.vii.2012, 0 m alt., within 10-20 m from the coast, on the surface of the sea (Fig. 1). The specimen was identified by C. Makris using the identification key of Alonso-Zarazaga and Sánchez-Ruiz (2009) and its identification was later confirmed by Mr G. Kakiopou-

[1] Department of Forests, Ministry of Agriculture, Rural Development and Environment, 26 Louki Akrita, 1414, Nicosia, Cyprus.
[2] Ethnikis Antistaseos 21, Lemesos, CY-3022, Cyprus.
[3] Section of Ecology and Systematics, Department of Biology, National and Kapodistrian University of Athens, GR-157 84 Athens, Greece.
[4] Joint Services Health Unit Cyprus, BFC RAF Akrotiri BFPO 57, Akrotiri, Cyprus.
[5] Enalia Physis Environmental Research Centre, Acropoleos 2, Aglantzia 2101, Nicosia, Cyprus.
* Corresponding author: jakovosdemetriou@gmail.com

los. The specimen is deposited in the private collection of C. Makris.

This record constitutes the first report on *S. placidus vestitus* from the island. The circumstances under which the specimen was collected do not permit any identification of host-plants or other ecological parameters. However, as Protaras is a famous touristic resort situated along the coastline which maintains open areas covered by imported turfgrass, the specimen most probably originated from the coast of Protaras. As the main introduction pathway for the species has been related to the import of turfgrasses (Aletby *et al*., 2015), an accidental introduction to the island via the horticultural pathway is hypothesised.

Sphenophorus placidus vestitus has been identified as a major pest of turfgrasses in both its native range and invaded countries, commonly found in golf courses (Hatsukade, 1997; Yang *et al*., 2009; León-García *et al*., 2012) and orchardgrass (Kuhn *et al*., 2013). The distribution of the species within Cyprus, the presence of established populations and any adverse economic impact on the island are currently unknown, as the presented specimen stands out as the sole individual of *S. placidus vestitus* detected on the island. Nevertheless, given the extensive coverage of urban and semi-urban sites in Cyprus with turfgrass (e.g., golf courses, football fields, hotels, municipal parks and other tourist sites), *S. placidus vestitus* could potentially inflict a serious economic burden towards the maintenance of turfgrasses in the tourism industry, such as hotels and sports facilities creating yellow spots due to the drying of lawn (Alonso-Zarazaga and Sánchez-Ruiz, 2009; Aletby *et al*., 2015).

Sphenophorus placidus vestitus raises the number of non-native weevils of the subfamily Dryophthorinae (tribe Rhynchophorini) in Cyprus to 7 species, namely: *Rhynchophorus ferrugineus* (Olivier, 1790) (Kontodimas *et al*., 2006); *Scyphophorus acupunctatus* Gyllenhal, 1838, (Vassilliou and Kitsis, 2015); *Sitophilus granarius* (Linnaeus, 1758) (Georghiou, 1977); *Sitophilus oryzae* Schoenherr, 1838 (Georghiou, 1977); *Sitophilus sculpturatus* (Gyllenhal, 1838) (Georghiou, 1977); *Sitophilus zeamais* Motschulsky, 1855 (Gözüaçık *et al*., 2015); *Sphenophorus venatus vestitus* Chittenden, 1904 (present study).

Figure 1. Habitus of *Sphenophorus placidus vestitus* Chittenden, 1904. Photo C. Makris.

We would like to kindly acknowledge Mr George Kakiopoulos for validating the identification of S. placidus vestitus as well as the two anonymous Reviewers for their corrections and comments on the manuscript.

Literature cited

Aletby, M.A.A.W., Kadhim, S. and Alhadalg, H. 2015. Two billbugs (Curculionidae; Coleoptera), first recorded from Iraq. *Journal of Purity, Utility Reaction and Environment*, 4(6): 190-193.

Alonso-Zarazaga, M.Á. and Sánchez-Ruiz, M. 2009. Presencia de dos nuevas plagas en el Mediterráneo occidental y las Canarias y datos sobre otras

(Coleoptera: Dryophthoridae). *Boletín Sociedad Entomológica Aragonesa*, 45: 519-523.

Alonso-Zarazaga, M.Á., Barrios, H., Borovec, R., Bouchard, P., Caldara, R., Colonnelli, E., Gültekin, L., Hlaváč, P., Korotyaev, B., Lyal, C.H.C., Machado, A., Meregalli, M., Pierotti, H., Ren, L., Sánchez-Ruiz, M., Sforzi, A., Silfverberg, H., Skuhrovec, J., Trýzna, M., Velázquez de Castro, A.J. and Yunakov, N.N. 2017. *Cooperative Catalogue of Palaearctic Coleoptera Curculionoidea*. Monografías electrónicas Sociedad Entomológica Aragonesa S.E.A. 8, 729 p.

Aoyagi, M., Ishii, M., Hirowatari, T. and Yasuda, T. 1990. Record of the zoysiagrass billbug, *Sphenophorus venatus vestitus* Chittenden, from the bank of the Yamato River in Osaka and Nara Prefectures. *Japanese Journal of Applied Entomology and Zoology*, 34(3): 253-254. https://doi.org/10.1303/jjaez.34.253.

Georghiou, G.P. 1977. *The Insects and Mites of Cyprus. With Emphasis on Species of Economic Importance to Agriculture, Forestry, Man and Domestic Animals*. Kiphissia, Athens: Benaki Phytopathological Institute, 347 p.

Gözüaçık, C., Güllü, M., Konuksal, A., Yücel, A. and Hekimhan, H. 2015. Insect species of stored cereal in Northern Cyprus. In *Proceedings of 2nd International Symposium for Agriculture and Food*, Ohrid, Republic of Macedonia, 7-9 Oct. 2015, poster presentation.

Hatsukade, M. 1997. Biology and control of the hunting billbug, *Sphenophorus venatus vestitus* Chittenden, on golf courses in Japan. *International Turfgrass Society Research Journal*, 8: 987-996.

INPN 2021. Inventaire National du Patrimoine Naturel. Available at: https://inpn.mnhn.fr/espece/cd_nom/593184?lg=en (Accessed 18 May 2021).

Kontodimas, D.C., Milonas, P.G., Vassiliou, V., Thymakis, N. and Economou, D. 2006. The occurrence of *Rhynchophorus ferrugineus* in Greece and Cyprus and the risk against the native greek palm tree *Phoenix theophrasti*. *ENTOMOLOGIA HELLENICA*, 16: 11-15. https://doi.org/10.12681/eh.11621.

Korotyaev, B.A. and Apt, A.S. 2018. The First Record of an Invasive Nearctic Weevil *Sphenophorus placidus vestitus* Chittenden (Coleoptera, Dryophthoridae) from Greece. *Entomological Review*, 98: 1157–1158, https://doi.org/10.1134/S0013873818080237.

Kuhn, W.R., Youngman, R.R., Wu, S. and Laub C.A. 2013. Ecology, Taxonomy, and Pest Management of Billbugs (Coleoptera: Curculionidae) in Orchardgrass of Virginia. *Journal of Integrated Pest Management*, 4(3): 1-5, https://doi.org/10.1603/IPM12022.

León-García, I., Rodríguez-Leyva, E., Equihua-Martínez, A., Valdez, J.M. and Jones, R.W. 2012. First record of the billbug, *Sphenophorus venatus* (Say) (Coleoptera: Curculionidae: Dryophthorinae), on turfgrass in Quintana Roo, Mexico. *The Coleopterists Bulletin*, 66(1): 34–36. https://doi.org/10.1649/072.066.0110.

Oberprieler, R.G., Anderson, R.S. and Marvaldi, A.E. 2014. *Curculionoidea Latreille, 1802: Introduction, Phylogeny*. In: LESCHEN, RAB and BEUTEL RG. (eds). *Handbook of Zoology, Arthropoda: Insecta; Coleoptera, Beetles, Vol. 3: Morphology and Systematics (Phytophaga)*. Walter de Gruyter, Berlin/Boston, p. 423–648.

Orousset, J., Osella, G., Pelletier, J. and Voisin, J.F. 2008. *Sphenophorus venatus* (Say, 1831), espèce nouvelle pour la faune de Corse et de France (Coleoptera, Curculionoidea). *Coleopteriste*, 11(3): 154-156.

Pelletier, J. 2005. *Sphenophorus venatus* (Say, 1831), espèce envahissante de la Région paléarctique (Coleoptera, Curculionoidea, Dryophthoridae). *Nouvelle Revue d'Entomologie*, 22(2): 189-190.

Prena, J. 2018. An Annotated Inventory of the Weevils (Coleoptera: Curculionoidea) Described by Thomas Say. *Bulletin of the Museum of Comparative Zoology* 161(9): 323–401. https://doi.org/10.3099/MCZ161-09.1.

Sauvard, D., Branco, M., Lakatos, F., Facolli, M. and Kirkendall, L.R. 2010. Weevils and Bark Beetles (Coleoptera, Curculionoidea). *BioRisk*, 4(1): 19–266. https://doi.org/10.3897/biorisk.4.64.

Vassilliou, V. and Kitsis, P. 2015. First record of the sisal weevil, *Scyphophorus acupunctatus*, in Cyprus. *ENTOMOLOGIA HELLENICA*, 24: 22-26. https://doi.org/10.12681/eh.11542.

Yang, S.W., Shim, G.Y., Kim, J.H., Hong, K.J. and Jeong, Y.G. 2009. Exotic pest, hunting billbug, *Sphenophorus venatus vestitus* Chittenden (Coleoptera, Dryophthoridae) in Korea. *Asian Journal of Turfgrass Science*, 23(2): 265-270.

Received: 18 June 2021; Accepted: 17 March 2022

ΣΥΝΤΟΜΗ ΑΝΑΚΟΙΝΩΣΗ

Πρώτη καταγραφή του *Sphenophorus placidus vestitus* (Coleoptera: Curculionidae: Dryophthorinae) στην Κύπρο

Η. Κακούρης, Χ. Μακρής και Ι. Δημητρίου

Περίληψη Το Βορειοαμερικανικό σκαθάρι *Sphenophorus placidus vestitus* Chittenden, 1904, ένα ξενικό έντομο που είναι γνωστό ότι προσβάλλει χλοοτάπητες, αναφέρεται για πρώτη φορά στην Κύπρο. Ένα μοναδικό δείγμα συλλέχθηκε από την Αμμόχωστο, στον Πρωταρά το 2012. Γίνεται αναφορά στη διασπορά του εντόμου και τις πιθανές οικονομικές επιπτώσεις. Παρουσιάζεται μια συνοπτική λίστα με τα ξενικά είδη Dryophthorinae της Κύπρου.

Hellenic Plant Protection Journal **15:** 72-75, 2022

sciendo

Hellenic Plant Protection Journal **15:** 76-79, 2022
DOI 10.2478/hppj-2022-0009

SHORT COMMUNICATION

First record of the Nearctic *Ozognathus cornutus* (LeConte, 1859) (Coleoptera: Ptinidae: Anobiinae) in Cyprus

J. Demetriou[1,2], G. Kakiopoulos[3] and A.F. Martinou[2,4*]

Summary The Nearctic spider beetle *Ozognathus cornutus* (LeConte, 1859) is recorded for the first time in Cyprus during entomological surveys on alien *Eucalyptus* spp. The biology of this alien species is still understudied. A short overview of the species distribution and ecology in the Mediterranean is presented, adding a new host plant for the beetle.

Additional keywords: alien species, Eastern Mediterranean, *Eucalyptus*, non-native, spider beetles

The New World genus *Ozognathus* LeConte, 1861(Ptinidae: Anobiinae) is represented by 11 species native to North and South America (White 1974; 1975; Zahradník and Mifsud, 2005; Trócoli *et al*., 2020). Originating from the Nearctic zoogeographic realm, *Ozognathus cornutus* (LeConte, 1859) has been accidentally introduced outside its native range to the Western Palearctic, where it has been recorded in France (Allemand *et al*., 2008), Germany (Allemand *et al*., 2008), Gibraltar (GONHS, 2017), Israel (Miłkowski, 2019), Italy (including Sardinia and Sicily) (Cusimano *et al*., 2015; Bazzato *et al*., 2021; Cerasa and Lo Verde, 2021), Latvia (Telnov *et al*., 2016), Malta (Zahradník and Mifsud, 2005), Portugal (Madeira) (Zahradník and Mifsud, 2005), Spain (including Canary Islands) (Bercedo *et al*., 2005; Viñolas, 2017; Trócoli *et al*., 2020), Switzerland (Germann and Schmidt, 2017), Tunisia (Zahradník and Mifsud, 2005) and the United Kingdom (Stenhouse, 2017) (Fig. 1).

During entomological field surveys concerning alien insects on *Eucalyptus* spp., three male *Ozognathus* specimens were collected and identified using species diagnoses of Zaradník and Mifsud (2005) and Stenhouse (2017). The specimens were deposited at the National and Kapodistrian University of Athens, Greece as part of the first author's MSc Thesis.

Material examined:
Cyprus, Limassol (Lemesos), Marina (Molos), (34.6750° N, 33.0475° E), 0 m alt., collected during beat-sheet sampling on *Eucalyptus* spp., urban area - municipal park by the sea, 23.iv.2021 and 14.v.2021 (Fig. 2), 2 males collected.
Cyprus, Limassol (Lemesos), Port, (34.6453° N, 33.0008° E), 0 m alt., collected during beat-sheet sampling on *Eucalyptus* spp., windbreaker near crop, 31.iii.2021, 1 male collected.

The ecology of *O. cornutus* is still rather unknown and the species has been recorded at a wide range of habitats including agricultural, coastal, urban and woodland areas (Zaradník and Mifsud, 2005; Miłkowski, 2019; Trócoli *et al*., 2020). *Ozognathus cornutus* is regarded as polyphagous, being associated with 38 different host plants (Bazzato *et al*., 2021). In Mediterranean countries, it has been collected from various plant spe-

[1] Department of Ecology and Systematics, Faculty of Biology, National and Kapodistrian University of Athens, GR-157 84 Athens, Greece.
[2] Joint Services Health Unit Cyprus, BFC RAF Akrotiri BFPO 57, Akrotiri, Cyprus.
[3] Ilidos 60-62 street, GR-115 27 Athens (Ampelokipi), Greece.
[4] The Cyprus Institute, Athalassa Campus, Aglantzia, Cyprus.
* Corresponding author: a.martinou@cyi.ac.cy

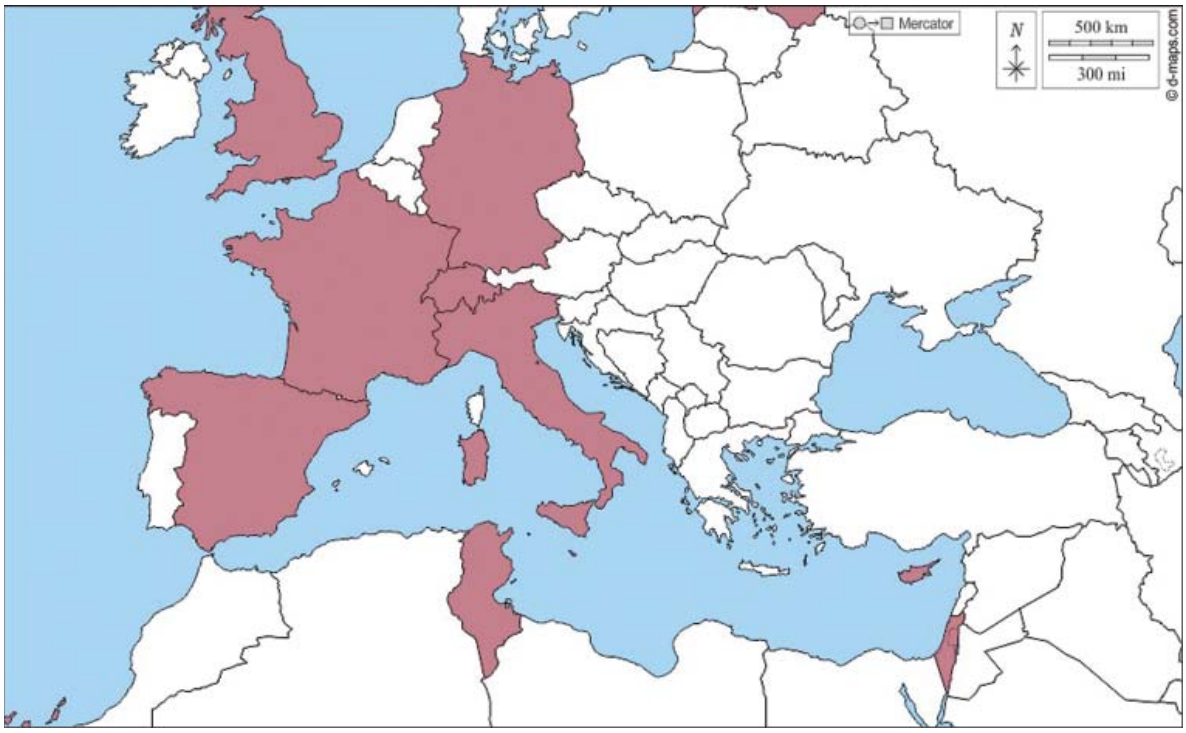


Figure 1. Distribution map of *Ozognathus cornutus* (LeConte, 1859) in the Western Palearctic.

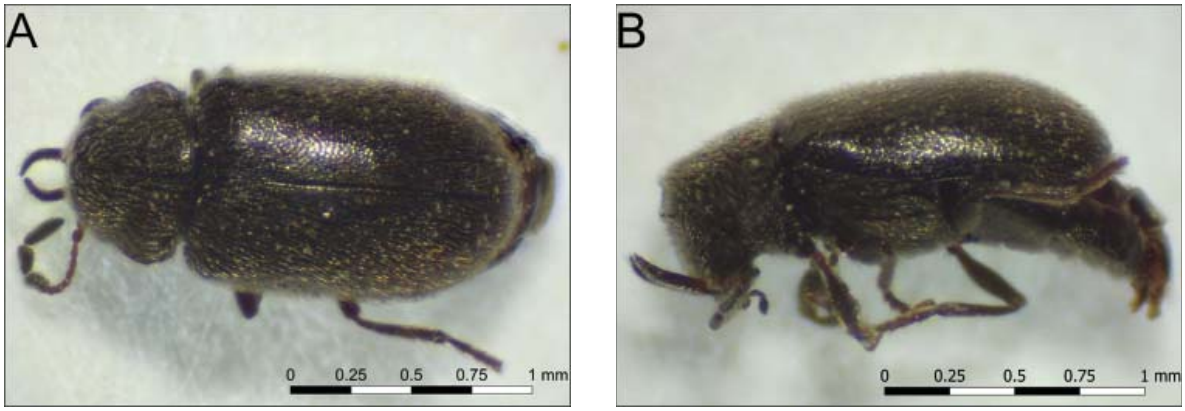


Figure 2. Male *Ozognathus cornutus* (LeConte, 1859) collected in Limassol city centre, dorsal (A) and lateral view (B).

cies such as *Allium sativum*, *Carduus* spp., *Eriobotrya japonica*, *Euphorbia characias*, *Ficus carica*, *Fraxinus angustifolia syriaca*, *Phoeniculum vulgare*, *Phoenix dactylifera*, *Retama monosperma*, *Robinia pseudocacia*, *Quercus suber*, *Schefflera arboricola* and *Scolymus hispanicus* (Bercedo *et al*., 2005; Miłkowski, 2019; Trócoli *et al*., 2020; Bazzato *et al*., 2021) as well as from dried fruit of *Prunus amygdalinus* (Allemand *et al*., 2008). The beetle has been regarded as saproxylophagous, feeding on decaying plant tissues such as dried fruit and woodborers' faeces in galleries as well as inhabiting galls of various Cecidomyidae and Cynipidae (Miłkowski, 2019; Trócoli *et al*., 2020; Cerasa and Lo Verde, 2021).

Although, eucalypts in the sampled areas were heavily infested with *Glycaspis brimblecombei* Moore, 1964 lerps (Limassol marina) as well as galls of *Leptocybe invasa* Fisher and La Salle, 2004 and *Ophelimus maskelli* (Ashmead, 1900) (Limassol marina and port), no associations or interactions with *O. cornutus* were observed.

According to Cusimano *et al.* (2014) more records are anticipated as the presence of *O. cornutus* in Europe has been associated with international trade. During our surveys, one specimen was collected from Limassol port which is the island's largest commercial port, while the other two male specimens were collected from the Limassol marina, approximately 3 km from Limassol port. This potentially pinpoints the original point of entry of *O. cornutus* to the country. The presence of *O. cornutus* in Cyprus does not come as a surprise, given its extended presence in the Mediterranean Basin and recent records from neighbouring Israel (Miłkowski, 2019). Due to the wide range of native and non-native host plant species, *O. cornutus* is expected to become widespread in Cyprus.

We are highly grateful to Prof. Margarita Arianoutsou and Dr Canella Radea (National and Kapodistrian Univeristy of Athens, Greece) for overseeing the MSc Thesis during which field surveys took place. We are also thankful to the two anonymous reviewers for their valuable comments and corrections to the manuscript. This work was funded by the State Scholarship Foundation of Cyprus (IKYK) during a postgraduate scholarship awarded to Mr Jakovos Demetriou for his MSc studies.

Literature cited

Allemand, R., Laclos, É., de Büche, B. and Ponel, P. 2008. Anobiidae nouveaux ou méconnus de la faune de France (3e note) (Coleoptera). *Bulletin de la Société entomologique de France*, 113(3): 397-402.

Bazzato, E., Marignaia, M., Ancona, C., Caria, M., Cillo, D. and Serra, E. 2021. First record of *Ozognathus cornutus* (LeConte, 1859) (Coleoptera Ptinidae) from Sardinia, Italy. *REDIA*, 104: 89-99. http://dx.doi.org/10.19263/REDIA-104.21.10

Bercedo, P., Arnáiz, L., Coello, P. and Baena, M. 2005. *Ozognathus cornutus* (LeConte, 1859), nuevo anòbido para la fauna Ibérica (Coleoptera: Anobiidae). *Boletín de la Sociedad Entomológica Aragonesa*, 37: 213-214.

Cerasa, G. and Lo Verde, G. 2021. Naturalization and spread of the alien species *Ozognathus cornutus* (LeConte, 1859) (Coleoptera: Ptinidae: Ernobiinae) in Italy. *Phytoparasitica*. https://doi.org/10.1007/s12600-021-00923-x

Cusimano, C., Cerasa, G., Lo Verde, G. and Massa, B. 2014. *Ozognathus cornutus* (LeConte, 1859) (Coleoptera Anobiidae), new record for Italy. *Il Naturalista siciliano, N. S.*, 38(1): 131-132.

Germann, C. and Schmidt, M. 2017. Erstes Auftreten von *Ozognathus cornutus* (LeConte, 1859) in der Schweiz (Coleóptera, Ptinidae). *Entomologische Nachrichten und Berichte*, 61: 151-153.

GONHS (Gibraltar Ornithological and Natural History Society) 2017. Provisional List of the Coleoptera of Gibraltar. Available at: https://www.gonhs.org/list/coleoptera (Accessed 15 May 2021).

Miłkowski, M. 2019. *Ozognathus cornutus* (LECONTE, 1859) (Coleoptera: Ptinidae) – nowy gatunek w faunie Izraela. *Wiadomości Entomologiczne*, 38(2): 87-90.

Stenhouse, D.A. 2017. *Ozognathus cornutus* (LeConte, 1859) (Ptinidae) in Britain. *The Coleopterist*, 26(2): 94-96.

Telnov, D., Bukejs, A., Gailis, J., Kalniņš, M., Kirejtshuk, A.G., Piterāns, U. and Savich, F. 2016. Contributions to the knowledge of Latvian Coleoptera 10. *Latvijas Entomologs*, 53: 89-121.

Trócoli, S., Tomás, M., Lencina, J.L., Torres, J.L., Vela, J.M., and Baena, M. 2020. Nuevos registros ibéricos de *Ozognathus cornutus* (LeConte, 1859) y notas sobre su biología y distribución (Coleoptera: Ptinidae). *Boletín de la Sociedad Entomológica Aragonesa*, 30: 83-95.

Viñolas, A. 2017. Nueva aportación al conocimiento de los Ptinidae (Coleoptera) de la Península Ibérica e Islas Canarias, con la descripción de un nuevo *Stagetus* Wollaston, 1861 de Navarra. *Arquivos Entomolóxicos*, 18: 137-148.

White, R.E. 1974. Type-species for World genera of Anobiidae (Coleoptera). *Transactions of the American Entomological Society*, 99: 415-475.

White, R.E. 1975. Sixteen new neotropical Anobiidae with a new genus and keys (Coleoptera). *Proceedings of the Entomological Society of Washington*, 77: 169-188.

Zahradník, P. and Mifsud, D. 2005. *Ozognathus cornutus* (LeConte) – new record for the Palaearctic Region (Coleoptera: Anobiidae). *Studies and reports of District Museum Prague-East Taxonomical Series*, 1(1-2): 141-143.

Received: 16 August 2021; Accepted: 17 March 2022

ΣΥΝΤΟΜΗ ΑΝΑΚΟΙΝΩΣΗ

Πρώτη καταγραφή του Βορειοαμερικανικού σκαθαριού *Ozognathus cornutus* (LeConte, 1859) (Coleoptera: Ptinidae: Anobiinae) στην Κύπρο

Ι. Δημητρίου, Γ. Κακιόπουλος και Α.Φ. Μαρτίνου

Περίληψη Το Βορειοαμερικανικό σκαθάρι *Ozognathus cornutus* (LeConte, 1859) καταγράφεται για πρώτη φορά στην Κύπρο κατά τη διάρκεια εντομολογικών ερευνών σε ξενικά είδη ευκαλύπτων. Η βιολογία του ξενικού αυτού είδους είναι ακόμη σε μεγάλο βαθμό άγνωστη. Παρουσιάζεται μια σύντομη επισκόπηση της κατανομής του είδους και των φυτών ξενιστών του στη Μεσόγειο, προσθέτοντας ένα νέο φυτικό είδος ξενιστή για το σκαθάρι.

Hellenic Plant Protection Journal **15:** 76-79, 2022

sciendo

Hellenic Plant Protection Journal **15:** 80-86, 2022
DOI 10.2478/hppj-2022-0010

Biological parameters of grape phylloxera, *Daktulosphaira vitifoliae*, on local grapevine varieties in central Syria – Implications on their susceptibility

I. Idris[1*], A. Asaad[1], K. Houssian[1] and I. Kalefa[2]

Summary Biological parameters of phylloxera local strains as well as infestation were studied on Syrian grapevine varieties including Baladi, Salamone, Karawane, Hafarzale, Gharbe and the American resistance rootstock (ARR-B41) (*Vitis vinifera* x *Vitis berlandieri*). Root artificial infestation method revealed significant differences in phylloxera population between the majority of tested varieties comparing to the ARR-B41 rootstock. Hafarzale and Karawane have shown the lowest population of grape phylloxera, with a similar level of resistance to rootstock ARR-B41. Furthermore, Salamone and Gharbe are more conducive to grape phylloxera reproduction. Field investigation has shown significant differences between the local varieties and ARR-B41 in terms of the average number of galls "nodosities". Overall, Hafarzale and Karawane tended to form significantly fewer galls than the other varieties.

Additional keywords: B41 rootstock, galls, grape varieties, nodosities, phylloxera, tuberosities

Introduction

Syrian vineyards (*Vitis vinifera*) cover more than 70,000 hectares, producing about 540,000 tons of grapes annually (Makee *et al*., 2010). Since the end of the 19th century and the beginning of the 20th century, the grape phylloxera *Daktulosphaira vitifoliae*, Fitch (Hemiptera: Phylloxeridae), has spread worldwide through plant materials (Granett *et al*., 1985; Triska *et al*., 2018). In Syria, phylloxera was first discovered on the local grapevine variety Doumani in 1935 and caused catastrophe in the Douma region near Damascus. Subsequently, it invaded Syrian nurseries and quickly settled in Syrian vineyards (Balachawsky and Mesnil, 1935; Idris and Arabi, 2014).

Grape phylloxera is considered to be the most harmful pest to grapes. In Dara Province located in southern Syria, it was estimated that 82.5% of vineyards were infested with phylloxera (Al-Chaabi *et al*., 2012). Granett *et al*. (1996) reported that once a vineyard is infested with grape phylloxera, it is estimated to stop its production in about two to five years. Grape roots are damaged by phylloxera through the formation of flashy galls, "nodosities" on immature roots by the expansion of the root cortex, while on mature roots they are known as "tuberosities", and the root system weakens over time as a result (Kellow *et al*., 2004). These galls are used by phylloxera to extract stored nutrients, such as sugar and amino acids, and can support populations with high reproduction rates (Omer *et al*., 2002; Lawo *et al*., 2011; Idris and Arabi, 2014). Thus, the infestation of phylloxera destroys the root system of grapevine, while the new phylloxera type may adapt to resistant rootstocks (i. e ARR-B41) (Granett *et al*., 2001).

Several control methods including pesticides, radiation and soaking in hot water would be only useful in the isolation and treatment of quarantine grape phylloxera (Granett *et al*., 2001; Makee *et al*., 2008; Makee *et al*., 2010, Adam *et al*., 2012; Adam *et al*., 2013; Adam *et al*., 2021). Yet none of these methods are effective to control the insect. Grafting susceptible species onto American phylloxera-resistant rootstocks remains the most effective phylloxera control method

[1] Department of Biotechnology, Atomic Energy Commission of Syria (AECS), P. O. Box 6091 Damascus, Syria.
[2] General Commission for Scientific Agricultural Research, Homs, Syria.
* Corresponding author: ascientific23@aec.org.sy

(Fossen, 2005). Noteworthy, some resistant rootstocks have either lost their resistance or became less resistant to phylloxera after years of use (Granett *et al*., 1985). For example, the rootstock ARR-B41 which is still resistant in France, it is not resistant in California vineyards, creating and urgent need to replant new vines with a suitable resistant rootstock (Granett *et al*., 1983; De Benedictis and Granett, 1993; Makee *et al.,* 2004). In most cases, rootstocks usually restrict the growth due to incompatibility of roots between rootstocks and grafted vines, reducing the vigor of grafted varieties (Makee *et al*., 2010; Idris and Arabi, 2014). According to previous research, in Dara Province (southern Syria), 89% of grapevine varieties grafted onto American rootstocks resistant were incompatible (Al-Chaabi *et al*., 2012).

This study examined the population dynamics of grape phylloxera on five local grapevine varieties planted in central region of Syria and the resistant rootstock ARR-B41. Biological parameters of phylloxera, i.e., fecundity, egg laying period, development time of nymphs, number of galls "tuberosities", and survival rate of adults were considered. In addition, the number of galls "nodosities" located on the roots during the growing season were evaluated in the field.

Materials and Methods

Establishment of phylloxera colonies

Grape phylloxera was originally collected from the infested roots of local grapevine varieties in the middle region of Syria. Colonies of phylloxera were established in our laboratory according to protocols of previous work (Idris and Arabi, 2014).

Biological parameters of phylloxera and infestation in the laboratory

Healthy fresh roots with a diameter of 4-7 mm and a length of 5-7 cm from local grape varieties (Baladi, Salamone, Karawane, Hfalzale, Gharbe) and the American phylloxera-resistant rootstock ARR-B41 were collected from the field. Twelve roots of each variety were cleaned with tap water and wrapped separately with wet cotton wool. Ten phylloxera eggs from the laboratory colony were placed on each root (total of 120 eggs in each local grape variety and ARR-41B). Three to four infested root pieces were transferred onto a wet paper in plastic Petri dishes (diameter=12 cm, depth= 1 cm) as described by Makee *et al*. (2010) thus there were four replicates per variety. Ventilation was possible through a cloth–screened hole (1-1.5 cm) at the Petri dish lid. The dish edges were fixed with parafilm, kept in plastic boxes with firmly fitting lids, and brooded in incubator at 25°C and 75% relative humidity.

The infested roots were examined under a microscope for newly hatched nymphs, and the survival rate of adults were calculated using the following formula: % = (number of eggs hatched - number of nymphs that died/ number of eggs hatched) x 100. A total of 25 females were selected from the four replicates of each variety group (5 root pieces of each group X 5 females on each root) and the roots were then transferred to other plastic Petri dishes, held in plastic boxes with tightly fitting lids under the same conditions as described above. The following reproductive parameters were measured for each female: fecundity (number of eggs), oviposition (period during which eggs are laid), and developmental time (from egg to egg). Observations were made of the number of tuberosities that formed on the roots.

Infestation in field conditions

The experiment was carried out at about 12 kilometers north of Homs (Al Mukhtareyah: 34°80'42. 37" N 36°73'96. 97" E) (approximately 539 mm of rainfall during the growing season from November 10 to May 30. Stems of local varieties Baladi, Salamone, Karawane, Hafarzale, Gharbe and ARR- B41 were selected (n=228). After immersion in a 2000 ppm solution of Indole Butyric Acid (IAA) for 2 minutes, 48 stems of each variety were planted in 10-L plastics pots with sterile moist soil. Four months later, plants infested with phylloxera eggs (50 eggs per plant) were planted in the field, one square

meter apart, line by line. In the following year, 12 plants of each variety were uprooted, root samples were collected randomly from each plant (about 100 cm), cleaned roughly on the spot and stored at 7°C for transportation to the laboratory. The number of nodosities was determined by a microscopic examination of each root.

Statistical analysis

The statistical analysis was done using the STATISTIC program version 6 (Statsoft, Inc. 2003). Data were subjected to analysis of variance at 5% significance level (P= 0.05). Data were transformed into square roots where appropriate. Significance of differences between means, were performed according to Tukey HSD test.

Results and discussion

Grape phylloxera infestation intensity is affected by environmental factors such as temperature, humidity, soil, grape variety and genotype (Makee *et al*., 2010; Powell, 2012; Hoffmann *et al*., 2016). The assessment of biological parameters is a good method of determining the grapevine root susceptibility to phylloxera (Idris and Arabi, 2014). The results showed significant differences between local grapevine varieties in Syria and the resistant ARR-B41 rootstock for biological parameters including, fecundity, oviposition (egg laying period), developmental time of nymphs, and number of tuberosities ($F= 441.35$, $df= 5$, $P<.005$; $F= 223.2$, $df= 5$, $P< 0.005$; $F=25.8$, $df= 5$; $P<0.005$; $F=55.6$; $df= 5$, $P<0.005$, respectively) (Fig. 1). These biological parameters were significantly lower in Akrawane and Hafarzale compared to the other local varieties. In contrast, on these varieties and ARR-B41, it took the same amount of time for phylloxera nymphs to reach adulthood. According, to the study by Makee *et al*. (2010) local varieties phylloxera reproduction abilities on their roots. The survival percentage of phylloxera adults on roots was not significantly different between the Hafarzale variety and ARR-B41, but it was low in comparison with the other local varieties ($F= 182.3$, $df= 5$, $P<0.005$) (Fig. 2).

The number of nodosities differed significantly between the local varieties and ARR-B41 in spring, summer and autumn ($F=179.08$, $df=5$, $P<0.0001$; $F=176.216$, $df=5$, $P<0.0001$; $F=102.96$, $df= 5$, $P<0.0001$, respectively), and Hafarzale was the variety with the lowest nodosities (Hafarzale, ARR-B41, Baladi, Gharbe, Karawane, Salamone). Root galls significantly increased in spring and summer but decreased in autumn (Fig. 3). In winter, none of the studied varieties formed nodosities on the roots (Fig. 3). There is a strong correlation between the adaptability of grapevine varieties to phylloxera reproduction and the number of nodosities (Lawo *et al*., 2011).

Grapevine genotype, virulence of phylloxera strains and growing season conditions, i.e., dominant temperature in winter, are key factors influencing the grapevine resistance to phylloxera. According to Hoffmannet *et al*. (2016), phylloxera forms galls on suitable vine roots due to the effect of two main parameters, the plant genotype and the growing season. No formation of root galls in the examined local grapevine varieties during winter indicates that local season conditions influence the insect distribution and lifespan. In central Syria, the temperature can drop to -10°C for many days during winter, and the mean temperature is 1-3°C for several months. These low winter temperatures may play a crucial role in preventing phylloxera from reproduction due to mortality from freezing (Korosi *et al*., 2012). In temperate climates, phylloxera can survive winter in high density on grapevine roots even on American resistant rootstocks (Hoffmannet *et al*., 2011; Hoffmannet *et al*., 2016).

Omer *et al*. (1997) suggest a reduced direct impact of environmental conditions, such as the impact of temperature on phylloxera's population dynamics. As noted by Eissenstat *et al*. (2005), the majority of root growth in grapevine occurs between spring and summer and the roots usually do not grow near the harvest time or the dormant

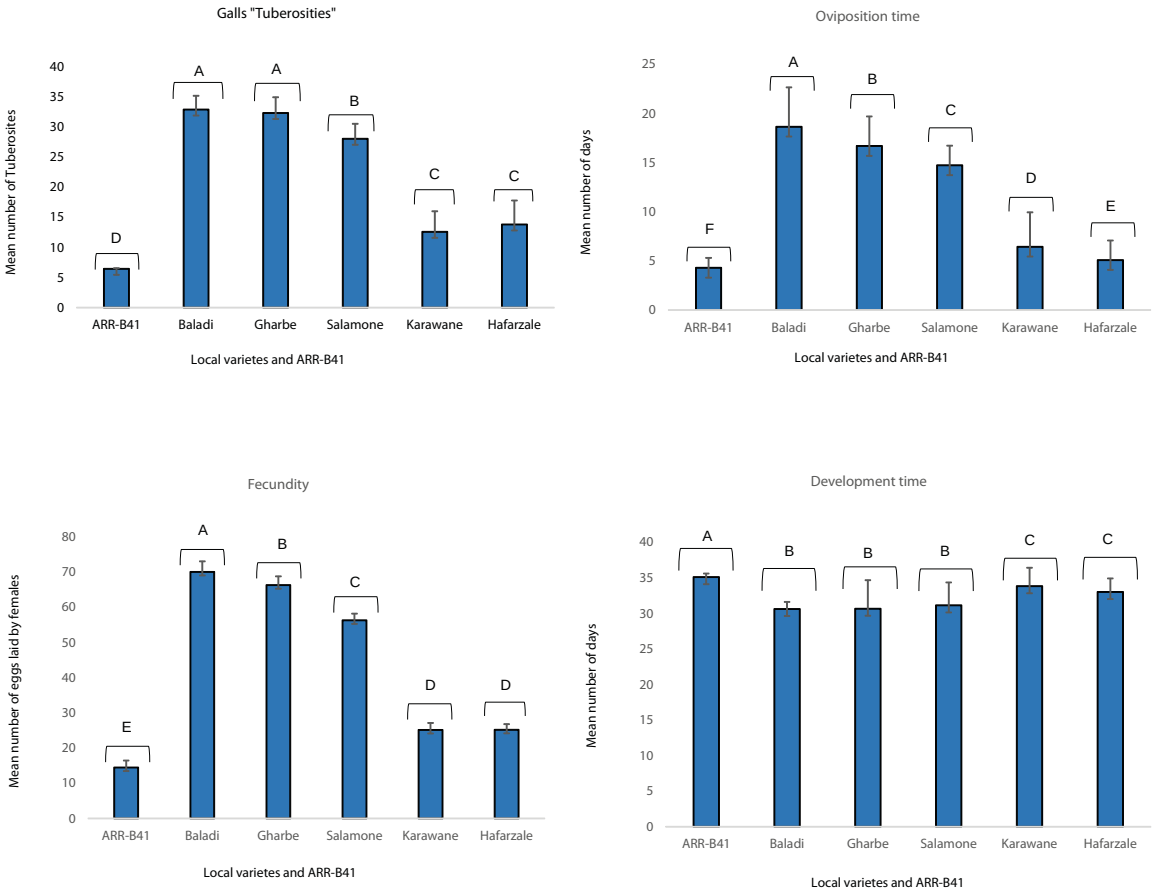


Figure 1. Biological parameters of phylloxera on local grapevine varieties in Syria and the phylloxera-resistant ARR-B41 rootstock. Different capital letters on columns indicate significantly different means ± standard deviation at $P < 0.05$ (Tukey HSD test).

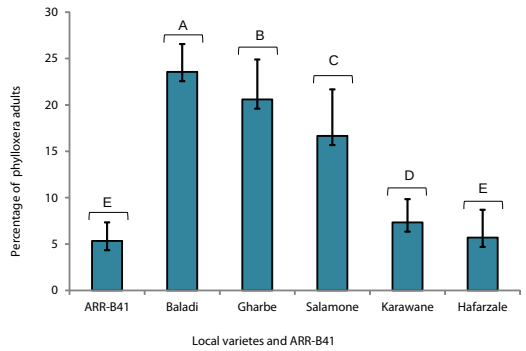


Figure 2. Survival percentages of phylloxera adults on local grapevine varieties in Syria and the phylloxera-resistant ARR-B41 rootstock. Different capital letters on columns indicate significantly different means ± standard deviation at $P < 0.05$ (Tukey HSD test).

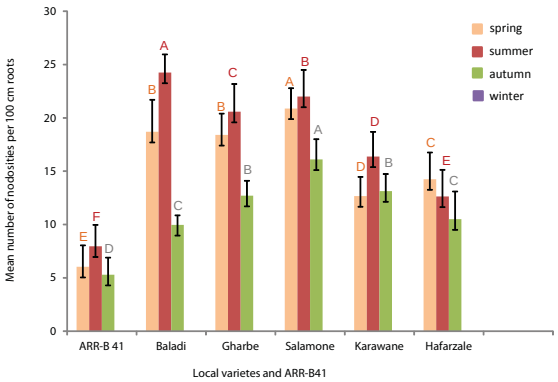


Figure 3. Number of galls "nodosities" on roots of local grapevine varieties in Syria and the phylloxera resistant ARR-B41 rootstock during the growing season. Different capital letters on columns of the same color indicate significantly different means ± standard deviation at $P < 0.05$ (Tukey HSD test).

season when the soil is cold. Grapevine is the only host plant for grape phylloxera, so its nutritional value and chemical composition changes during the season and consequently the composition of food sources may affect the life cycle of phylloxera. In addition, the plant-mediated life cycle stages may also be altered by the microbial root and soil community structure or symbiosis association (Huber *et al*., 2007; Vorwerk *et al*. 2007). Therefore, we believe that the formation of galls is affected by the growth time of roots (spring to summer) resulting in intensive propagation of phylloxera on new roots, and the development of phylloxera stages is related to the state of the root system (Balbiani, 1874; Cornu, 1878). The varieties Hafarzale and Karawane, which had fewer galls compared to the other local varieties, may be considered for use as rootstock vines resistant to phylloxera infestation and reproduction.

Conclusion

The assessment of sensitivity to grape phylloxera infestation of five native grape varieties in Syria in comparison to the resistant rootstock ARR-B41, based on biological parameters of the insect and gall formation capacity under the same conditions, suggests that Hafarzale and Karawane can be included in the list of resistant grape varieties against phylloxera.

The authors acknowledge Professor I. Othman, General Director of Syrian Atomic Energy Commission, and Dr N. Mirali, Head of the Molecular Biology & Biotechnology Department, for encouragement and supporting of present work. Thanks are extended to Dr H. Makee to initiation of the work for feedback and to Dr Mohamad Hawat for revising the manuscript.

Literature cited

Adam, A., Idris, I. and Ayyoubi, Z. 2013. In vitro *Pseudomonas putida* BTP1-induced systemic resistance in grapevine rootstocks against phylloxera (*Daktulosphaira vitifoliae*). *Advances in Horticultural Science*, 27(4): 137-142.

Adam, A., Idris, I., Asaad, A. and Houssian, K. 2021. Impact of *Bacillus subtilis* strains on survival and reproduction of grapevine phylloxera. *Hellenic Plant Protection Journal*, 14(1): 24-30.

Adam, A., Makee, H. and Idris, I. 2012. The influence of a non-pathogenic *Pseudomonas putida* strain BTP1 on reproduction and development of grape phylloxera. *Advances in Horticultural Science*, 26(2): 75-80.

Al-chaabi S., Abu-Fadelt, Arabi S., Farawati, F., Matrod, L., Akeel, S., Al-Zoubi, M., Abo-Ruckbieh, F. and Rahaal, A.F. 2012. Spread of Phylloxera pest on grapevine in Dara'a governorate, Syria, and its management. *Arab Journal of Plant Protection*, 30(1): 55-69.

Balbiani, M. 1874. Observations sur la reproduction du phylloxera de la vigne. *Académie des sciences (France),*1371-1384.

Balachowsky, A. and Mesnil, L. 1935. Les insects nuisibles aux plantes cultivées. *Paul Lechevalier, Paris, France*: 697.

Cornu, M. 1878. Etudes sur le *Phylloxera vastatrix*. *Impr. nationale. Paris*.

De benedictis J. A., and Granett J. 1993. Laboratory evaluation of grape roots as hosts of California grape phylloxera biotypes. *American Journal of Enology and Viticulture*, 44(3): 285-291.

Eissenstat, D.M., Bauerle, T.L., Comas, L.H., Lakso, A.N., Neilsen, D., Neilsen, G.H. and Smart, D.R. 2005. Seasonal patterns of root growth in relation to shoot phenology in grape and apple, *International Symposium on Mineral Nutrition of Fruit Plants*, 721: 21-26.

Fossen, M.A. 2005. Biological constraints affecting root damage on grapevines infested with grape phylloxera (*Daktulosphaira vitifoliae* (Fitch) [Hemiptera: Phylloxeridae]), University of California, Davis.

Granett, J., Timper, P., and Lider, L.A. 1985. Grape phylloxera (*Daktulosphaira vitifoliae*) (Homoptera: Phylloxeridae) biotypes in California. *Journal of Economic Entomology*, 78(6): 1463-1467.

Granett, J., Walker, A., De Benedictis, J., Fong G., Lin, H. and Weber, E. 1996. California grape phylloxera more variable than expected. *California Agriculture*, 50(4): 9-13.

Granett, J., Bisabri-Ershadi, B. and Carey, J. 1983. Life tables of phylloxera on resistant and susceptible grape rootstocks. *Entomologia Experimentalis et Applicata*, 34(1): 13-19.

Granett, J., Walker, M.A., Kocsis, L. and Omer, A.D. 2001. Biology and management of grape phylloxera. *Annual Review of Entomology*, 46(1): 387-412.

Hoffmann, M., Ruehl, E.H., Eisenbeis, G. and Huber, L. 2016. Grape root as habitat: overwintering

and population dynamics of grape phylloxera (*Daktulosphaira vitifoliae* Fitch) in temperate climate viticulture. *Australian Journal of Grape and Wine Research,* 22(2): 271-278.

Hoffmann, M., Ruehl, E.H., Huber, L., Eisenbeis, G. and Kirchmair, M. 2010. A scanner based approach to assess grape root infesting parasites in field. *International Phylloxera Symposium*, 904: 101-109.

Huber, L., Hoffmann, M., Rühl, E.H. and Kirchmair, M. 2007. Disease suppressiveness of vineyard soils infested with grape phylloxera. *International Phylloxera* Symposium, 816: 41-52.

Idris, I. and Arabi, M.I.E. 2014. The relationship between grape phylloxera and Fusarium root infection. *Advances in Horticultural Science*, 28 (1): 14-19.

Kellow, A.V., Sedgley, M. and Van Heeswijck, R. 2004. Interaction between Vitis vinifera and grape phylloxera: changes in root tissue during nodosity formation. *Annals of Botany,* 93(5): 581-590.

Korosi, G.A., Mee, P.T. and Powell, K.S. 2012. Influence of temperature and humidity on mortality of grapevine phylloxera *Daktulosphaira vitifoliae* clonal lineages: a scientific validation of a disinfestation procedure for viticultural machinery. *Australian Journal of Grape and Wine Research*, 18(1): 43-47.

Lawo, N.C., Weingart, G.J., Schuhmacher, R. and Forneck, A. 2011. The volatile metabolome of grapevine roots: first insights into the metabolic response upon phylloxera attack. *Plant physiology and biochemistry*, 49(9): 1059-1063.

Makee, H., Ammounha, H. and Idris, I. 2010. Development and reproduction of phylloxera on some local grape varieties in Syria. *Advances in Horticultural Science*, 1: 169-175.

Makee, H., Charbaji, T., Ayyoubi, Z., Idris, I. 2004. Evaluating resistance of some rootstocks to grape phylloxera with in vitro and excised root testing systems. *In Vitro Cellular and Developmental Biology*, 40(2): 225-229.

Makee, H., Charbaji, T., Idris, I. and Ayyoubi, Z. 2008. Effect of gamma irradiation on survival and reproduction of grape phylloxera *Daktulosphaira vitifoliae* (Fitch). *Advances in Horticultural Science*, 1: 182-186.

Omer, D.A., Granett, J. and Walker, M.A. 2002. Influence of plant growth stage on grape phylloxera (Homoptera: Phylloxeridae) populations. *Environmental Entomology*, 31(1): 120-126.

Omer, A.D., Granett J., Downie, D.A. and Walker, M.A. 1997. Population dynamics of grape phylloxera in California vineyards., *Vitis*, 36: 199-206.

Powell, K.S. 2012. A holistic approach to future management of grapevine phylloxera. - *Arthropod Management in Vineyards,* Springer, Dordrecht, pp. 219-251.

Triska, M.D., Powell, K.S., Collins, C., Pearce, I. and Renton, M. 2018. Accounting for spatially heterogeneous conditions in local-scale surveillance strategies: case study of the biosecurity insect pest, grape phylloxera (*Daktulosphaira vitifoliae* (Fitch)). *Pest Management Science*, 74(12): 2724-2737.

Vorwerk S., Martinez-Torres, D. and Forneck, A. 2007. Pantoea agglomerans-associated bacteria in grape phylloxera (*Daktulosphaira vitifoliae*, Fitch). *Agricultural and Forest Entomology*, 9 (1): 57-64.

Received: 28 September 2021; Accepted: 23 June 2022

Βιολογικές παράμετροι της φυλλοξήρας της αμπέλου, *Daktulosphaira vitifoliae*, σε τοπικές ποικιλίες αμπέλου στην κεντρική Συρία – Επιπτώσεις στην ευαισθησία των ποικιλιών

I. Idris, A. Asaad, K. Houssian και I. Kalefa

Περίληψη Μελετήθηκαν οι βιολογικές παράμετροι τοπικών στελεχών της φυλλοξήρας της αμπέλου καθώς και η προσβολή στις ποικιλίες αμπέλου της Συρίας, Baladi, Salamone, Karawane, Hafarzale, Gharbe και στο Αμερικανικό ανθεκτικό υποκείμενο ARR-B41 (*Vitis vinifera* x *Vitis berlandieri*). Η τεχνητή προσβολή στις ρίζες έδειξε σημαντικές διαφορές στον πληθυσμό της φυλλοξήρας μεταξύ της πλειονότητας των ελεγχόμενων ποικιλιών σε σύγκριση με το υποκείμενο ARR-B41. Οι ποικιλίες Hafarzale και Karawane είχαν τον χαμηλότερο πληθυσμό φυλλοξήρας, με επίπεδο αντοχής παρόμοιο με αυτό του υποκειμένου ARR-B41. Επιπλέον, οι ποικιλίες Salamone και Gharbe φαίνεται να ευνοούν περισσότερο

την αναπαραγωγή της φυλλοξήρας. Το πείραμα στον αγρό έδειξε σημαντικές διαφορές μεταξύ των τοπικών ποικιλιών και του ARR-B41 όσον αφορά τον μέσο αριθμό όγκων. Συνολικά, οι ποικιλίες Hafarzale και Karawane έτειναν να σχηματίζουν σημαντικά λιγότερους όγκους από τις άλλες ποικιλίες.

Hellenic Plant Protection Journal **15:** 80-86, 2022

Hellenic Plant Protection Journal
Volume 15, 2022

Contents

A. Majeed, S. Siyar and S. Sami
Late blight of potato: From the great Irish potato famine to the genomic era – An overview 1-9

Gh.A. Amirkyaei, S. Mousanejad, N. Safaie and S.A. Khodaparast
Temporal development of stem rot caused by *Athelia rolfsii* in peanut fields in Iran 10-20

F. Khorrami, A. Soleymanzade, H. Batmani and Y. Ghosta
Entomopathogenic fungus, *Metarhizium anisopliae* anchored onto MCM-41 silica nanomaterial: A novel and effective insecticide against potato tuber moth in stored potatoes 21-29

F. Elhouiti, K.H. Benabed, D. Tahri, M. Ouinten and M. Yousfi
Antioxidant and antifungal activities of essential oils from Algerian spontaneous plants against five strains of *Fusarium* spp. 30-39

F. Malhat, Ch. Anagnostopoulos, S. El-Sayed and S. Abdelsalam Shokr
Dissipation of spiroxamine residues in open field cucumber and dietary risk assessment 40-48

S. Palavouzis, A. Triantafyllopoulou, A.K. Tzima and E.J. Paplomatas
Differentiation between *Botryosphaeria dothidea* and *Neofusicoccum* spp. based on a single nucleotide polymorphism in the ITS region 49-56

M.M. Abdel-Kader, M.S.A. Khalil and N.S. El-Mougy
Efficacy of fungicide alternatives against late wilt disease of maize and their influence on plant morphogenesis and yield character 57-71

E. Kakouris, C. Makris and J. Demetriou
First record of *Sphenophorus placidus vestitus* (Coleoptera: Curculionidae: Dryophthorinae) in Cyprus 72-75

J. Demetriou, G. Kakiopoulos and A.F. Martinou
First record of the Nearctic *Ozognathus cornutus* (LeConte, 1859) (Coleoptera: Ptinidae: Anobiinae) in Cyprus 76-79

I. Idris, A. Asaad, K. Houssian and I. Kalefa
Biological parameters of grape phylloxera, *Daktulosphaira vitifoliae*, on local grapevine varieties in central Syria – Implications on their susceptibility 80-86

Hellenic Plant Protection Journal
Τόμος 15, 2022

Περιεχόμενα

A. Majeed, S. Siyar και S. Sami
Περονόσπορος της πατάτας: Από τον Ιρλανδικό λιμό της πατάτας στη γονιδιωματική εποχή - Μια ανασκόπηση 1-9

Gh.A. Amirkyaei, S. Mousanejad, N. Safaie και S.A. Khodaparast
Ανάπτυξη σήψης στελέχους *Athelia rolfsii* σε καλλιέργεια αραχίδας στο Ιράν 10-20

F. Khorrami, A. Soleymanzade, H. Batmani και Y. Ghosta
Σκεύασμα του εντομοπαθογόνου μύκητα *Metarhizium anisopliae* με νανοϋλικό πυριτίου MCM-41: Ένα νέο και αποτελεσματικό εντομοκτόνο κατά της φθοριμαίας της πατάτας σε αποθηκευμένους κονδύλους 21-29

F. Elhouiti, K.H. Benabed, D. Tahri, M. Ouinten και M. Yousfi
Αντιοξειδωτική και αντιμυκητιακή δράση αιθερίων ελαίων από αυτοφυή φυτά της Αλγερίας έναντι πέντε στελεχών *Fusarium* spp. 30-39

F. Malhat, Ch. Anagnostopoulos, S. El-Sayed και S. Abdelsalam Shokr
Μελέτη διασποράς υπολειμμάτων σπιροξαμίνης σε υπαίθρια καλλιέργεια αγγουριού και εκτίμηση διατροφικής επικινδυνότητας 40-48

Σ. Παλαβούζης, Α. Τριανταφυλλοπούλου, Α.Κ. Τζίμα και Ε.Ι. Παπλωματάς
Διαφοροποίηση μεταξύ B*otryosphaeria dothidea* και ειδών του γένους *Neofusicoccum* με βάση πολυμορφισμό ενός νουκλεοτιδίου στην περιοχή ITS 49-56

M.M. Abdel-Kader, M.S.A. Khalil και N.S. El-Mougy
Αποτελεσματικότητα εναλλακτικών μυκητοκτόνων κατά του φυτοπαθογόνου μύκητα *Magnaporthiopsis maydis* στον αραβόσιτο και επίδραση στη μορφογένεση των φυτών και σε χαρακτηριστικά της απόδοσης 57-71

Η. Κακούρης, Χ. Μακρής και Ι. Δημητρίου
Πρώτη καταγραφή του *Sphenophorus placidus vestitus* (Coleoptera: Curculionidae: Dryophthorinae) στην Κύπρο 72-75

Ι. Δημητρίου, Γ. Κακιόπουλος και Α.Φ. Μαρτίνου
Πρώτη καταγραφή του Βορειοαμερικανικού σκαθαριού *Ozognathus cornutus* (LeConte, 1859) (Coleoptera: Ptinidae: Anobiinae) στην Κύπρο 76-79

I. Idris, A. Asaad, K. Houssian και I. Kalefa
Βιολογικές παράμετροι της φυλλοξήρας της αμπέλου, *Daktulosphaira vitifoliae*, σε τοπικές ποικιλίες αμπέλου στην κεντρική Συρία – Επιπτώσεις στην ευαισθησία των ποικιλιών 80-86

Τόμος 15, Τεύχος 2, Ιούλιος 2022
ISSN 1791-3691 (Print)
ISSN 2732-656X (OnLine)

Περιεχόμενα

Σ. Παλαβούζης, Α. Τριανταφυλλοπούλου, Α.Κ. Τζίμα
και Ε.Ι. Παπλωματάς
Διαφοροποίηση μεταξύ B*otryosphaeria dothidea* και ειδών του γένους *Neofusicoccum* με βάση πολυμορφισμό ενός νουκλεοτιδίου στην περιοχή ITS 49-56

M.M. Abdel-Kader, M.S.A. Khalil και N.S. El-Mougy
Αποτελεσματικότητα εναλλακτικών μυκητοκτόνων κατά του φυτοπαθογόνου μύκητα *Magnaporthiopsis maydis* στον αραβόσιτο και επίδραση στη μορφογένεση των φυτών και σε χαρακτηριστικά της απόδοσης 57-71

Η. Κακούρης, Χ. Μακρής και Ι. Δημητρίου
Πρώτη καταγραφή του *Sphenophorus placidus vestitus* (Coleoptera: Curculionidae: Dryophthorinae) στην Κύπρο 72-75

Ι. Δημητρίου, Γ. Κακιόπουλος και Α.Φ. Μαρτίνου
Πρώτη καταγραφή του Βορειοαμερικανικού σκαθαριού *Ozognathus cornutus* (LeConte, 1859) (Coleoptera: Ptinidae: Anobiinae) στην Κύπρο 76-79

I. Idris, A. Asaad, K. Houssian και I. Kalefa
Βιολογικές παράμετροι της φυλλοξήρας της αμπέλου, *Daktulosphaira vitifoliae*, σε τοπικές ποικιλίες αμπέλου στην κεντρική Συρία – Επιπτώσεις στην ευαισθησία των ποικιλιών 80-86

Περιεχόμενα Τόμου 15 (2022)

Volume 15, Issue 2, July 2022
ISSN 1791-3691 (Print)
ISSN 2732-656X (OnLine)

Hellenic Plant Protection Journal

Contents

S. Palavouzis, A. Triantafyllopoulou, A.K. Tzima and E.J. Paplomatas
Differentiation between *Botryosphaeria dothidea* and *Neofusicoccum* spp. based on a single nucleotide polymorphism in the ITS region 49-56

M.M. Abdel-Kader, M.S.A. Khalil and N.S. El-Mougy
Efficacy of fungicide alternatives against late wilt disease of maize and their influence on plant morphogenesis and yield characters 57-71

E. Kakouris, C. Makris and J. Demetriou
First record of *Sphenophorus placidus vestitus* (Coleoptera: Curculionidae: Dryophthorinae) in Cyprus 72-75

J. Demetriou, G. Kakiopoulos and A.F. Martinou
First record of the Nearctic *Ozognathus cornutus* (LeConte, 1859) (Coleoptera: Ptinidae: Anobiinae) in Cyprus 76-79

I. Idris, A. Asaad, K. Houssian and I. Kalefa
Biological parameters of grape phylloxera, *Daktulosphaira vitifoliae*, on local grapevine varieties in central Syria – Implications on their susceptibility 80-86

Contents of Volume 15 (2022)

www.hppj.gr