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MYCOLOGY

ENTOMOLOGY

Photo of the cover page: *Chrysoperla lucasina* (Courtesy *Wejdène Karouia*)

Guest Editorial

The Pesticides and the Alternative Control Methods

Nowadays, the usefulness of pesticides no longer needs to be demonstrated. Without them, half of the harvest would be lost, the sanitary quality of agricultural products would be compromised and we would not be able to meet global food demand. But their irrational and abusive use is likely to present, without doubt, a major risk of toxicity for man and his environment.

Despite the regulations relating to the use of pesticides, the conscience of both producers and the majority of users and consumers, the anarchic and abusive use remains the rule in most cases. Indeed, for a better yield, many farmers generally resort firstly to the use of pesticides. At home and to live in an atmosphere with the slightest source of nuisance from flies, mosquitoes, cockroaches and other animals, many people rely on the use of pesticide products, sometimes even that for agricultural use.

Faced with this unanimous observation, debating the problem of the use of pesticides has already become an absolute priority for research and the implementation of alternative methods. And it is in this context that in Tunisia, within the framework of the project "Support for official control services for Animal and Plant Products (ASCO)" financed by the European Union (EU) and implemented by the United Nations Organization for Food and Agriculture (FAO), the General Directorate of Plant Health and Control of Agricultural Inputs (DGSVCIA) organized a national symposium which took place on April 25 and 26, 2023, at Hammamet, Tunisia, on the theme "Pesticides for agriculture and alternative control methods".

It becomes urgent to carry out a collective expertise with all the national partners, taking stock of the available knowledge regarding the conditions of use of pesticides in agriculture, the means of reducing their use and to limit their environmental and health impacts. This is to be achieved by creating multi-stakeholder plant protection synergies, by promoting scientific research and innovation in the field of sustainable alternatives to pesticides, by promoting new alternatives to pesticides, and finally by encouraging meaningful action for the use of biological control agents. The major goal id to debate the non-rational use of pesticides and how to increase awareness of the risks they can generate for the environment safety and the human health.

It is clear that despite the mass of results of research and experimentation that are of practical significance, few techniques are adopted in practice by operators. Thus, the implementation of a strategy for the valorization of research achievements based on IPM is becoming more than necessary.

Aware of the problems posed by the use of pesticide products, sometimes

sold on the parallel market, both in agricultural production and for home use, it is time to combine efforts to raise awareness among users and promote by all means the alternatives methods to pesticides.

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Review on Resistance of Lentil Varieties to the Blight Disease Caused by *Ascochyta lentis*, with Emphasis on Genetic Aspects

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ABSTRACT

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Lentil, *Lens culinaris* (syn: *Lens esculenta*), is one of the most important annual legumes from the Fabaceae family, which is widely cultivated throughout Asia, Europe, Northern America, Australia, and North Africa. Lentil seeds are mostly used in food industries to produce soups and its fodder is used as livestock feed. Ascochyta blight of lentil (ABL), which is caused by the pathogenic fungus *Ascochyta lentis* (teleomorph *Didymella lentis*), is one of the important diseases of this crop worldwide which causes serious damage to it. Resistance of different lentil varieties to this disease is variable. For this purpose, different studies have been performed on resistance in cultivated and wild varieties against this disease; some of them have focused on ecological aspects, others on genetics, and few on pathogen virulence. In this review, we have outlined the advantages of each background along with latest research. The present review, due to its unique characteristics which has been done to our knowledge for the first time, can be considered as valuable regarding the management of this dangerous disease in lentil.

Key words: *Ascochyta lentis*, blight, lentil, pathogen, resistance, variety

INTRODUCTION

Botany of lentil.

Lentil, *Lens culinaris* (syn: *Lens esculenta*), is one of the annual edible legumes that are famous for its lentil-shaped seed (Figs. 1, 2). Height of the mature plant when its seeds grow as pods is about 40 cm (Yadav et al. 2007). Its stem is angular with many hairs. The leaves are compound, alternate, and have up to 7 pairs of oval leaflets without petiole. The plant has flowers with different colors (white, pink, purple, etc.) that are gathered in groups of up to 4 on a long stem. Its fruits are pods with 1.5 to 2 cm long, inside which there are two expanded seeds about 0.5 cm wide. Lentil is cultivated in many parts of the world; although, wild species are also found in nature (Bayaa et al. 1994). In 2020, global production of lentil reached 6.5 million tons where Canada is the top with 45%, followed by India with 18% (Erskine 2009).

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Lentil diseases.

The fungal diseases on lentil are the most important biological limitation for its productivity in different parts of the world. Among them, two phytopathogens known as *Ascochyta lentis* (teleomorph *Didymella lentis*) that causes Ascochyta blight of lentil (ABL) (Fig. 3) and

Fusarium oxysporum f. sp. *lentil* that causes Fusarium wilt, can generate severe damage in most regions of the world. The other fungal diseases including gray mold (*Botrytis cinerea, B. fabae*), rust (*Uromyces viciae-fabae*), Stemphylium blight (*Stemphylium botryosum*), and Anthracnose (*Colletotrichum truncatum*) also occur in some seasons with special environmental conditions. Furthermore, lentil can be infected by many of plant viruses; but their pathogenicity is less than the pathogenic fungi. Lentil yellow diseases caused by Bean Leaf Roll Virus (BLRV), Beet Western Yellow Virus (BWYV), and Clover Red Leaf Virus (SCRLV) are prevalent worldwide. The other important virus diseases in lentil include as Bean Yellow Mosaic Virus (BYMV), Pea Seed Mosaic Virus (PSbMV), Cucumber Mosaic Virus (CMV), Alfalfa Mosaic Virus (AMV), and Broad Bean Spot Virus (BBSV) (Taylor et al. 2007). The class Dothideomycetes includes a variable group of fungal species that has a wide range of pathogens while many of them cause disease on important plants (Ohm et al. 2012). *A. lentis* is an infectious fungus from the class of Dothideomycetes. It causes severe blight on aerial parts in lentil plants (Fig. 4). The damage caused by the disease is directly related to temperature and relative humidity (RH) (Sambasivam et al. 2017). In high humidity, disease damage is very severe, but in high temperatures, the pathogen activity is low or stops. Due to the limited area of lentil cultivation in Iran, there is very limited information about the pathogenicity mechanism of the disease on its host, which needs to be investigated especially in areas with high infection rate (Henares et al. 2023b). Integrated disease management (IDM) includes use of resistant varieties, good agricultural practices, and application of fungicides which can reduce severity of lentil diseases (Taylor et al. 2007).

Fig. 1. Mature plant of lentil (*Lens culinaris*) (Yadav et al. 2007).

Fig. 2. Seeds of some lentil varieties (Yadav et al. 2007).

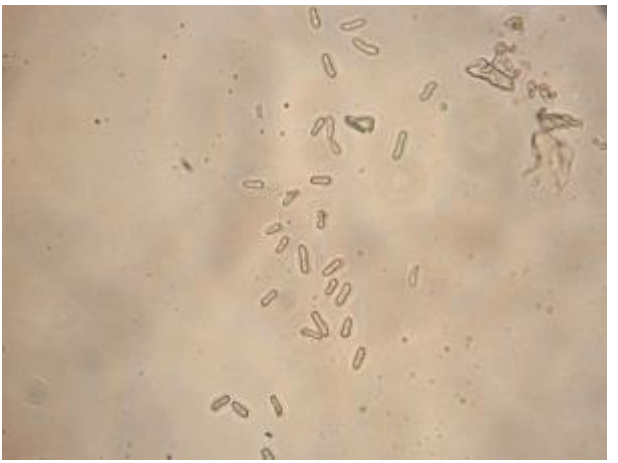

Fig. 3. Conidia of the pathogenic fungus, *Ascochyta lentis* (Henares et al. 2023b).

Fig. 4. Ascochyta blight disease on aerial parts of lentil (Henares et al. 2023b).

Plant variety and resistance mechanisms.

Variety refers to plant species that naturally grow in the environment. Unlike a cultivated plant, a variety does not require human intervention to grow and reproduce. The seeds of a particular variety often grow according to type meaning that their offspring retain unique characteristics from the mother plant. In the classification system of plants, a variety is a taxon which is ranked as subspecies (Clausen 1941). Different plants have developed a wide defense system against different pathogens. When pathogens cross mechanical barriers in the host, plant receptors start signaling that induces defense response genes. The immune system in plants has abilities to recognize foreign molecules, signal transmission, and defense response through pathways which include many genes and their products. Pathogens actively try to escape from these response pathways or interfere in them or choose a multicomponent immune system for pathogenicity. Molecular advances have increased scientists' understanding on plant immunity, resulting its suitable potential for application in agricultural systems (Rodda et al. 2017; Andersen et al. 2018).

Resistance in plants is divided into two main categories; ecological and genetical (Ahmad et al. 1997; Antonio and Thomsen 2004). Ecological resistance refers to the role of living and non-living factors which limit growth of a pathogen. There are interests in applying this method for management of invasive species; but, practical problems in restoring resistance prevent it being investigated on large scales. Although temporal and spatial fluctuations in resistance are common, this concept provides a valuable foundation for a more sustainable approach to long-term management. This aim should be achieved through identifying processes involved in natural communities (Antonio and Thomsen 2004). Genetic resistance and tolerance have been highly importance in plant research since the past decades. In recent years, providing genetic solutions for diseases and pests has become

increasingly important following loss of plant protection and disease evolution. Genetic resistance is one of the important principles in integrated pest management (IPM) programs while it depends on plant genetic features. The resistance originated by resistance gene (R) which recognizes the product of an insensitive gene (Avr) in pathogens and starts plant defense. Mutations in the gene Avr of pathogens have allowed it to escape from detection and cause disease in plants that are apparently resistant. When an R gene is widely developed, there is a strong selection pressure in pathogen strains that can overcome the plant resistance, referred to as resistance breakdown. When a variety is lost, it is due to change in frequency of Avr alleles in pathogens and does not depend on changes in plants' genetics (Samuel and Dines 2023).

Research aims.

ABL is one of the most important diseases in Iran when comprehensive investigation on it was felt. In this review, plant immune systems related to pathogen resistance as well as control mechanisms are investigated. Also, main components of the immune system in plants and future directions on interactions between plant and pathogen will be investigated.

Methods.

To conduct the present review, after determining the topic, a comprehensive search was conducted in three main databases including Google Scholar, Scopus, and Science Direct during January and February 2023. The search strategy was designed based on three main criteria's including "Ascochyta", "Lentil Blight", and "Resistant Variety", and the search time was not limited to a specific period. Finally, the articles related to lentils were extracted and after screening, 20 articles were selected for use in the present review. In these articles, the ecological aspect such as role of nutrition in occurrence of ABL, pathogenicity of the fungus, status of wild varieties, and breeding works of the local varieties for resistance against ABL were discussed. Limited studies have been conducted on resistance of different lentil varieties to ABL. In the following, we briefly review the most important results of each study.

Results of latest research.

Plant nutrition and disease outbreak. There are variations in content of micro- and macro-elements from resistant and sensitive lines. Based on this, plant nutrition has an important role in resistance of plants to ABL and should be taken in future researches. In this direction, Sahi et al. (2007) studied the effects of nitrogen, phosphorus, and potassium on lentil resistance to ABL where they reported that nitrogen and phosphorus in non-infected susceptible plants had higher content than the resistant. Moreover, potassium in resistant lines was more than sensitive. After pathogen infection, nitrogen and potassium increased in both resistant and sensitive lines meaning that phosphorus increased in the inoculated sensitive group and decreased in the resistant lines. In general, plants contained lower nitrogen and phosphorus showed resistance to ABL, while higher nitrogen and phosphorus made it more susceptible. in another study of Sahi et al. (2010), the effect of ABL on mineral elements in the sensitive and resistant lentil plants with inoculation by pathogen were investigated when content of sodium, calcium, zinc, copper, and iron increased steadily in both groups (sensitive and resistant). In another aspect, magnesium increased in the sensitive group and decreased in the resistant one.

Virulence of pathogen. There are biochemical differences in interactions of post-inoculation by ABL with slow response in susceptible genotypes. Rapid releases of reactive oxygen species (ROS) and hypersensitive reactions (HR) have been determined as first defense lines in point penetration from resistant lentil genotypes. Also, pathogen intensity in all genotypes was seen. In this context, histopathological studies were needed for accurate understanding of differences between isolate-host combination and improving knowledge on resistance mechanisms in lentil against ABL. Sambasivam et al. (2017) studied variation in disease, pathogenicity, and response from the lentil and found that there were six pathogenicity patterns identified for ABL when pathotype I (AL4) being the most virulent and causing disease in all genotypes except ILL7537 and pathotype VI (Kewell). In another study by Khorramdelzad et al. (2018), transcriptomic profile of lentil during 24 h after infection by pathogen was studied. They concluded that for discovering genetic characteristics in lentil resistance to ABL, expressed genes appear for a long time. In early infection, resistant and susceptible genotypes of lentil including ILL7537 and ILL6002 were analyzed at 2, 6, and 24 h by using high-throughput RNA sequencing. Genotyping and time-dependent expression analysis identified the genes that play key roles in defense response, recognition of fungal factors, and signaling. Structural and biochemical responses, transcriptional regulators, hypersensitivity reaction, death of cell, systemic resistance, and resistant genotype showed earlier and faster detection of response signals to pathogens with high expression levels from the genes related to plant defense against it.

Sahi et al. (2018) investigated different levels of lentil infection to ABL on growth and yield of lentil. they concluded that height of plant was minimum if there are 5 spores/ml in all four lines. Also, the number of leaflets, plant pods, seeds per pod, and weight of 100 seeds decreased with increasing concentration of spore from 10^3 spores/ml to 5×10^4 spores/ml. Finally, frequency of lesions/pod showed positive correlation with spore concentration. Henares et al. (2023a) investigated profiles of virulence and genome-wide association from ABL in Australia and concluded that loci AlAvr1 on chromosome 3 was severely related with PBA Hurricane XT in ABL responses from Indianhead and Nipper, but one genomic region on chromosome 11 was associated to Nipper. Their results confirmed the role of identified AlAvr1 locus for field collected isolates with release period and widespread adoption of PBA Hurricane XT. PCR assay was developed to differentiate AlAvr1-1 and AlAvr1-2 for predicting PBA Hurricane XT as virulence and pathotype designation in a diversity panel. High numbers of PBA Hurricane XT-virulent pathotype 2 isolates across the time indicates strong selection for isolates including AlAvr1-2 allele. Furthermore, one region of the ABL genome contributes to pathogen-host interaction in lentil.

Resistance in wild varieties. Resistance to ABL of native varieties was higher than breeds, which should be considered in future. Bayaa et al. (1994) studied response of wild lentil varieties to ABL in Syria while concluding that 24 of 86 from *L. culinaris* subsp. *orientalis*, 12 of 35 from *L. culinaris* subsp. *odemensis*, 3 of 35 from *L. nigricans* subsp. *nigricans*, 36 of 89 from *L. nigricans* subsp. *ervoides*, and 3 from *Vicia montbretii* had similar resistance. In their study, 64% of identified resistant sources were identified from Syria and southeast of Turkey. Moreover,

prevalence of ABL was not related to height of plant and average of annual rainfall. Furthermore, there was a significant correlation between plant leaf width and ABL reaction, but it was not related to the number of morphological features. Ahmad et al. (1997) studied genetic features from resistance sources of ABL in the genus *Lens* and concluded that resistance can be controlled by two pairs of dominant genes in two wild species including *L. ervoides* and *L. odemensi.* For occurrence of resistance, these two genes must be dominant in homozygous or heterozygous forms. Whenever a pair of these genes is homozygous, they cover the effect of the dominant gene pair that causes sensitivity to ABL. According to this, wild species have been considered to improve genetic resistance of cultivated lentil against ABL. In the end, it is suggested that interspecies hybrids should be included in lentil breeding programs.

Identification of resistance genes, characterization of host-pathogen system, and molecular markers associated with resistant genes are considered as main agents. Bedasa (2021) evaluated genotypes from lentil for resistance to ABL and reported that 7 genotypes being resistant, 15 were moderately resistant, and the others being susceptible or highly susceptible which included 10 and 30 lines at Alem Tena. In another aspect, 1 was resistant, 8 moderately resistant, 12 susceptible, and 41 genotypes were highly susceptible at Minjar. Promising genotypes are considered as parental material in the breeding process.

Breeding in varieties and masses. Determining the resistance against ABL is not an easy task as mentioned in various researches. Rubiales et al. (2018) reported that resistant varieties are an effective and consistent method of control of this disease. Breeding for resistance to ABL has been considered as priority for breeders around the world while a number of resistant sources have been identified and widely exploited. Combination of genomic resources, efficient tools on molecular genetics, and high-resolution phenotyping tools can improve selection efficiency of resistance and accelerate diversity development. Disease resistance in lentil is mainly controlled by major genes. However, minor genes also play an important role. Ye et al. (2002) studied breeding for resistance against ABL and identified 6 pathotypes that many varieties have shown relative resistance against them. For this reason, breeding programs are based on resistant cross-breeding with high-yield varieties and multilocus genetic testing. Gene pyramiding, exploration of slow blight, partial resistance, and use of genes in wild relatives are effective methods in the future. Furthermore, identification of resistance genes, appropriate description of the host-pathogen system, and identification of molecular markers related to genes can be considered as key fields in this direction.

Resistant and sensitive lentil varieties were not only different in expressed gene, but also differ in time and expression levels, which can be effective in response to ABL from resistant varieties. Mustafa et al. (2009) used cDNA microarray for investigating lentil resistance to ABL inoculation. Accordingly, they determined highly resistant (ILL7537) and highly sensitive (ILL6002) varieties. Moreover, 90 and 95 genes were expressed in ILL7537 and ILL6002, respectively. The expression from two varieties showed significant difference in type and time of expressed genes in response to ABL. The resistant variety showed early regulation of PR4 with 10 proteins and the other genes

related to resistance. Finally, sensitive genotype showed fast decreasing of defense-related genes. Lentil hybridization programs are developed against ABL using resistant varieties with high yield potential. Based on the linear relationship between screening methods, it was suggested to screen the lentil germplasm at seedling in the greenhouse and then resistant and tolerant accessions at flowering stage or mature plants under field conditions. Performing these methods can save time and work. In another study, Iqbal et al. (2010) identified ABL resistant sources from local lentil germplasm and concluded that among all populations, 5473, 5490, 5499, 5569, 5548, 5547, 5545, and 5570 can resist to the disease and 5570 should be re-evaluated for resistance.

The flanking markers can be useful tools for genes pyramiding involved in resistant crops. According to this, Gupta et al. (2012) investigated integration of EST-SSR markers of *Medicago truncatula* to linkage map of lentil and identification of Quantitative Trait Locus (QTL) conferring resistance to ABL at seedling and pod stages. They reported 196 markers including new 15M and truncatula EST-SSR/SSR which was mapped by using of 94 F_5 recombinant population inbred lines produced from a cross between variety Northfield (ILL5588) and variety Digger (ILL5722) when clustered into 11 linkage groups (LG) covering 1156.4 cM. Subsequently, size and effects of QTL caused ABL resistance at seedling and pod/maturity stages. Three QTL were detected for seedling resistance on LG1 and LG9 and further 3 for pod/maturity resistance on LG1, LG4, and LG5. Totally, these accounted 34% and 61% of total phenotypic variation and demonstrated that resistance at different growth stages when potentially conditioned by different genomic regions. Influence of dominant varieties in cultivation with loss of effective resistance was discussed by researchers. In this context, Davidson et al. (2016) studied ABL in southern Australia and showed that a small percentage of isolates collected before commercial release of variety Nipper were able to infect it, indicating natural pathogenic variation. It has been selected in response to high cultivation intensity of the variety Nipper. Spore release studies in infected lentil collected from commercial crops resulted high infection percentages in previously resistant varieties including Nipper and Northfield. Additionally, lower than 10% occurred on resistant differentials ILL7537 and Indianhead. Also, variation of pathogenicity in seasonal populations was not affected by varieties which were indicated variation in natural pathogenicity.

Sari et al. (2018) investigated responses of lentil genotypes carrying non-allelic ABL resistance genes to infection by the pathogen and found that differences were reflected in downstream expression of response such as pathogenesis-related proteins (PR proteins) and genes related with cell death induction and reinforcement. A significant correlation between genes selection levels based on quantitative real-time PCR and their expression estimated RNA-seq demonstrated technical and analytical accuracy of RNA-seq for identification of expressed genes in genotypes. Presence of resistance mechanisms in 964a-46 and CDC robin indicates their role in pyramiding genes caused durable resistance to ABL. Dadu et al. (2019) investigated *A. lentis* resistance in global collection by using a germplasm and reported that significant resistance variation was detected by using controlled climate bioassay with highly virulent in Australian isolate known as FT13037. Genotype IG 207 expressed the lowest

area of symptomatic tissue and 12 genotypes showed moderate resistance. Also, IG 207 recorded lowest disease against 4 aggressive isolates and performed better than used resistance sources, ILL7537 and Indianhead. In addition, delayed pre-penetration of isolate FT13038 on IG207 into leaflets indicated discovery through germplasm technique of novel ABL resistance source. In various surveys, screening methods with new breeding technologies have been investigated on decreasing damage of disease and progress in making lentil species more flexible against its spread under climatic changes. Rodda et al. (2017) investigated molecular breeding for ABL resistance and concluded that a range of genetic linkage maps were available on DNA-based markers and QTL for resistance to the pathogen. Molecular markers associated with QTLs can potentially be applied for pyramiding of resistance genes. Finally, Roy et al. (2022) studied different technologies to improve resistance and reported that extensive hybridization and seed rescue techniques helped to introduce resistance features into cultivated lentil. In addition, induced mutagenesis has contributed to ABL diversity tolerance with several disease-resistant mutants. However, advances in molecular marker and genomic technologies have helped to develop disease-resistant and climate-tolerant lentil varieties with high efficiency.

DISCUSSION

Lentil is a valuable crop all over the world with high nutritional value. ABL is one of the common diseases on this product, which causes quantitative and qualitative damage to it (Erskine 2009). There are very few studies on lentil resistance to ABL. In our search, we found less than 20 articles about it with emphasis on pathogenicity and characteristics of resistant lentil varieties. Nutrition in plants has a very important role in resistance to disease and should be seriously considered. About lentil varieties, macro-elements can affect resistance or sensitivity to ABL. Nitrogen and phosphorus increase in non-infected plants had high content compared to sensitive group, while potassium is high from resistant plants. After infection with ABL, nitrogen and potassium increases in resistant plants means that phosphorus increased in inoculated sensitive plants and decreased in resistant plants. In general, plants containing less nitrogen and phosphorus with high potassium were resistant to ABL, while higher nitrogen and phosphorus made it more sensitive. For this reason, it is important to consider balance in plant nutrition with use of elements that make resistant in lentil to ABL (Sahi et al. 2007). Micro elements are also effective. When the plant was inoculated with pathogen, the content of sodium, calcium, zinc, copper, and iron in both groups (sensitive and resistant plants) increased when magnesium increased in the sensitive plant and decreased in the resistant. For this reason, paying attention to nutritional needs with a resistance approach is a new aspect of these studies (Sahi et al. 2010).

About the pathogen virulence, there are differences in interactions caused by development of disease in susceptible genotypes. Two main mechanisms including reactive oxygen species production and hypersensitive reaction are the most important factors in lentil genotypes. Furthermore, histopathological studies are needed to understand isolate-host composition differences and improve the knowledge on resistance mechanisms in lentil against the pathogen (Sambasivam et al. 2017). In general, six pathogenic patterns have been identified for ABL, of which pathotype I (AL4) is the

most destructive on all lentil genotypes. Discovery of genetic characteristics from pathogen-resistant lentil varieties showed that the expressed genes appear for a long time. Based on studies, resistant and sensitive genotypes of lentil including ILL7537 and ILL6002 have genes with key roles in defense response, detection of fungal agents, and fast signaling. Biochemical responses, regulator of transcription, reaction of hypersensitivity, death of cell, systemic resistance, and resistant genotype are also found to plant defense against ABL (Khorramdelzad et al. 2018). About the pathogenicity, plant height, number of leaflets, pods, and seeds per pod with weight of 100 seeds decreased with increasing spore concentration, but frequency of lesions/pod showed a positive correlation with it (Sahi et al. 2018). In these studies, a region of the pathogen genome may contribute to their interaction in lentil (Henares et al. 2023b).

The response of wild lentil varieties to ABL in Syria and Turkey showed that most resistant sources with high diversity are available which can be applied for identifying pathogen-resistant interactions. There was not high annual rainfall in these countries and significant correlation was observed between plant leaf width and ABL reaction (Bayaa et al. 1994). Resistance in lentil can be controlled by two pairs of genes while these two genes must be dominant in each form (homozygous or heterozygous). When a pair of them is homozygous, it covers the effect of the dominant pair and makes the lentil sensitive to ABL. Based on this, wild species are considered to improve genetic resistance of cultivated lentil against ABL. Promising genotypes are considered as a parent source in the breeding process. In African countries such Ethiopia, there are various opportunities to improve lentil productivity, including diverse agro-ecologies, legumes, and grains that need to pay attention more in the future (Bedasa 2021). Resistant varieties are an effective method known for control of ABL, but determining the lentil resistance to it is not an easy task. Breeding for ABL resistance has been considered as a priority for breeders worldwide while a number of resistant sources have been widely exploited. Combination of genomic resources, efficient molecular genetics tools, and high-resolution phenotyping tools can improve resistance efficiency and enhance diversity development. Disease resistance in lentil is mainly controlled by major genes. However, minor genes also play an important role (Rubiales et al. 2018). Breeding has been studied for resistance to ABL and six pathotypes have been identified. For this reason, breeding programs based on resistant crosses, high-yielding varieties, and multilocus genetic testing are recommended. Gene pyramiding, slow development of ABL, partial resistance, and use of genes in wild relatives can be effective methods in the near future. In addition, identification of resistance genes, proper characterization of the host-pathogen system, and identification of molecular markers associated with genes can be considered as key agents for this purpose (Ye et al. 2002).

The effect of dominant varieties with loss of effective resistance has been discussed by researchers. Spore propagation studies in infected lentil collected from commercial crops showed high infection percentage in resistant varieties. Also, pathogenicity in from of seasonal populations was not affected by derived varieties, indicating natural pathogenicity changes (Davidson et al. 2016). Resistant and sensitive lentil varieties are different in terms of expressed genes, but time and expression

amount are also different and when be effective in responding to the ABL in resistant varieties. CDNA microarray can be used for resistance checking in lentil against inoculation of ABL. Based on this, highly resistant (ILL7537) and highly sensitive (ILL6002) varieties were determined. Also, 90 genes were expressed in ILL7537 with 95 genes in ILL6002. Expression of the two varieties showed significant difference in terms of type and time of expression in response to ABL. The resistant variety showed early regulation of PR4 with 10 proteins and the other genes were related to resistance. Finally, susceptible genotype showed rapid regulation of defense-related genes. In another study, disease-resistant resources were identified from local germplasm, among all populations, 5473, 5490, 5499, 5569, 5548, 5547, 5545, and 5570 were resistant when 5570 should be re-examined for resistance (Iqbal et al. 2010). Molecular breeding for ABL resistance had a range of genetic linkage on DNA-based. A significant correlation between genes selection based on PCR and their expression estimated RNA-seq technical accuracy of RNA-seq for expressed genes in genotypes (Rodda et al. 2017). Markers can be useful tools for future genes involved in resistant crops. Based on this, integration of EST-SSR markers with linkage map of QTL provides resistance to ABL in seedling and pod stages when clustered in different linkage groups. These accounted for 34% and 61% of total phenotypic variation, suggesting that resistance at different stages is potentially conditioned by different genomic regions (Gupta et al. 2012). Markers and QTL which are applied for pyramiding of resistance genes differences reflected in response expression such as pathogenesis-related proteins and genes related with death of cell and reinforcement (Sari et al. 2018).

It is suggested that interspecies hybrids can be included in lentil breeding programs. Identification of resistance genes, host identification, and molecular markers associated with resistance genes are considered as key factors (Ahmad et al. 1997). Hybridization programs of lentil against ABL in resistant varieties with high potential for yield have been developed based on linear relationship with germplasm screening in seedlings to flowering stage or mature plants under field condition (Mustafa et al. 2009). Resistance to ABL in global collection by using a germplasm showed significant variation by using controlled climate bioassay with highly virulent in Australian isolate known as FT13037. Genotype IG 207 had the lowest symptomatic tissue area and 12 genotypes showed moderate resistance. Also, IG207 has the lowest disease against 4 aggressive isolates and performed better than used best resistance sources, ILL7537 and Indianhead. In various surveys, screening methods have been investigated on decreasing ABL damage and progress of lentil species more flexible against its spread under climatic changes (Dadu et al. 2019). Different technologies try to improve resistance with extensive hybridization and seed rescue techniques that helped to introduce resistance features in lentil. In addition, induced mutagenesis has contributed to tolerance diversity with several disease-resistant mutants. However, advances in molecular markers and genomic technologies have helped to develop disease-resistant and climate-tolerant lentil varieties with high efficiency (Roy et al. 2022).

CONCLUSION

The balance of nutrients in lentil and use of appropriate fertilizers including micro- and macro-elements are very effective in plant freshness and resistance

against ABL. Excessive use of some fertilizers causes sensitivity of plants to this disease, which is closely related to some agricultural traits such as plant growth, leaf size, number of pods, etc. Regarding this disease, there are six pathogenic patterns when some lentil varieties showed suitable resistance determined as AL4, VI, ILL7537, and IG207. Resistance to ABL is controlled by two dominant genes, although minor genes play an important role in this case. It is very important to identify wild species that have good diversity in some countries and cross them with native varieties under hybridization. Moreover, use of molecular tools and determination of host-pathogen relationships can be effective in molecular techniques and germplasm application in this direction. Authors of this article invite other researchers to investigate different aspects of this little-known disease in Iran, so that a comprehensive understanding can be obtained by collecting various studies for determining of resistant and sensitive varieties in Iran.

RESUME

Badri H. et Simorgh S. 2023. Revue bibliographique sur la résistance des variétés de lentilles à la maladie de l'ascochytose causée par *Ascochyta lentis*, en mettant l'accent sur les aspects génétiques. Tunisian Journal of Plant Protection 18 (1): 1-14.

La lentille, *Lens culinaris* (syn: *Lens esculenta*), est l'une des légumineuses annuelles les plus importantes de la famille des Fabacées, qui est largement cultivée en Amérique, Europe, Asie, Australie et Afrique du Nord. Les graines de lentilles sont principalement utilisées dans les industries alimentaires pour produire des soupes et son fourrage est utilisé comme aliment pour le bétail. L'ascochytose de la lentille, causée par le champignon pathogène *Ascochyta lentis* (teleomorph *Didymella lentis*), est l'une des principales maladies de cette culture dans le monde, qui lui cause de graves dégâts. La résistance des différentes variétés de lentilles à cette maladie est variable. A cet effet, diverses études ont été réalisées sur la résistance des variétés cultivées et sauvages contre cette maladie; certains d'entre elles se sont concentrées sur les aspects écologiques, d'autres sur la génétique et peu sur la virulence des agents pathogènes. Dans cette revue, nous avons décrit les avantages de chaque aspect ainsi que les recherches récentes. La présente revue, en raison de ses caractéristiques uniques qui, à notre connaissance, a été réalisée pour la première fois, peut être considérée comme précieuse en ce qui concerne la gestion de cette maladie dangereuse chez les lentilles.

Mots clés : Ascochytose, lentille, agent pathogène, résistance, variété

ملخص

بدري، هاجر وصبا سمرغ. 2023. مراجعة لمقاومة أصناف العدس لمرض اللفحة التي تسببها *Ascochyta lentis*، مع التركيز على الجوانب الجينية. Tunisian Journal of Plant Protection 18 (1): 1-14.

العدس، *Lens culinaris* (syn: *Lens esculenta*)، هو أحد أهم البقوليات السنوية من عائلة البقوليات/الفوليات، والتي تزرع على نطاق واسع في أمريكا وأوروبا وآسيا وأستراليا وشمال إفريقيا. تستخدم بذور العدس في الغالب في الصناعات الغذائية لإنتاج الحساء وعلفها يستخدم كعلف للماشية. لفحة الأسكوكيتا للعدس، التي يسببها الفطر *Ascochyta lentis* (teleomorph *Didymella lentis*)، هي واحدة من الأمراض الهامة لهذا المحصول في جميع أنحاء العالم، والتي تسبب أضرارًا جسيمة لها. تختلف مقاومة أنواع أصناف العدس لهذا المرض. لهذا الغرض، تم إجراء مسح لدراسات مختلفة على مقاومة الأنواع المزروعة والبرية ضد هذا المرض؛ ركز البعض منها على الجوانب البيئية، والبعض الآخر على علم الوراثة، وقليل منها على ضراوة العوامل الممرضة. في هذه البيبليوغرافيا، حددنا مزايا كل خلفية حسب أحدث الأبحاث. يمكن اعتبار هذه المراجعة، نظرًا لخصائصها الفريدة التي تم إجراؤها لأول مرة حسب علمنا، بأنها ذات قيمة فيما يتعلق بإدارة هذا المرض الخطير للعدس.

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First Attempt to Develop a Rearing Method for the Native Green Lacewing *Chrysoperla lucasina* in Tunisia

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https://doi.org/10.52543/tjpp.18.1.2 (*Tunisia*)

ABSTRACT

Karouia, W., Hamdi, F., and Boulahia-Kheder, S. 2023. First attempt to develop a rearing method for the native green lacewing *Chrysoperla lucasina* in Tunisia. Tunisian Journal of Plant Protection 18 (1): 15-27.

Green lacewing (*Chrysoperla lucasina*) is well known for its polyphagous predator larvae which are commonly used in pest's biological control worldwide. In Tunisia, this auxiliary occurs in the nature in association with several pests but it has never been mass reared and released in biological control programs. Hence, the chrysopid species *C. lucasina* was chosen for an attempt of rearing in order to strengthen its natural populations. The study was carried out in the insectarium of the company ControlMed specialized in *Trichogramma* parasitoid production. All equipment used for green lacewing larvae and adult rearing was newly designed for this study. Two diets were given to larvae and adults to assess their biological performances. The first diet was based on pollen for adults and eggs of *Ephestia kuehniella* for larvae. The second diet consisting of a pasty mixture of honey, yeast and eggs of *E. kuehniella*, was supplied to adults and larvae. The laboratory rearing conditions were kept constant with 26 ± 2°C, 65 ± 5% RH and a photoperiod of 16L: 8D. The diets based on pollen and *E. kuehniella* eggs gave the best biological parameters for both adults and larvae. This study which allowed to design a simple technique to rear green lacewing based on affordable material, is a first step for developing a mass rearing of chrysopids in Tunisia. However, further improvements are required for adult and larvae diets to increase reproductive and developmental performance, as well as an economic feasibility study.

Keywords: Artificial diets, biological performances, *Chrysoperla lucasina*, green lacewing, low-cost material, insect rearing

In agroecosystems of temperate regions, the larvae of lacewings *Chrysoperla* spp. are well-known as effective biological control agents of a wide variety of pests such as aphids, and other small soft-bodied arthropods as well as lepidopteran eggs and larvae (Devetak and Klokokovnic 2016; Huang and Enkegaard 2010; Loru et al. 2014; Rosenheim et al. 1999; Villenave-Chasset 2007). Adults of the genus *Chrysoperla* Steinman (1964) are palyno-glycophagous feeding on sugar-rich food such as honeydew, nectar and plant fluids combined with pollen from a wide range

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of plant families (Devetak and Klokokovnic 2016). Among the most common species of the *Chrysoperla* complex occurring in the vicinity of crops, are *Chrysoperla carnea*, and *Chrysoperla lucasina* (Villenave-Chasset et al. 2006).

Identification and genetic structure of lacewing species are important issues for mass rearing and biological control purpose (Lavagnini et al. 2015). However, identification of the cryptic species of the *carnea* complex is sometimes problematic based on the morphology (Canard et al. 2007; Henry et al. 2002; Tauber et al. 2000).

The species *C. lucasina* (Lacroix) (Neuroptera: Chrysopidae), green lacewing, is often dominant in Mediterranean region (Thierry et al. 1996). In Tunisia, it is relevant in fruit and citrus orchards and as predator of several pests such as mealybugs, citrus leafminer and whiteflies (Boukhris-Bouhachem 2011). Fortunately, *C. lucasina* has characteristics making it reliably identifiable (Henry et al. 1996). It remains green during winter diapause while the other species change to brownish or reddish (Henry et al. 2002).

The green lacewing is a good candidate as biological control agent and its predation performance could be improved by several approaches. For example, biological conservation control by supplying supplemental food (Messelink et al. 2016) or introduction of certain plants could enhance chrysopid regulation action. Indeed, *C. lucasina* adults were mostly attracted by the Brassicaceae *Capsella bursapastoris* and *Biscutella auriculata* as well as the Asteraceae *Anthemis arvensis*, which could be introduced to enhance predation (Alcala Herrera et al. 2020).

The second approach is the classical biological control by mass rearing and releasing of biological control agents (Pappas et al. 2011). *C. lucasina*, *C. carnea* and *C. externa* are among the most studied species for this use (Bezerra et al. 2016; Pappas et al. 2011). Several mass rearing approaches of chrysopids have been developed mostly based on factitious preys such as *Ephestia kuhniella* or *Sitotroga cerealella* (Albuquerque et al. 1994; Haramboure et al. 2016). However, this type of rearing is very costly due to the production cost of factitious preys, that makes it difficult to sustain for a wide agricultural use. Several artificial diets were tested in combination or not with factitious preys to reduce the cost of prey production with various results (Haramboure et al. 2016; Jokar and Zarabi 2014; Polak et al. 1998; Ye et al. 2017). The genus *Chrysoperla* became commercially available since the 90's in the USA, Europe and China (Loru et al. 2014) with *C. carnea* and *C. externa* sold in Europe, Latin America and Asia (Tauber et al. 2000). However, in the Maghreb region (North Africa), Chrysopidae have been little studied for mass rearing to control crop pests (Limem-Sellami and Chermiti 2015). In Tunisia, so far, no specific action has been conducted to enhance local lacewing populations. Mass rearing of lacewings has never been achieved, while in the citrus agroecosystems of Cap Bon, several species were identified as autochthon natural enemies. The species *C. lucasina*, *C. carnea* and *C. affinis* were the most frequent in Tunisian citrus orchards with 53%, 24% and 18% respectively. However, lacewings were generally present with low frequency (Limem-Sellami 2017). This can be explained by the overuse of broad-spectrum insecticides against citrus pests. For example, neonicotinoid insecticides which are very commonly sprayed against aphids, are very likely to affect populations of *C. carnea* (Rogers et al. 2007).

In order to reduce pesticide use to control crop pests, various environmentally friendly methods are increasingly used or are under investigation in fruit orchards in Tunisia (Bachrouch et al. 2008; Boulahia-Kheder et al. 2012). In a framework of promoting conservation biological control, our study aimed to develop for the first time in Tunisia, an affordable and easy approach to rear the native green lacewing *C. lucasina* using a factitious prey and other nutritional supplements, to enhance its natural populations. This could be a first step for a mass rearing of green lacewings in Tunisia. In this paper we describe (1) the device designed to rear larvae and adults as an outcome, and report (2) the biological performances of two diets supplied to larvae and adults.

MATERIALS AND METHODS

Study site.

The study was carried out in the insectarium of a private company (ControlMed) specialized in the mass-rearing of beneficial insects for biological control of crop pests. Currently, this company which is located at Sidi-Thabet region (36º 54′ 31″ North, 10º 02′ 33″ East) (Governorate of Ariana) mainly produces the parasitoid *Trichogramma cacoeciae* (Hymenoptera: Trichogrammatidae) used for the biological control of *Ectomyelois ceratoniae* (Lepidoptera: Pyralidae) in pomegranate orchards. In this study, we aimed to optimize the exploitation of the eggs of *E. kuehniella* as food already produced for *T. cacoeciae* rearing.

Collection and identification of green lacewing adults.

The first step to start the rearing process, was the collection of adults of chrysopids. Adults have been collected using an aerial net and a small aspirator, mainly from pear, peach and citrus in six orchards located in Sidi-Thabet (36° 54′ 31″ N, 10° 02′ 33″ E) during the period 23/05- to 05/07-2017. The summer season was favorable for the availability of green lacewing associated with *Myzus persicae* aphids and citrus leafminer *Phyllocnistis citrella* populations. Taxonomic identification of collected adults was achieved using a binocular microscope based on the keys of Henry et al. (1996) and Mazel et al. (2006).

Rearing conditions and diets.

After being identified, the adults were maintained in a climatic chamber at 26 ± 2°C temperature, 65 ± 5% relative humidity and 16:8 h light:dark photoperiod, as recommended in Loru et al. (2014).

Six adult units were performed according to Loru et al. (2014) with some modifications. A 20 cm high and 9 cm diameter cylinder made from a plastic water bottle was used. The upper opening was closed with tulle mesh and secured with rubber bands. The unit was kept vertically fixed on the closed end (Fig. 1A). Dark Bristol paper or Kraft paper (10 cm/10 cm) cardboard were accordion fold lined in the inner part of the cylinder and fixed by clips to play the role of a laying substrate. Food was presented in a half Petri dish (90 cm diameter × 1.5 cm height) and water was supplied by damping cotton in distilled water with making sure that water did not drip down (Fig. 1A). The sex ratio was 1:1 as recommended in Mudassar et al. (2013) so each unit hosted 3 couples of green lacewings. The selection of males and females for mating is not recommended for a mass-rearing as it could affect genetic variability. However, we did it because our goal was mainly to test the experimental device.

The eggs laid on Bristol paper in each adult unit were transferred in 6 larval

units daily. Each larval unit contained eggs laid on the same day from the same adult unit. Then the newborn larvae were placed in appropriate units, until adult stage. Each larval stage (1st, 2nd, or 3rd) was placed in separated rearing units to avoid cannibalism. During larval development, each stage duration (days) was recorded. The larval rearing unit was a 16.4 cm × 6.5 cm rectangular transparent plastic food box with a plastic lid, available in supermarkets (Fig. 1B). Tiny holes have been made for the ventilation of rearing units. After emergence, the new adults were sexed based on the genitalia and put into the adult's containers.

In this study, the rearing units used for adults and larvae were very small for mass-rearing (Fig. 1). However, they could give the first guidelines for a larger rearing.

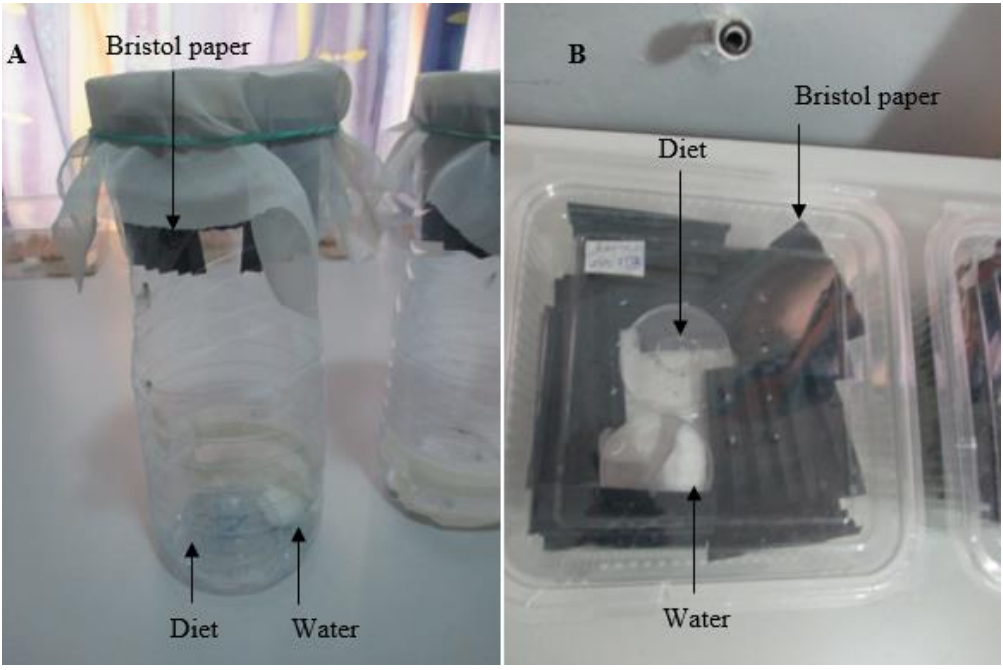

Fig. 1. Rearing units of green lacewing (*Chrysoperla lucasina*): For adults (A) and for larvae (B).

Rearing units for adults and larvae, Petri dishes and the climatic chamber have been cleaned regularly to avoid any type of contamination (mites, fungi, bacteria). Rearing units were serviced three times a week while Petri dishes and the climatic chamber were cleaned every day. The paper towels were changed daily and the pollen was renewed twice a week. The cotton was refilled with distilled water or renewed every day.

Two diets were given to adults and two to larvae (Table 1). Adults collected from orchards (F_0) were divided into two groups, one fed with the diet D_1 which was pure multi-floral honeybee pollen (Royaume des abeilles®, origin: Tunisia) and the second group was fed with the diet D_2 which is a pasty mixture of multi-flowered honey + beer yeast (Phytothera®) + *E. kuehniella*'s eggs (Table 1). This diet was obtained by mixing the 3 ingredients manually with a spoon and was preserved 7 days in a refrigerator at 4°C. The *E. kuehniella*'s eggs were provided by the hosting company which produces them throughout the year to rear *T. cacoeciae*. The larvae originated from the adult green lacewing fed with pollen, were supplied with eggs of *E. kuehniella* (diet D_3) until they completed their development (Table 1). The adults (F_1) were supplied with pollen and their progeny with eggs of *E. kuehniella* until they reached the adult stage (F_2).

The larvae originated from the adults fed with the diet D_2, were supplied with the same diet (D_2) until they finished their development. The obtained adults (F_1) were fed again with D_2.

For each diet, 3 replicates were carried out. Then, performances of the adults and larvae were compared for each diet in order to determine the best one for both stages. The evaluated parameters are those of the generation (F_1).

Table 1. Composition of the 3 diets (D_1, D_2, and D_3) used for rearing of *Chrysoperla lucasina*

Adults		**Larvae**
D_1	**D_2**	**D_3**
Honeybee pollen (2 g)	• 0.03 g beer yeast • 0.03 g *Ephestia kuehniella*'s eggs • 0.3 g honey	*Ephestia kuehniella*'s eggs (0.5 g)

Biological parameters assessed.

For all stages of development of the green lacewing, some biological parameters were evaluated. The performances resulted from each diet for both adults and larvae, were evaluated and compared. For adults (F_1), the parameters recorded were: longevity (days), mortality rate ((number of dead adults/total number of adults) × 100) (per week), duration of development time from eggs to adults (days), without distinguishing sexes, and daily fecundity per female (mean number of eggs laid/day), total fecundity per

female (average daily fecundity × longevity).

Egg fertility was estimated each day by collecting and counting eggs laid from the adult units, and then transferring them to the larval units. On each unit, a label indicated the origin of eggs and their laying date. Thus operating, we followed the development of each larvae and evaluated the biological parameters. The parameters recorded for eggs and larvae were the fertility, the pupation rate (percentage of larvae reaching the pupal stage) and the adult emergence rate (percentage of pupae reaching the adult stage). The statistical analysis was performed by using the XLSTAT software of Microsoft Excel. This program was adopted for analysis of variance (ANOVA) between the tested diets.

Predation test.

Preliminary tests were carried out to assess the predatory capacity of green lacewing larvae reared under controlled conditions. The objective was to determine whether larvae fed on factitious diets alone (eggs of *E. kuehniella)* would easily accept natural preys. For this, first and third instars larvae of green lacewing were exposed to one kind of prey in a Petri dish (90 cm diameter × 1.5 cm height): aphid *M. persicae* adults or citrus leafminer *P. citrella* larvae. Both of these preys were available in the orchards during the study period. The test was carried out on 4 young larvae (2 replicates) and 6 older ones (3 replicates) and the total number of preys consumed by larvae was counted.

RESULTS

Design of the green lacewing rearing device.

The green lacewing species collected for rearing was identified as *C. lucasina*. In our study, the experimental device setup for rearing green lacewing was designed using inexpensive equipment available in Tunisia (see Material and methods). In our rearing device, some strengths and weaknesses can be highlighted.

The most interesting findings are the ease of design and low cost of adult and larva rearing units. The major difficulty was avoiding larval cannibalism which increased with lack of food, number of larvae per unit and temperature. Thus, it would be more appropriate to use larger equipment for mass rearing. This is also suggested for adult units to help feed insects and facilitate the cleaning of units, while adults are flying.

Regarding the diets for adults, the diet D_1 composed of honeybee pollen was appreciated and did not pose issues of cleanness. However, the diet D_2 where one of the ingredients is honey, required frequent cleaning. It caused fouling of adult's wings, legs and abdomen. So, insects became unable to move and this can lead to adult death. For larvae, the same problem was faced with the diet D_2.

Biological performance according to diet.

Based on the biological parameters evaluated for one generation of green lacewing, the diets that produce the best results were D_1 for adults and D_3 for larvae (Tables 2, 3). Indeed, for adults the diet D_1 allowed a significantly higher fecundity and longevity than D_2 and the shortest duration of development from egg to adult (Table 2). The consumption of food provided to adults was estimated at about 0.1 g per day for D_1 and D_2 and per rearing unit housing 6 individuals.

For larvae, the diet D_3 allowed a significantly better pupation and adult emergence rates, and the shortest larval development (Table 3).

Table 2. Biological parameters of *Chrysoperla lucasina* adults fed on two diet

Biological parameter	Diet		*p* value	Degree of freedom	F value
	D_1	D_2			
Fecundity (eggs/female/day)	9.08 ± 6.13 a	3.64 ± 4.13(b	0.00	120	29.78
Total fecundity (eggs/female)	236.19 a	69.13 b	0.00	120	47.70
Longevity (days)	26	19	-	-	-
Mortality (%)	32.72 ± 0.15 a	24.07 ± 0.18 a	0.29	16	1.19
Duration of development time (days)	24.25 ± 7.80 a	39.67 ± 12.22(b	0.03	5	4.23
Egg hatching rate (%)	24.49 ± 0.20 a	20.82 ± 0.12 a	0.40	59	0.71

Data are mean values ± SD (standard deviation). Values within each line followed by the same letter are not significantly different ($p < 0.05$).

Table 3. Biological parameters of *Chrysoperla lucasina* larvae fed on two diet

Biological parameter	Diet		*p* value	Degree of freedom	F value
	D_2	D_3			
Pupation rate (%)	4.30 ± 0.11 b	23.64 ± 0.17 a	0.000002	59	24.45
Adult emergence (%)	30.36 ± 0.48 b	60.95 ± 0.32 a	0.05	58	16.58
Duration of larval development (days)	9.00 ± 4.42 a	7.71 ± 2.27 a	0.34	27	0.95

Data are mean values ± SD (standard deviation). Values within each line followed by the same letter are not significantly different ($p < 0.05$).

Preliminary test for predation ability.

The predation behavior of larvae of *C. lucasina* from our rearing against natural preys was tested. The average consumption of a young green lacewing larva (L_1) was 3 adults of *M. persicae* aphids per hour. Third instar larvae were more voracious than young ones, with an average consumption of 9.67 aphids/hour (Table 4). Regarding the citrus leafminer *P. citrella*, the consumption of green lacewing larvae was reduced. It was 3 larvae/hour for the third instar of *C. lucasina* larvae. Moreover, a very young larva of green lacewing (L_1) was not able to even consume a whole old leafminer larva.

Table 4. Predation ability of 1st and 3rd instars larvae of *Chrysoperla lucasina*

Prey species (number consumed/hour)	Predator stage	
	1st instar larva	3rd instar larva
***Phyllocnistis citrella* larvae**	0.2±0.28	2±3
***Myzus persicae* adults**	3±2.83	9.67±2.08

Data are mean values of three replicates ± SD (standard deviation).

DISCUSSION

Currently in Tunisia, the biological control of crop pests is little applied at the farmer scale due to the unavailability of entomophagous with an affordable cost. This study aimed to develop a simple and low-cost device to rear green lacewing, as a first step that could be replicated in a bigger scale for a mass rearing. Specifically, the objective was to take benefit of *E. kuhniella*'s eggs as ingredients of the diets for larvae and adults. Especially that Lepidopteran eggs are one of the preferred foods to green lacewing larvae (Legaspi et al. 1994) but their production is relatively costly (Tauber et al. 2000). The availability of an egg production should optimize the very costly larvae production of green lacewing because the three stages are predators (Tauber et al. 2000).

The species *C. lucasina* was chosen for a rearing attempt in order to be released in fields to enhance its natural populations. This species was selected because it was the most abundant one among the specimens collected and it seems well adapted to Tunisian climatic conditions. Our observations agree with Limem-Sellami and Chermiti (2015). Such experiment was carried out by Polak et al. (1998) for *C. chaquensis* collected from cotton fields in Argentina, mass-reared and released to increase their regulation action.

The major results of this study are the design of a simple and inexpensive rearing device for *C. lucasina* and the selection of two diets that ensure the development or larvae and adults. However, for mass rearing purpose, development and reproduction parameters need to be improved.

Rearing device and diets.

The rearing conditions were a constant temperature of 26 ± 2 °C, a relative humidity of 65 ± 5% and a photoperiod maintained on 16:8. These conditions are close to those used in most rearings of chrysopid species and allowed the best performance (Albuquerque et al. 1994; Atlihan et al. 2004; Huang and Enkegaard 2010; Jokar and Zarabi 2012; Liu and Chen 2001).

The rearing units designed for larvae and adults were made with cheap equipment locally available. It consisted of a cylinder (20/9 cm) cut from a plastic water bottle covered with a tulle mesh. The height of the cylinder (20 cm) was to increase the space flight for adults and the only one opening at the top of the bottles (instead of 2) was to reduce mesh tulle handling and fastening. In each unit, an accordion-like strip of Kraft or Bristol paper was placed as an egg-laying site. Both papers were equally used by females to lay eggs; so Kraft paper can be adopted because it's less expensive than Bristol.

Then eggs laid were separated daily by cutting Kraft paper to isolate newly emerged larvae individually. The rearing unit for larvae was a rectangular transparent plastic food box with a plastic lid (16.4 cm / 6.5 cm). Each day, the eggs laid were placed in the same box. The three larval stages were separated in order to avoid cannibalism. At the end of the larval development, the obtained adults were sexed and put into the adult's unit.

Four diets were proposed, two for adults and two for larvae, (1) a multi-floral honeybee pollen and (2) a mixture of eggs of *E. kuehniella* + honey + beer yeast, to adults and (3) this same mixed diet and (4) eggs of *E. kuehniella* to larvae. The amount of food consumed by adults per day for both diets was about 0.1 g/unit.

Biological performance.

All diets have ensured surviving of *C. lucasina*, but did not provide the same biological performance. The pollen

for adults and *E. kuehniella* eggs for larvae, gave the best biological parameters. This result matches the objective of this study that was to take advantage of the egg production of *E. kuehniella*, as this factitious prey is known to be very expensive: around 500-600 euros/kg in 2008 (Vandekerkhove et al. 2008).

The pollen-based diet for adults and *E. kuehniella* eggs for their offspring allowed the significantly highest fecundity, the highest hatchability, the highest longevity and the significantly shortest development duration time: around 236 eggs, 24%, 26 days and 24 days respectively. However, the mortality was higher than with the mixed diet: 33% vs. 24%. Compared to other studies on chrysopid rearing, but with other species than *C. lucasina*, some adult parameters are considered encouraging: longevity, mortality and development duration. However, fecundity and hatching ability particularly need to be improved. For instance, the fecundity of females fed with pollen (236 eggs in total) is considered low compared to other findings. Indeed, the total fecundity reached was in average of 400-500 to 1000 eggs depending on the species and the diet (Albuquerque et al. 1994; Alghamdi and Sayed 2017; Hagen and Tassan 1970 in Tauber et al. 2000; Haramboure et al. 2016; Jokar and Zarabi 2014; Polak et al. 1998; Ye et al. 2017). The parameter "fecundity" is very important as it is a start-reproduction parameter that will influence the productivity of the mass rearing. Our findings were close to that of Jokar and Zarabi (2012) which varied from 7 to 10 eggs/female/day. The diet that ensured this fecundity contains a high amount of vitamin E (vitamins and chicken egg yolk) which is appropriate for rearing of adults of *C. carnea* (Balouch et al. 2016)

The egg hatchability is also a critical reproduction parameter for the mass rearing of *C. lucasina* as a biocontrol agent. In our study, this parameter was around 24% which is considered very low compared to 85% for *Dichochrysa tacta* fed by *E. kuhniella eggs* (Alghamdi and Sayed 2017) and to 70% at least, depending on diets for *C. externa* (Haramboure et al. 2016) and also to 92% for *C. chaquensis* fed with a mixture of honey, yeast, wheat germ and water (Polak et al. 1998).

Hence, honeybee pollen alone did not allow a satisfactory reproduction potential for a mass rearing. Consequently, adults should be supplied with vitamins in addition to pollen in order to increase fecundity and fertility. Our choice of a diet based on honeybee pollen was motivated by its nutritive value, as it is rich in carbohydrates, proteins and amino acids (Villenave-Chasset 2007). Furthermore, it is the essential diet for green lacewing, ladybugs and hoverflies (Leroy et al. 2008). The slight disadvantage of using pollen is the availability of various kinds of pollen derived from different plants (Loru et al. 2014).

The longevity of adults fed with pollen obtained in our rearing (26 days) was close to that recorded by Balouch et al. (2016) for *C. carnea* with a diet based on water, sugar and yeast (30 days) and that achieved by Alghamdi and Sayed (2017) for the species *Dichochrysa tacta* where larvae were fed on *E. kuhniella* eggs (27.7 days in average). However, 26 days is very short compared to that obtained with *Mallada basalis* (45 days) on an artificial diet (Ye et al. 2017).

Regarding the mortality of adults, in our rearing we obtained 32% on average without distinguishing between sexes, which is too high. The survival of adults should be studied according to age and sex of adults as it is known that in chrysopids,

females live more than males, in the *C. carnea* group.

For larvae, the diet based on *E. kuhniella* eggs allowed the shortest larval development and significantly the highest pupation and adult emergence rates: respectively around 7.7 days, 23.6% and 61%. A short larval development is a relevant factor for a species to be mass reared and the value found in our rearing is considered interesting compared to other studies in close conditions but with other species and different diets: 12.2 days for *C. externa* (Albuquerque et al. 1994); 12.83 days for *M. basalis* (Ye et al. 2017); 13.5 days for *C. paraguaria*, 11 days for *C. externa* and 9 days for *C. chaquensis* (Polak et al. 1998).

However, the parameters "pupation rate" and "adult emergence rate" obtained with the retained diets, respectively 23.64% and 60.95%, were not enough high to ensure an efficient mass rearing. The pupation rate was 45% with minerals and vitamins (Balouch et al. 2016) but only 10.1 % with diet based on *E. kuehniella* (Alghamdi and Sayed 2017). Hence, it is important to add vitamins and minerals to larvae diet to improve pupation rate. In the research result of Balouch et al. (2016), the adult emergence rate did not exceed 30% with a diet containing water, sugar and yeast. Moreover, a cost-analysis should be conducted for *C. lucasina* reared on pollen for adults and *E. kuhniella*'s eggs for larvae, in order to estimate the economic feasibility as achieved by Mudassar et al., (2013) who found that *C. carnea* mass production was more economically efficient on *Sitotroga cerealella* eggs than on *Phenacoccus solenopsis* crawlers.

Predation ability.

The average consumption of a young *C. lucasina* larva was 2 adults of *M. persicae*/hour, while the third instar larvae were more voracious with an average consumption of 9.67 aphids/hour. This same instar consumed 3 larvae of *P. citrella*/hour, while a younger *C. lucasina* larva cannot even consume an entire old leafminer larva. These preliminary results only gave an idea about acceptation of the natural prey for larvae reared on *E. kuhniella* eggs. They cannot indicate the real predatory capacity because of the low number of replicates and also because *C. lucasina* larvae were not starved. Polak et al. (1998) reported that the third instar of green lacewing was the most voracious, suggesting a high number of preys being consumed during the larval development. This characteristics of Chrysopidae, with their polyphagy and great mobility (Polak et al. 1998), make their interest as predators.

In summary, the device designed in our study is quite satisfactory for a small rearing for experimental purpose. However, this device as well as the diets based on pollen for adults and *E. kuhniella*'s eggs for larvae, have to be improved to produce a higher yield. Indeed, for a mass rearing, more space is recommended that means a whole room dedicated to rearing with spacious units. Regarding diets for adults, honeybee pollen must be complemented by vitamins and amino acids to improve fecundity and fertility of eggs. The preoviposition period should also be checked. For *C. lucasina* larvae, eggs of *E. kuhniella* are a good diet, but should be complemented with other ingredients to increase pupation rate. With these improvements, *C. lucasina* should have the potential to be a candidate for mass rearing and release as a biocontrol agent against several crop pests in Tunisia. This work is nevertheless a first step for an application at larger scale.

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RESUME

Karouia W., Hamdi F. et Boulahia-Kheder S. 2023. Première tentative de développement d'une méthode d'élevage de la chrysope verte autochtone *Chrysoperla lucasina* en Tunisie. Tunisian Journal of Plant Protection 18 (1): 15-27.

La chrysope verte (*Chrysoperla lucasina*) est bien connue pour ses larves prédatrices polyphages qui sont communément utilisées dans la lutte biologique contre les ravageurs dans le monde entier. En Tunisie, cet auxiliaire est présent dans la nature en association avec divers ravageurs mais il n'a jamais été élevé en masse et lâché dans le cadre de programmes de lutte biologique. Ainsi, l'espèce de chrysope *C. lucasina* a été choisie pour une tentative d'élevage afin de renforcer ses populations naturelles. L'étude a été réalisée dans l'insectarium de la société ControlMed spécialisée dans la production des parasitoïdes du genre *Trichogramma*. Tout le matériel utilisé pour l'élevage des larves et des adultes de la chrysope verte a été nouvellement conçu pour cette étude. Deux régimes alimentaires ont été donnés aux larves et adultes pour évaluer leurs performances biologiques. Le premier régime était à base de pollen pour les adultes et d'œufs d'*Ephestia kuehniella* pour les larves. Le deuxième régime fourni aux adultes ainsi qu'aux larves, était un mélange pâteux de miel, levures et œufs d'*E. kuehniella*. Les conditions d'élevage au laboratoire ont été maintenues constantes avec 26 ± 2°C, 65 ± 5% HR et une photopériode de 16L:8D. Les régimes à base de pollen et d'œufs d'*E. kuehniella* ont donné les meilleurs paramètres biologiques respectivement pour les adultes et les larves. Cette étude ayant permis de concevoir une technique d'élevage simple de *C. lucasina* basé sur du matériel à prix abordable, constitue une première étape pour le développement d'un élevage de masse des chrysopes en Tunisie. Cependant, des améliorations des régimes alimentaires des adultes et des larves sont nécessaires pour augmenter les performances de reproduction et de développement, ainsi qu'une étude de faisabilité économique.

Mots clés: Chrysope verte, *Chrysoperla lucasina*, élevage d'insectes, matériel à bas coût, performances biologiques, régimes artificiels

ملخص

قروية، وجدان وفاتن حمدي وسندة بولحية-خذر. 2023. أول محاولة لتطوير طريقة تربية الحشرة الخضراء شبكية الأجنحة *Chrysoperla lucasina* المحلية في تونس.

Tunisian Journal of Plant Protection 18 (1): 15-27.

تُعرف الحشرة الخضراء شبكية الأجنحة أو أسد المنّ (*Chrysoperla lucasina*) بيرقاتها المفترسة المستخدمة عبر العالم في المكافحة البيولوجية. في تونس، توجد هذه الحشرة طبيعيا مقترنة بعديد الآفات، لكن لم يتم مطلقاً اكثارها ونثرها في في إطار المكافحة البيولوجية. لذلك، تم اختيار هذه الحشرة لمحاولة إكثارها كميا من أجل تعزيز مجتمعاتها الطبيعية. احتضنت مخابر شركة "كنترولماد ControlMed" المختصة في إكثار شبه الطفيليمن جنس *Trichogramma*. في هذه الدراسة، تم اختبار نظامين غذائيين للبالغين واليرقات لحشرة أسد المنّ. يستند النظام الغذائي الأول بالنسبة إلى البالغين على حبوب اللقاح. ويتكون النظام الغذائي الثاني من خليط من بيض عثّة الطحين (*Ephestia kuehniella*) والعسل والخميرة. وبالنسبة إلى اليرقات، كان النظام الغذائي الأول هو بيض عثّة الطحين والنظام الغذائي الثاني هو خليط من بيض عثّة الطحين والعسل والخميرة .تم الحفاظ على ظروف ثابتة للتكاثر، تتلخص في درجة حرارة 26 ± 2 ºس، نسبة رطوبة 65 ±5 %، 16 ساعة إضاءة / 8 ظلام في اليوم. وفقا للنتائج التي تم الحصول عليها، تم اختيار حبوب اللقاح للبالغين وبيض عثّة الطحين لليرقات كأفضل أنظمة غذائية. هذه الدراسة التي مكنت من تصميم تقنية إكثارأسد المنّ، تعد خطوة أولى يمكن تبنيها لتطوير إنتاج هذه الحشرة في تونس لكن يبقى ضروري إدخال تحسينات على النظام الغذائي للرفع من كفاءة الحشرة في التكاثر والتطور.

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Effects of Five Plant Essential Oils on the Protein Content and Digestive Enzymes of *Ephestia kuehniella*

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ABSTRACT

Asadi, M. 2023. Effects of five plant essential oils on the protein content and digestive enzymes of *Ephestia kuehniella*. Tunisian Journal of Plant Protection 18 (1): 29-39.

The Mediterranean flour moth or mill moth *Ephestia kuehniella* is a common pest of cereal grains, especially flour. In this research, the sublethal effects of essential oils isolated from five medicinal plants including *Allium sativum*, *Glycyrrhiza glabra*, *Rosmarinus officinalis*, *Salvia officinalis*, and *Piper nigrum* were investigated on the protein content and digestive enzymes activity of the fifth instar larvae, under laboratory conditions. The GC-MS analysis of the essential oils showed that tetracosamethyl cyclododeca siloxan, aristolene, α-pinene, β-thujone, and caryophyllene were the dominant constituents for each essential oil, respectively. The enzymatic activity was investigated by the treatment using the LC_{30} concentration against the larvae that were 2.86, 12.03, 2.19, 7.84, and 9.39 µl/l air, respectively. Results revealed that there were significant differences among the treatments on total protein content and enzymatic activities ($F_{5,12}$ = 2.95, 3.56, and 7.07). About the total protein content, the control and treatment with *R. officinalis* essential oil showed the highest and lowest concentrations (1.0288 ± 0.0212 mg/ml versus 0.7333 ± 0.0329 mg/ml). The highest amylolytic activity was also seen in the control (0.0551 ± 0.0025 mU/mg) and the lowest being in *R. officinalis* oil treatment (0.0373 ± 0.0009 mU/mg). Moreover, the highest and lowest proteolytic activities were observed in the control (5.5063 ± 0.1086 U/mg) and *R. officinalis* essential oil (3.3028 ± 0.1077 U/mg). Accordingly, *R. officinalis* essential oil could be applied for the control of *E. kuehniella*.

Keywords: Activity, amylase, enzyme, *Ephestia kuehniella*, protease, protein content

Protecting of agricultural products from the pests' attacks during their storage is a guarantee for food security and healthy foods for humans. Inability to control pests causes loss of food resources and economic dependence of countries. Regardless of economic aspects, the arrival of pests can be a serious threat to food and health security. For this reason, it seems necessary to create suitable infrastructure for storing primary food outside the growing season and managing pests using new methods. Due to construction method and lack of proper urban infrastructure, food storages are exposed to the invasion of various pests (Jacob and Cox 1976).

The Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), is one of the important pests of stored products especially flour in most parts of the world (Ahmadpour 2021; Asadi et al. 2018a). This pest has global distribution and its adult has a short life

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span about one week. Females lay an average of 250 eggs in small batches (up to 20) on various food sources. The emerged larvae are reddish pink in color, but feeding type and host has great effect on their color (Jacob and Cox 1976). The number of generations varies according to living environment. In relatively warm storages, this moth continues to feed and reproduce without interruption in all seasons. In these conditions, it can develop six or more generations during a year. In opposite, under cold environmental conditions (temperature less than 10°C), activity of larvae is stopped (Sarmadi et al. 2010).

The effective role of food in growth, reproduction, immune parameters, and the activity of digestive enzymes has been proven. If insects benefit from good food with higher nutritional value, they have stronger immune systems and can better deal with external factors. In addition, sufficient nutrition during the larval period leads to production of insects with appropriate reproductive capacity (Ajamhassani and Amiri Jami 2018). In insects' midgut, the main digestive enzymes included amylases, glycosidases, lipases, and proteases, which are similar in hydrolytic nature. These enzymes play a significant role in digestion and absorption of food. Life of various insects depends on their existence (Terra 1990). Amylases are important enzymes for carbohydrate metabolism in insects (Horie and Watanabe 1980). Many insects, especially larval stages, feed on rich sources of polysaccharides and extensively use these enzymes to break down polysaccharide and starch chains (Valencia et al. 2008). α-amylases are enzymes that are abundant in nature and their main role is to catalyze the joining of α-D-(1,4)-glucan chains in starch and the other carbohydrates (Franco et al. 2000; Stroble et al. 1998). Food sources of insects have a great effect on the activity of their digestive amylases. The activity of this enzyme is very low in wool-eating insects, but it is very abundant in oligophagous and polyphagous insects (Slansky and Scriber 1981). Proteases are also considered as the most important enzymes in insects and their role is to supply necessary amino acids and energy from food sources for growth and development. In many insects, especially in the order Lepidoptera, serine proteases are the most important group (Telang et al. 2005).

Essential oils are a group of plant volatile metabolites, some of which have fumigant, contact, repellent, and anti-feeding properties against pests (Asadi et al. 2019, 2021). Due to high insecticidal activity, they are often recommended to control insect pests. On the other hand, the activity of digestive enzymes in insects, especially herbivorous insects, is influenced by metabolites present in various plants, which are known as plant secondary metabolites (Parsia Aref and Valizadegan 2015). Proper nutrition and getting adequate energy from food resources is ultimately related to egg production and the other biological activities. Therefore, it is necessary to investigate the changes of these enzymes in pest management. One of the obvious effects of plant essential oils is their anti-nutritional activity, which is very intense in some plant species. This characteristic of essential oils is very effective in reducing damage of pests due to low or no production of digestive enzymes (Parsia Aref and Valizadegan 2015; Razmjou et al. 2018). Due to importance of digestive enzymes and nutrition as important features in pests, the present research was performed on the sublethal effects of essential oils isolated from five medicinal plants on the protein content with amylolytic and proteolytic activity of *E.*

kuehniella. The main aim of this research was to determining effective plant compounds on this important pest for applying in pest management designs.

MATERIALS AND METHODS

Rearing of *Ephestia kuehniella.*

The flour moth *E. kuehniella* eggs were obtained from a private insectarium (registered name: Jalilian) from Eslamabad-e Gharb city, Kermanshah province, Iran, during 2018. Then, 0.25 g of the eggs were distributed on 750 g of wheat flour with 250 g of wheat bran, under the laboratory conditions including 23 ± 2°C, 50 ± 5% relative humidity (RH), and 24 h darkness (Abedi et al. 2012, 2014; Mahdavi and Saber 2013).

Extraction of essential oils.

The selected medicinal plants including garlic (*Allium sativum*), rosemary (*Rosmarinus officinalis*), black pepper (*Piper nigrum*), sage (*Salvia officinalis*), and liquorice (*Glycyrrhiza glabra*) that was available in the Iranian flora and contained suitable amount of essential oil, were collected from different regions of Eslamabad-e Gharb city (34.11° N, 46.53° E) in Kermanshah Province, Iran, during May 2017. The aerial parts (leaves and seeds) of the plants were powdered. Then, 50 g of their powder was mixed with 500 ml of distilled water into a 1-liter balloon of the Clevenger apparatus (Asadi et al. 2018a). After 4 h, the essential oil appeared as a pale green layer on the water. To remove excess water and purify the essential oil, sodium sulfate (Na_2SO_4) was used (Asadi et al. 2018b). Finally, the essential oils were stored in special containers covered with the aluminum foil, inside the refrigerator at 5°C, (Asadi et al. 2019; Negahban et al. 2007; Parsia Aref and Valizadegan 2015). To determine the quantitative and qualitative components of the essential oils, it was used the Agilent technologies 7890B (Manufactured by the USA) gas chromatography with HP-5MS column, length 30 m, diameter 0.25 mm, and film thickness 0.25 mm. Temperature program was 350°C. Determining ranges was also done using a data bank of mass, Kovats index, and retention time. Each essential oil components were detected based on patterns of ranges refraction compared to standard by using of reputable sources (Adams et al. 2007).

Bioassay.

To investigate the fumigant toxicity of essential oils on fifth instar larvae of *E. kuehniella*, different concentrations which lead to mortality rate between 20% and 80% were placed on filter paper (2 × 2 cm) using a microapplicator, in 70 ml glass Petri dishes as fumigation chamber. The distilled water was used in the control treatment (Parsia Aref and Valizadegan 2015). Then, 15 larvae were placed inside each Petri dish that were immediately sealed with parafilm tape to prevent evaporation of essential oils. Each essential oil concentration was assayed in 4 replicates. After 24 h of exposure, the number of dead larvae was recorded. Accordingly, insects are considered dead when no movements have been observed when stung with a needle (Shahriari and Sahebzadeh 2016).

Preparation of enzymatic mixture.

Fifth instar larvae of *E. kuehniella* were exposed for 24 h to the LC_{30} values (as sublethal concentration based on bioassay experiments analysis) of the five essential oils (2.86, 12.03, 2.19, 7.84 and 9.39 µl/L air). Then, the alive treated larvae were carefully dissected in distilled water using a stereomicroscope. The digestive tubes of the larvae were carefully separated and placed inside 5 ml

microtubules containing NaCl 1.5%. Then, all parts of digestive tube were homogenized with a manual homogenizer on ice and the homogenized mixture was centrifuged at 15,000 rpm for 15 min (4°C). Finally, its supernatant was stored at -15°C for enzymatic analyses (Bidar et al. 2016). For each treatment, ten digestive tubes were isolated.

Protein assay.

Protein concentration is one of the most important components in the formula for determining the specific activity of digestive enzymes (U), which is measured based on the method of Bradford (1976). Based on the studies, absorption rate at 595 nm wavelength indicates protein concentration in each sample. In this study, 600 µl of Bradford's reagent was added to 20 µl of enzymatic mixture inside 5 ml microtubes and three repetitions were considered for each treatment and the control. Finally, the protein concentration was determined using the standard equation and comparing of absorbance in the samples (Bidar et al. 2016).

α-Amylase activity.

To determine the α-amylase activity, each experimental unit containing 20 µl of enzyme mixture, 50 µl of 1% starch substrate, and 250 µl of phosphate buffer was mixed and placed at room temperature for 30 min. Then, 50 µl of 3,5-dinitrosalicylic acid was added as an inhibitor of enzyme activity and then placed in a water bath at a temperature of 90°C (10 min). Finally, after 5 min of centrifugation at 16,000 rpm (4°C), absorbance of the samples was measured at 540 nm wavelength using a spectrophotometer. In the studies, a blank sample was used for all treatments. All assays were performed in triplicate (Bernfeld 1955).

General protease assay.

To study this enzyme in the midgut of *E. kuehniella* larvae, 1.5% azocasein was used as main substrate (Elpidina et al. 2001). Accordingly, 50 µl of the enzyme mixture and 80 µl of its substrate were placed in an appropriate volume of phosphate buffer at room temperature for 60 min. Then, 100 µl of 30% trichloroacetic acid compound was added as enzyme activity inhibitor. Then, the undigested azocasein was removed and the mixture was centrifuged at 15,000 rpm for 15 min (4°C). Finally, 100 µl of 2 M NaOH compound was added to 100 µl of the prepared mixture and its absorbance was measured at 405 nm wavelength by using of spectrophotometer. Each treatment and the control were measured in three replicates (Bidar et al. 2016).

Statistical analysis.

In this research, three repetitions were considered for each essential oil treatment with the control, then the data were examined for normality. Finally, the data were analyzed using one-way ANOVA and their means compared with Tukey's test ($p < 0.05$) using SPSS software, version 22.

RESULTS

Essential oils compounds.

The results of GC-MS analysis from the five essential oils revealed eleven main compounds in *A. sativum*, forty-four compounds in *G. glabra* and *S. officinalis*, and forty-three compounds in *S. officinalis* and *P. nigrum*. These results were previously published in the article by Asadi et al. (2021), but their five dominant combinations are again presented in Table 1.

Table 1. Five dominant constituents in each essential oil of different medicinal plants (Asadi et al. 2021)

Medicinal plant	Family	Compound	Retention Time (Min)	Percentage of Total
Allium sativum	Alliaceea	Tetracosamethyl cyclododeca siloxan	38.274	15.824
		Anhydro 5-hydroxy-3-piperonyl-1	38.137	13.344
		1-Amino-1-prtho-chlorophenyl-2	38.016	12.872
		1, 4-Cyclohexadiene, 1, 3, 6-tris	37.92	10.573
		4-Methoxy-3-(3-methoxyphenyl)	37.802	10.201
Glycyrrhiza glabra	Fabaceae	Aristolene	25.58	20.135
		2, 6-Octadien-1-ol, 3, 7-dimethyl	24.704	8.544
		Butanoic acid, 3, 7-dimethyl-2	23.588	7.337
		Lavandulyl acetate	18.293	6.257
		3-Hexen-1-ol, benzoate	23.889	4.373
Rosmarinus officinalis	Lamiaceae	α-Pinene	5.448	9.985
		Bicyclo [2.2.1] heptan-2-one	9.847	7.775
		Bicyclo [3.1.1] hept-3-en-2-one	11.768	7.242
		1, 8-Cineole 2-oxabicyclo	7.256	6.111
		Bicyclo [2.2.1] heptan-2-ol	14.503	5.71
Salvia officinalis	Lamiaceae	β-Thujone	8.903	25.626
		Bicyclo [2.2.1] heptan-2-one	9.865	16.463
		1, 8-Cineole 2-oxabicyclo	7.248	10.556
		Thujone	9.109	6.538
		Veridiflorol	24.33	4.705
Piper nigrum	Piperaceae	Caryophyllene	19.512	36.031
		L-Limonene cyclohexene	7.191	7.02
		3-Carene	6.836	5.167
		Cyclohexene, 1-methyl-4-(5-Methyl)	22.082	5.125
		2-β-Pinene bicyclo	6.222	4.343

Bioassay.

The obtained values of LC_{30}, LC_{50}, and LC_{90}, probit line, slope slope ± error with $\chi 2$ value for five plant essential oils on *E. kuehniella* larvae are shown in Table 2. Results from bioassays revealed that the acute toxicity of *R. officinalis* essential oils on the larvae was more than others. Also, *G. glabra* essential oil showed the lowest acute toxicity in this research.

Table 2. Acute toxicity of the essential oils on the last instar larvae of *Ephestia kuehniella* (Insect number: 360)

Medicinal plant	Slope ± E	LC_{30} µl/l air (95% CL)	LC_{50} µl/l air (95% CL)	LC_{90} µl/l air (95% CL)	χ^2
Allium sativum	1.03 ± 0.21	2.86 (0.91 – 4.88)	9.14 (5.58 – 12.69)	156.39 (74.65 – 815.48)	5.47 ns
Glycyrrhiza glabra	0.83 ±0.13	12.03 (4.76 – 20.63)	51.18 (32.42 – 77.49)	1761.33 (715.73 – 9656.30)	7.63 ns
Rosmarinus officinalis	1.71 ± 0.28	2.19 (0.95 – 3.41)	4.48 (2.65 – 5.98)	24.92 (18.70 – 40.69)	7.67 ns
Salvia officinalis	1.10 ± 0.21	7.84 (3.06 – 12.42)	23.41 (15.76 – 31.77)	339.23 (164.31 - 1652.50)	4.62 ns
Piper nigrum	0.91 ± 0.15	9.39 (3.57 - 15.73)	35.32 (29.97 - 51.58)	905.44 (379.59 - 5356.93)	4.14 ns

CL: Confident limit, LC: Lethal Concentration, E: Error, $\chi 2$: Chi-Square value, ns: non-significant.

Protein concentration in the digestive tube.

The results of determining protein concentration in the digestive tube of *E. kuehniella* larvae are shown in Table 3. The highest protein concentration in the control group was 1.0288 ± 0.0212 mg/ml and the lowest being in *R. officinalis* treatment as 0.7333 ± 0.0329 mg/ml. Also, there was no significant difference among the essential oils of *A. sativum*, *G. glabra*, *S. officinalis*, and *P. nigrum* ($F_{5,12} = 2.95$). In this case, the decreasing trend for all treatments was as follows: the control, *P. nigrum*, *S. officinalis*, *G. glabra*, *A. sativum*, and *R. officinalis*.

Table 3. Protein content in the digestive tube of *Ephestia kuehniella* under essential oils treatment

Treatment	Protein (mg/ml)
Control	1.0288 ± 0.0212 a
Allium sativum	0.7695 ± 0.0.0663 ab
Glycyrrhiza glabra	0.8153 ± 0.0397 ab
Rosmarinus officinalis	0.7333 ± 0.0329 b
Salvia officinalis	0.8424 ± 0.0688 ab
Piper nigrum	0.8446 ± 0.0955 ab

Different letters following the values indicate significant differences (Tukey's test, $p < 0.05$).

Amylolytic activity assay.

The results of amylolytic activity in the digestive tube of *E. kuehniella* larvae are shown in Table 4. Accordingly, the highest activity was observed in the control (0.0551 ± 0.0025 mU/mg) and the lowest being in *R. officinalis* essential oil (0.0373 ± 0.0009 mU/mg). Also, there was no significant difference between the control and *S. officinalis* essential oil, as well as among the essential oil of *A. sativum*, *G. glabra*, and *P. nigrum* ($F_{5, 12}$= 3.56). Besides, the decreasing trend in all treatments was as follows: the control, S. officinalis, G. glabra, A. sativum, P. nigrum, and R. officinalis.

Table 4. Amylolytic activity in digestive tube of *Ephestia kuehniella* under treatment of essential oils

Treatment	Protein (mg/ml)
Control	0.0551 ± 0.0025 a
Allium sativum	0.0471 ± 0.0047 ab
Glycyrrhiza glabra	0.0491 ± 0.0032 ab
Rosmarinus officinalis	0.0373 ± 0.0009 b
Salvia officinalis	0.0547 ± 0.0027 a
Piper nigrum	0.0435 ± 0.0056 ab

Different letters following the values indicate significant differences (Tukey's test, $p < 0.05$).

Proteolytic activity in the alimentary canal.

The results of proteolytic activity in the digestive tube of *E. kuehniella* larvae are shown in Table 5. Based on this, the highest and lowest activity of this enzyme was determined in the control and *R. officinalis* essential oil (5.5063 ± 0.1086 to 3.3028 ± 0.1077 U/mg). Also, the differences among the control, *G. glabra*, and *S. officinalis*, as well as the difference between *A. sativum* and *P. nigrum* were not significant ($F_{5,12}$ = 7.07). The decreasing trend of all treatments was as follows: the control, *S. officinalis*, *G. glabra*, *A. sativum*, *P. nigrum*, and *R. officinalis.*

Table 5. Proteolytic activity in the digestive tube of *Ephestia kuehniella* under essential oils treatments

Treatment	Protein (mg/ml)
Control	5.5063 ± 0.1086 a
Allium sativum	4.5347 ± 0.3238 ab
Glycyrrhiza glabra	5.4353 ± 0.2540 a
Rosmarinus officinalis	3.3028 ± 0.1077 b
Salvia officinalis	5.6223 ± 0.4775 a
Piper nigrum	4.4516 ± 0.5100 ab

Different letters following the values indicate significant differences (Tukey's test, $p < 0.05$).

DISCUSSION

The secondary compounds of plants known as essential oils are the most popular group. They have different effects with important role especially in defense mechanisms of plants against the herbivores (Parsia Aref and Valizadegan 2015; Rafiee-Dastjerdi et al. 2013; Razmjou et al. 2018). These compounds are present in most plant families, especially in the Asteraceae, Lamiaceae, Lauraceae, and Myrtaceae, in which the variety and amount of these volatile compounds are greater. For this reason, these families are called aromatic plants (Bakkali et al. 2008). In the present study, *R. officinalis* and *S. officinalis* are from the family Lamiaceae and the three other plants, *A. sativum*, *G. glabra*, *P. nigrum*, belong to the families Alliaceae, Fabaceae, and Piperaceae, respectively. Based on essential oil analysis, number of secondary compounds in *S. officinalis* and *P. nigrum* was more than the other investigated plants. The essential oils that have more effective compounds made greater insecticidal effects (Asadi et al. 2018b). In this study, this was well observed in the case of *R. officinalis.* In addition, essential oils with less insecticidal compounds showed insignificant effects on this pest. This was observed in the case of essential oils isolated from *S. officinalis* and *G. glabra* (Asadi et al. 2018a, 2018b, 2019, 2021).

Regarding the direct effects of plant essential oils on the digestive enzymes of *E. kuehniella*, Teodoro Martinez et al. (2012) studied the insecticidal effect of labramin which is a lectin-like protein isolated from the seeds of the *Labramia bojeri*, on *E. kuehniella* when reported that it caused 90% mortality on larvae under 1% (w/w) treatment. The presence of 0.25% labramin in the diet caused high larval period, less pupal development, and low percentage of emerged adults. The treatments in

increased levels of trypsin indicated that labramin could affect enzymatic mechanisms by disrupting the peritrophic membranes in the midgut of *E. kuehniella* larvae. Results of nutrition experiments with *E. kuehniella* larvae revealed a decrease in conversion efficiency of ingested and digested food with an increase in approximate digestibility and metabolic cost (Teodoro Martinez et al. 2012). In the present study, purification and investigation of dominant constituents from the essential oils on the digestive properties of larvae were not investigated, but the main effect of essential oils due their presence is argued. Further work on the purification of the dominant constituents from five medicinal plants and the investigation of their effects on different pest will be planned.

Shahriari and Sahebzadeh (2016) investigated the effects of diallyl disulfide on physiological characteristics of *E. kuehniella* and found that the concentration (0.31%) was determined as LC_{50}. Also, activity of digestive enzymes including α-amylase and proteases significantly decreased in the treated larvae. Their results confirmed that diallyl disulfide interrupted larvae digestion with less digestive enzymes activity which showed a significant effect on its metabolism. Our results are in agreement with this research because with application of various treatments, significant changes have been made on various enzymes, which indicate effect of these factors on the digestion process. In another study, Shahriari et al. (2022) studied the response of *E. kuehniella* larvae to toxicity of trans-anethole and found that its LC_{50} value on the larvae was determined as 7.03 $\mu l/g^{-1}$. Also, a significant decrease in activity of digestive enzymes (α-amylase, α and β-glucosidase, and lipase) and proteases (trypsin, chymotrypsin, elastase, amino, and carboxypeptidase) was observed.

Zallaghi et al. (2020) studied the combined effect of *Lavandula angustifolia* essential oils with γ irradiation treatment on some biological aspects of *E. kuehniella* when concluded that there was a significant increase in conversion effect of food consumed and feed inhibition index due to irradiation and essential oil application. The experiment showed that insecticidal effect of essential oils can be increased by primary irradiation. Ajamhassani (2021) studied the effects of ethanolic extracts from *Ferula assa-foetida* and *Aloysia citriodora* on mortality and physiological characteristics of *E. kuehniella* larvae and concluded that *F. assa-foetida* extract significantly reduced the activity of lipase and β-glucosidase enzymes compared to the control, but the effect of *A. citriodora* extract on activity of these enzymes was insignificant than the control. The extracts had also no significant effects on α-glucosidase enzyme activity. The results of the present study are in agreement with the above work because changes in the physiological characteristics of the pest have been observed with the application of treatments. However, chemical compounds of medicinal plants were different.

Studies related to the direct effects of plant essential oils or their isolated compounds on the digestive enzymes of *E. kuehniella* are very limited. Therefore, this study provided some additional data in this field. The Mediterranean flour moth is one of the most important storage pests. For this, use of plant essential oils is a good solution due to their low effects on foods.

RESUME

Asadi M. 2023. Effets de cinq huiles essentielles végétales sur les modifications de la teneur en protéines et des enzymes digestives chez les larves d'*Ephestia kuehniella*. Tunisian Journal of Plant Protection 18 (1): 29-39.

La teigne méditerranéenne de la farine ou teigne du moulin *Ephestia kuehniella* est un ravageur commun des grains de céréales, en particulier de la farine. Dans cette recherche, les effets sub-létaux des huiles essentielles isolées de cinq plantes médicinales, *Allium sativum*, *Glycyrrhiza glabra*, *Rosmarinus officinalis*, *Salvia officinalis* et *Piper nigrum* ont été étudiés concernant la teneur en protéines et l'activité des enzymes digestives chez les larves de ce ravageur, sous des conditions de laboratoire. L'analyse GC-MS des huiles essentielles a montré que le tétracosaméthyl cyclododéca siloxane, l'aristolène, l'α-pinène, la β-thuyone et le caryophyllène étaient respectivement les constituants dominants de chaque huile essentielle. L'activité enzymatique a été étudiée par le traitement utilisant la concentration LC30 contre des larves de troisième stade qui étaient respectivement de 2,86, 12,03, 2,19, 7,84 et 9,39 µl/l d'air. Les résultats ont révélé qu'il y avait des différences significatives entre les traitements concernant la teneur en protéines totales et les activités enzymatiques ($F_{5,12}$ = 2,95, 3,56 et 7,07). Concernant la teneur en protéines totales, le témoin et le traitement avec l'huile essentielle de *R. officinalis* ont été associés aux concentrations les plus élevées et les plus faibles (1,0288 ± 0,0212 mg/ml contre 0,7333 ± 0,0329 mg/ml). L'activité amylolytique la plus élevée a été observée avec le témoin (0,0551 ± 0,0025 mU/mg) et la plus faible avec le traitement à l'huile de *R. officinalis* (0,0373 ± 0,0009 mU/mg). De plus, les activités protéolytiques les plus élevées et les plus faibles ont été observées avec le témoin (5,5063 ± 0,1086 U/mg) et l'huile essentielle de *R. officinalis* (3,3028 ± 0,1077 U/mg). En conséquence, l'huile essentielle de *R. officinalis* pourrait être appliquée pour lutter contre *E. kuehniella*.

Mots clés: Activité, amylase, enzyme, *Ephestia kuehniella*, protéase, teneur en protéines

ملخص

أسدي، محمد. 2023. تأثير خمسة زيوت نباتية أساسية على التغيرات في محتوى البروتين والإنزيمات الهاضمة لدى حشرة *Ephestia kuehniella*.

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تعتبر عثة الطحين المتوسطية أو عثة المطحنة *Ephestia kuehniella* من الآفات الشائعة على بذور الحبوب، وخاصة على الدقيق. في هذا البحث، تم التعرف على التأثيرات شبه المميتة للزيوت الأساسية المستخلصة من خمس نباتات طبية هي *Allium sativum* و *Glycyrrhiza glabra* و *Rosmarinus officinalis* و *Salvia officinalis* و *Piper nigrum* حيث تم تقييم محتوى البروتين ونشاط الإنزيمات الهاضمة لدى هذه الآفة تحت الظروف المختبرية. أظهر تحليل GC-MS للزيوت الأساسية أن tetracosamethyl cyclododeca siloxane و aristolene و α-pinene و β-thujone و caryophyllene كانت على التوالي المكونات المهيمنة لكل زيت أساسي. تمت دراسة فعالية الإنزيمات باستخدام تركيز LC_{30} ضد يرقات الطور الثالث والتي كانت 2.86 و 12.03 و 2.19 و 7.84 و 9.39 ميكرولتر/لتر من الهواء، على التوالي. أوضحت النتائج وجود فروق معنوية بين المعاملات فيما يتعلق بمحتوى البروتين الكلي وأنشطة الإنزيمات ($F_{5,12}$ = 2.95 و 3.56 و 7.07). فيما يتعلق بمحتوى البروتين الكلي، كان الشاهد والمعاملة بالزيت الأساسي لنبتة *R. officinalis* مرتبطان بأعلى وأدنى تركيزات (1.0288 ± 0.0212 مغ/مل مقابل 0.7333 ± 0.0329 مغ/مل). ولوحظ أعلى نشاط للمحلل النشوي مع الشاهد (0.0551 ± 0.0025 ميليوحدة/مغ) والأدنى مع زيت النبتة *R. officinalis* (0.0009 ± 0.0373 ميليوحدة/مغ). زيادة على ذلك، لوحظ أعلى وأدنى نشاط للمحلل البروتيني مع الشاهد (5.5063 ± 0.1086 وحدة/مغ) والزيت الأساسي لنبتة *R. officinalis* (3.3028 ± 0.1077 وحدة/مغ). بالتالي، يمكن استخدام الزيت الأساسي لنبتة *R. officinalis* لمكافحة البكتيريا *E. kuehniella*.

كلمات مفتاحية: الإنزيم، المحتوى البروتيني، المحلل البروتيني، المحلل النشوي، النشاط، عثة الطحين المتوسطية، *Ephestia kuehniella*

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Plant Protection Events

Report

on

The 13th Arab Congress of Plant Protection (ACPP)

Hammamet, Tunisia, 16-21 October, 2022

The 13th Arab Congress of Plant Protection (*ACPP* 2022) *has been held, for the first time in Tunisia, during the period 16-21 October, 2022.*

The Organizing Committee is extremely happy with the great success of this scientific event organized by the Arab Society for Plant Protection (*ASPP*) *and the Tunisian Ministry of Agriculture represented by the National Institute of Agronomic Research of Tunisia* (*INRAT*).

The congress was attended by more than 270 participants from Arab and non-Arab countries.

The Congress started on Monday 17th October with an opening ceremony under the patronage of Prof. Mahmoud Elyes Hamza, Minister of Agriculture, Water Resources and Fisheries of Tunisia,followed by a keynote address by Dr. Sophien Kamoun (Norwich Research Park, UK) entitled "Plant Health Vision for the 21st Century".

The scientific program included four plenary symposia covering the following themes:

1. Plant health for food security and safety.
2. Advances in molecular plant protection and its applications in pest management.
3. Research and innovation for sustainable crop protection.
4. Application of behavioral control tools as a safe and effective alternative in pest management.

The symposia plenary sessions included presentations by eminent invited speakers from well-known Research Centers or Universities (Italy, Germany, France,

United Kingdom, and Switzerland) or from International Organizations (FAO, ICARDA, ICIPE, CIMMYT, EPPO, CIHEAM, AOAD, and IOFS). These sessions included presentations on important plant protection topics such as:

- Mycotoxins as a hidden threat for food and feed safety.

- Importance of phytosanitary regulations and international standards for plant health to enhance food security.

- Conservation and use global plant genetic resources for enhancing insect pest and disease resistance: major foliar diseases of barley as an example.

-"Tomato plant-Trichoderma-Phytophthora nicotianae*", a complex of interaction system for understanding plant defense.*

- Parasitoid pre-adaptation improves biological control of symbiont-protected aphids.

- Integrated modern systematics and applications for mite biodiversity characterization.

- Challenges of automatic counting and identification of insect threats using smart technology.

- How to cope with resistance to insecticides to improve pest management.

- Role of pheromone applications in sustainable crop protection.

- Manipulation of plant pests host-finding and acceptance behavior for practical applications in integrated pest management.

In addition to the symposia, around 40 concurrent oral sessions were organized in different conference rooms, focusing on specialized topics within the plant protection field such as:

- *Soil-borne pathogens,*
- *Red palm weevil,*
- *Fungal diseases,*
- *Bacterial diseases,*
- *Virus diseases and phytoplasma,*
- *Biological control,*
- *Economic entomology,*
- *Plant extracts,*
- *Food security and plant protection,*
- *Chemical pesticides,*
- *Climate change and plant protection,*
- *Transboundary pests,*
- *Nematodes*
- *Diseases of olives,*
- Tuta absoluta,
- *Research coordination in plant health,*
- *Beneficial insects.*

Moreover, along with this rich and diversified program, more than 100 posters on the same above mentioned topics were presented.

Additionally, the ACPP 2022 organized a touristic and agricultural one day trip during which participants visited two private companies specialized in nursey plant propagation materials production, followed by a visit to some historical sites close to Tunis city.

During the congress, a new ASPP Executive Committee for the 2023-2025 period was elected and is composed as follows:

- *Dr. Safaa Kumari (Syria):* ***President***
- *Dr. Ahmad M. Katbeh-Bader (Jordan):* ***Vice President,***
- *Dr. Zinette Melhem Moussa (Lebanon):* ***Secretary-Treasure,***
- *Dr. Emad M. Ghalib Al-Maaroof (Iraq):* ***Member & Chairman of Translation Committee,***
- *Dr. Houda Boureghda (Algeria):* ***Member & Chairman of Publication Committee,***
- *Dr. Hassan Dahi (Egypt):* ***Member & Chairman of Membership Committee,***
- *Dr Asma Najar (Tunisia):* ***Member & Chairman of Honour and Awards Committee,***
- *Dr. Ibrahim Al-Jboory (Iraq):* ***Member & Editor-in-Chief, ANEPPB,***
- *Dr. Khaled Makkouk (Lebanon):* ***Member & Editor-in-Chief, AJPP.***

Acknowledgements: *The Organizing Committee would like to appreciate the financial support of all the sponsors that contributed toward the success of the ACPP 2022. Support was offered by international organizations, governmental and non-governmental institutions, and private companies.*

Prof. Asma Najar
INRAT, University of Carthage, Tunis, Tunisia
Chairperson of the Organizing Committee of the 13th ACPP 2022

Recent Doctorate Theses in Plant Protection (2022/23)

Hlaiem, Sawssen. 2023. Investigation and characterization of pathogenic fungi associated with forest species in Northern coastal dunes of Tunisia. Doctorate Thesis in Agronomic Sciences (Phytiatry), INAT, University of Carthage, Tunis, Tunisia, 195 pp. (*Public Defense*: *09 January 2023*).

Pathogenic fungi are amongst the main causes of forest trees and shrubs diseases. This study aims to investigate the dieback disease in two forests in the north-east (Henchir Kort) and north (Rimel) of Tunisia. The identification of the fungi was carried out by means of morphological and molecular features; then the pathogenicity of the isolates was evaluated and the fungal growth was evaluated at temperatures ranging from 5 to 40 °C on PDA medium. Antagonism tests were performed using direct and indirect confrontations between pathogenic fungi and the antagonist strain of *Trichoderma* obtained in this study. A collection of 115 fungal isolates was obtained from symptomatic branches of *Pinus* trees (*Pinus halepensis* and *P. pinea*) and shrubs species (e.g. *Juniperus oxycedrus*, *Tetraclinis articulata*, *Pistacia lentiscus*, *Olea europeae*, *Erica arborea*, *Retama raetam*, *Quercus coccifera*). Five different genera have been characterized: *Botyosphaeria* spp., *Pestalotiopsis* spp., *Heterotruncatella* spp. *Alternaria* spp. and *Fusarium* spp. The results showed that *Botryosphaeria* spp. were the most frequently isolated fungi, followed by *Pestalotiopsis* spp. Their isolation frequency has been noticed to be significantly correlated to the dendrometric parameters and ecological factors ($p < 0.000$). Morphological identification and genetic analysis of internal transcribed spacer region (ITS) of rDNA, and partial sequencing of the translational elongation factor 1-alpha (Tef-1 α) and β-tubulin (TUB) genes identified 12 fungal species: *Diplodia pseudoseriata*, *D. seriata*, *D. scrobiculata*, *D. africana*, *Neofusicoccum luteum*, *Pestalotiopsis biciliata*, *P. chamaeropis*, *Heterotruncatella spartii*, *Alternaria alternata*, *A. infectoria*, *A. tenuissima* and *Fusarium oxysporum*. The evaluation of the pathogenicity of the isolates confirmed their aggressiveness towards their host plants. *Diplodia scrobiculata* isolate was the most aggressive species. The results of tested temperatures suggested that the optimum growth temperature of the tested isolated was 25°C. The temperatures of 5°C and 40°C inhibited the mycelial growth of all the examined fungi except *D. seriata*, which could grow at 40°C. The genus *Trichoderma* is the most fungal used as biocontrol agent. Morphological and molecular characteristics of the ITS region allow to identify 15 isolates as *Trichoderma harzianum* isolated from asymptomatic branches of *P. pinea* trees. Direct (on PDA medium) or indirect (remote) confrontation tests between fungal isolates and *T. harzianum* showed that the antagonist has inhibited mycelial growth of the pathogenic fungus compared to the untreated control. In conclusion, this study indicated that the majority of the investigated forest types are threatened by dieback, and the forest in northern Tunisia (Rimel) appears to be the most affected. This decline in forests could be mainly linked to the impacts of climate change.

Hanafi, Marwa. 2023. Detection and identification of viruses infecting *Musa* spp. using polymerase chain reaction and high throughput sequencing technologies. Doctorate Thesis in Agronomic Sciences and Biological Engineering, Gembloux Agro-Bio Tech, University of Liege, Belgium, 212 pp. (*Public Defense*: *03 July 2023*).

Banana mild mosaic virus (BanMMV) (Betaflexiviridae, Quinivirinae, unassigned species) is a filamentous virus infecting *Musa* spp. with a very wide geographic distribution. It is defined as a significant risk for the banana sector in particular in the various DROMs, and seems to fulfil the criteria of a regulated non-quarantine pest. Therefore, it is important to establish appropriate control measures based on the setting-up of specific and sensitive diagnostic techniques. BanMMV displays a very high genome variability which makes its molecular detection by specific primers particularly challenging, and requires the development of diagnostic tests with high inclusivity. BanMMV detection and/or sanitation remains labor- and time-consuming. The current BanMMV indexing process for an accession requires the testing of no less than four plants cultivated in a greenhouse for at least 6 months and causes a significant delay for the distribution of the germplasm. The main objective of this thesis was to improve the diagnostic of BanMMV from *Musa* accessions. This study aimed to design new diagnostic primers in order to improve detection of BanMMV, following the identification of novel isolates of the virus. It also aimed to test the use of banana in vitro plants in order to accelerate the testing process and evaluate virus therapy success. In this context, a discrepancy in test result was observed between electron microscopy and immunocapture (IC) reverse transcription (RT) polymerase chain reaction (PCR) test results for one asymptomatic banana accession. The absence of molecular detection and the presence of filamentous particles suggested the presence of a new variants or new virus. The accession underwent high throughput sequencing which allowed the identification of two complete genomes of BanMMV with high nucleotide identity that constitute the two novel isolates of BanMMV found in this accession. These findings triggered the development of a new diagnostic primer based on these two new sequences and the BanMMV CP sequences already published in GenBank. A retrospective analysis of 110 different germplasm accessions from diverse origins was performed in order to compare BanMMV CP9 and BanMMCP2 primers. Five accessions showed contrasting results. The new primer missed the detection of BanMMV infection from three accessions. Similarly, BanMMCP2 failed to detect BanMMV infection from two banana accessions. Interestingly, the analytical sensitivity was better with BanMMV CP9 comparing to BanMMCP2. Through this study, we recommended the use of the two primers successively to improve the inclusiveness of the protocol. HTS technologies are one of the most important advances that have revolutionized molecular diagnostics. Their adoption in plant pest diagnostics, in particular plant virus diagnostics, has been growing steadily in the last decades. In same line, this study showed the improved performance of these technologies in BanMMV detection in comparison to the conventional molecular techniques from the same plants (in absence of therapy). These without a priori technologies were much more sensitive than RT-PCR from the same in vitro plants with 100% of diagnostic sensitivity (DSE) for HTS comparing to a DSE of 65% for RT-PCR. Interestingly, HTS technologies allowed the identification of a new species from these samples, with a genome of 7,364 bp, presenting a typical genome organisation of Betaflexiviridae members after annotation of its new contig. Thus, it could be interesting to consider the in-depth biological characterization of this new species. This would help to know more about the symptomatology, transmission, host range

of the new species, and its potential impact on either banana industry and/or the environment. In summary, this study suggested two tests for the detection of BanMMV from in vitro plants. HTS technologies could be performed from RNA extracts of pooled leaves or bases. If these technologies are not available or too expensive, RT-PCR could be applied instead on individual RNA extracted from at least four plants. This proposed methodology helps to avoid the greenhouse cultivation and thus can save time and space.

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o of the cover page: *Chrysoperla lucasina* (Courtesy *Wejdène Karouia*)

A Tunisian Half-Yearly Journal of Plant Health Sciences (TJPP)

Plantae Senae in Terra Sena