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CHARCOAL ROT OF THE SUNFLOWER ROOTS AND STEMS (*MACROPHOMINA PHASEOLINA* (TASSI) GOID.) - AN OVERVIEW

Otilia COTUNA[1,2], Mirela PARASCHIVU[3], Veronica SĂRĂȚEANU[1]

[1]Banat`s University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania", 119 Calea Aradului, Timisoara, Romania, Phone: +400256277007; Mobile phones: +0722527504; +0723153457; +0722800338; Emails: otiliacotuna@yahoo.com; veronica.sarateanu@gmail.com
[2]Agricultural Research and Development Station Lovrin, Timiş County, Romania, Phone: +40 0256381401; Mobile phone: +0722527504; Email: otiliacotuna@yahoo.com
[3]University of Craiova, Faculty of Agronomy, 19 Libertății Street, Craiova, Dolj county, Romania, Phone: +400251418475, Mobile: +40773818957; Email: paraschivumirela@yahoo.com

Corresponding author: paraschivumirela@yahoo.com, veronica.sarateanu@gmail.com

Abstract

Charcoal rot of the sunflower roots and stems is present worldwide in all the areas with arid climate where is determining major loses. The etiologic agent of this disease is the fungus Macrophomina phaseolina considered as invasive due to its great range of host plants (over 500 species) and the very high harvest loses of the cultivated crops, mostly sunflower. The present climate changes are favouring the spread of the disease in areas where it wasn't present before. The climatic simulations realised until now in Europe shows the adaptation trend of the fungus in areas from Central and Southern Europe. The survival of the pathogen Macrophomina phaseolina in soil for long time periods makes its control almost impossible. In this work it was brought to attention the state of the art of the researches regarding the symptomatology, pathogeny, epidemiology and the control of this pathogen in conjunction with the climate change. The control strategies should highlight the prevention methods and the biological ones and less attention to be addressed to the chemical ones. The fungal and bacterial agents can be in future a viable alternative for the control of the pathogen M. phaseolina.

Key words**:** *Macrophomina phaseolina, Rhizoctonia bataticola, sunflower, charcoal rot, climate change*

INTRODUCTION

The fungus *Macrophomina phaseolina* (Tassi) Goidanich is framed from taxonomic point of view in the family *Botryosphaeriaceae*, genus *Macrophomina*, species *phaseolina.* Even the teleomorph form of the fungus isn't known, Crous *et al.* [21] have demonstrated that *Macrophomina phaseolina* belongs to the family *Botryosphaeriaceae*. The microsclerotial form of the fungus is *Rhizoctonia bataticola* (Taubenhaus) E. J. Butler. The microsclerotia are forming on host plants stems and inside the stem [27][46][22].
Macrophomina phaseolina is a polyphagous fungus able to infect more than 500 cultivated and wild plants species [26][27][30][28][35]. The great number of host plants shows that it is a non-specific pathogen [35]. Due to the great number of hosts and the great capacity of producing yield loses, sometimes even 100 % loss, the pathogen is considered by the specialists as being "invasive" [26][24].
In sunflower *M. phaseolina* is a very important pathogen, able to produce great yield damages and even to compromise entire crop. The economic importance of this pathogen for sunflower is given by the severe symptoms produced as flower heads with reduced diameter, low seed weight, low oil quality and even the death of the plants in case of the massive infections [27][46][8].
The pathogen is extremely dangerous in the arid areas of the world (mainly the tropical and subtropical ones) where constantly produces damages in crops [26][32][49]. Farr and Rossman [31] show that the appearance of the fungus in different species of plants increases constantly at worldwide level.

According with other researchers, yield loses due to the charcoal rot can reach even to 60% [53]. Others have reported sunflower yield loses comprised between 20 and 36 % [35] [10]. In the years with favourable conditions for the pathogeny of this fungus there were reported total harvest loses of the sunflower crops [35][36].

The charcoal rot has been reported in numerous countries as are: Hungary, Romania, Spain, Serbia, Italy, Bulgaria, Portugal, Russia, U.S.A., Czech Republic, Turkey and Slovakia [23].

In Romania are few studies regarding the presence of the pathogen in the sunflower crops. In the year 1982 Comes *et al*. [19] doesn't describe this pathogen in the book "*Phytopathology*" even during 1981-1983 period this had created problems in numerous European countries [55]. Bontea [14][15] describes later the pathogen in the book "*Ciuperci parazite și saprofite din România*" (*En. Parasyte and Saprophyte Fungi from Romania*). In 1990, Docea *et* Severin [27] describe charcoal rot in three crops (maize, sunflower and soybean) in the book"*Ghid practic pentru recunoașterea și combaterea bolilor plantelor agricole*" (*En. Practical Guide for the Recognition and Control of the Crop Diseases*). An interesting study was published in 1996 by Ioniță *et al*. [36] regarding the presence of this pathogen in different agricultural crops from Romania (soybean, sunflower, sugar beet, bean and colza). In this study the authors have reported high attack frequencies of the fungus *Macrophomina phaseolina* in sunflower during 1992-1994, they being comprised between 46.5 % and 92.7 %. In the year 2021, the pathogen has been reported in several sunflower crops from Western Romania where it has produced great yield loses, some crops being compromised in totality [20].

The present climatic changes (mainly the increase of the temperatures) could influence positively the pathological - system *Macrophomina phaseolina* (Tassi) Goid. - *Helianthus annus* L. in the areas with moderate climate. In the countries with temperate climate *Macrophomina phaseolina* produces infections only in the years when there are registered high temperature and dryness. These types of situations have been reported during 1981 – 1983 period in almost all European countries, less in Poland [55]. Coakley *et al.* [17], claim that climate changes can have direct impact on the pathogens from sunflower crop, favouring the infections. The warm and dry weather is stimulating the pathogen *Macrophomina phaseolina*. According with Sarova *et al*. [50], the warm and dry weather with temperatures comprised between 28 – 30 ºC and the deficit of the water from soil are favouring the infection with this fungus.

In general, the fungi that are resisting in soil as sclerotia for long time periods could tolerate easier the unfavourable climatic conditions (e.g. drought). The absence of water in soil could predispose the sunflower plants to the attack of the systemic pathogens that are destroying and blocking the transport tissue [57][25].

In Romania, the fungus *Macrophomina phaseolina* could be present every year from now in sunflower crops, on the background of the climatic changes, respectively the increase of the temperatures over the multiannual averages. This isn't a good perspective having in view that the fungus is difficult to control. To the climate conditions can be added the soil conditions, improper rooting of the plants, boron deficiency, being known that the pathogen is infecting easier the plants affected by physiological disorders [46].

Nowadays climate change is an important global issue threatening plants health, particularly by unfavourable temperatures and precipitations leading more and more to food insecurity worldwide. However, climate change is challenging scientists to look for new methods to cope with negative impact of climate change on crops, breeding for more tolerant varieties to abiotic stresses, improving cropping technologies and controlling biotic constrainers (pests, diseases and weeds) [11][12][13][42][45][54].

The present work is an overview over the fungus *Macrophomina phaseolina* that is spreading in new areas where was present only sporadically, on the background of the climate change. The present climatic changes could bring this pathogen in actuality in many areas with moderate climate, threatening the sunflower crops in future. The formulation of some conclusions on the background of new researched from this field is necessary.

MATERIALS AND METHODS

This work is an overview on the present state of the art of the researches regarding the fungus *Macrophomina phaseolina* in sunflower crop and the spreading capacity in new areas due to the climate change.

For the achievement of this overview there were used several approaches (methods) specific for the realisation of such article: systematic, semi-systematic, bibliometric and integrative. In this way there were identified with careful attention the most relevant articles, reports and scientific works from the field for the chosen topic. These were analysed, compared, synthesised and integrated in this overview paper. The semi-systematic review highlights usually the progress achieved in the researches from the analysed field [61][52]. At the methods used is added a simple technique of text analysis (*text mining*) to identify the best scientific contributions in the field [52].

The review papers are recognized in general as research method more and more relevant, because identifies the eventual lacks and synthesizes the relevant published literature for a certain topic, thus coming to help the young researchers too [48][52].

RESULTS AND DISCUSSIONS

Symptomatology

In sunflower, the fungus *M. phaseolina* infects the plants during the first development stages. With all of these, the symptoms appear only near to the end of flowering stage [27]. The fact that the first symptoms appear at the plant maturity stage indicates a latent infection. Often, the plants that apparently show a good development evolution in the first vegetation stages will develop severe symptoms at the maturity. The plants will maturate early due to the infection and they will have smaller flower heads, sometimes deformed, and a low number of achenes. In the central part of the flower head many flowers are aborted [28]. Near to the end of flowering stage of the sunflower there appear the first symptoms produced by the pathogen on stems and roots. The stems are usually affected in the basal area in their inferior third part [27][46]. On the attacked stem surface appear as greyish discolouring, sometimes with silvery reflections, characteristic for this pathogen (Figure 4a). The fungus will form numerous black microsclerotia in the attacked tissues, that confer a grey-black colour, compared by some authors with a fine charcoal powder. The pith from the inferior part of the stem becomes blackish due to the microsclerotia [60][39][38] (Figure 6a). Sometimes in the affected part the stem it is without pith and in other cases the pith isn’t totally destroyed, being separated in disk-like segments parallelly disposed as” *tiered plates*” [27][46] (Photo 1).

Photo 1. Sunflower stem pith attacked with ”*tiered plates*” like symptom; the microsclerotia are visible (in Timiș County, Romania).
Source: Original photo by Cotuna O. (2021) [20].

The diseased epiderma is detaching easily from the stem. On the surface of the affected epiderma, and below the surface are forming from abundance black microsclerotia that are determining the charcoal-like aspect [39] (Photo 4b and Photo 5a). According with Csüllög *et al*. [24] the attacked stems have charred aspect and the epiderma is detaching.

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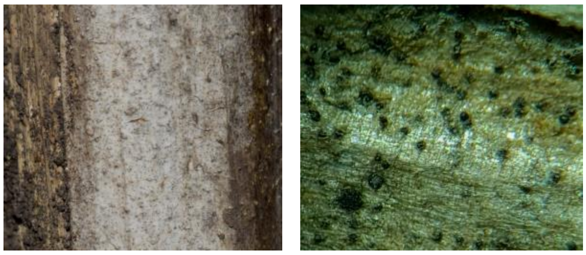
In the same time with the existence of the microsclerotia the fungus can produce pycnidia on the stems, but this happens more rarely in natural conditions. The same authors show that in the first infection stage the sunflower plants are manifesting fading symptoms, then appear the yellowing and senescence of the leaves that are remaining attached to the stems [51].

Photo 2. Fallen of the sunflower crop affected by charcoal rot attack (in Timiș County, Romania).
Source: Original photo by Cotuna O. (2021) [20].

Photo 3. Fallen sunflower plants due to the charcoal rot attack (in Timiș County, Romania).
Source: Original photo by Cotuna O. (2021) [20].

In the same way are infected the roots too. The fungus enters in the secondary and tertiary roots and after that will reach the primary root. In this way the fungus infects the fibro-vascular system of the roots and the basal internodes blocking the transport of the water and of the nutrients. Due to the destroyed root system the diseased plants can be easily uprooted from the soil and they die lastly (Photo 2 and Photo 3). On the diseased roots are forming black microsclerotia [3][27] (Photo 5b and Photo 6b).

Plant fading can start in the flowering stage and continues till to they get mature. In such situations the yield loses can be very high [40] [47].



a b

Photo 4. a) Greyish discolouring with silvery reflexions on sunflower stem; b) microsclerotia on the sunflower stem epiderma surface.
Source: Original photo by Cotuna O. (2021) [20].

a b

Photo 5. a) microsclerotia below the sunflower stem epiderma; b) microsclerotia on sunflower diseased root.
Source: Original photo by Cotuna O. (2021) [20].

a b

Photo 6. a) Microsclerotia in sunflower stem pith; b) sunflower root totally browned.
Source: Original photo by Cotuna O. (2021) [20].

Pathogeny and epidemiology

The charcoal rot fungus can resist as microsclerotia in soil on the litter but also it can resist on the seed mass [28][23][46][4]. There are researches that show positive correlations between the level of the inoculum source from the seed mass and infection severity [4][38]. The microsclerotia can survive in soil from two to 15 years [9][3][23].

The microsclerotia of the charcoal rot fungus can have spherical, oval or elongate form. The colour differs depending by the age of the microsclerotia (Photo 7). In the first development stages they are light brown and they become black once they are getting mature [43]. According with Docea *et* Severin (1990) the microsclerotia are usually ovoid shaped and have a diameter comprised between 50 - 300 μm.

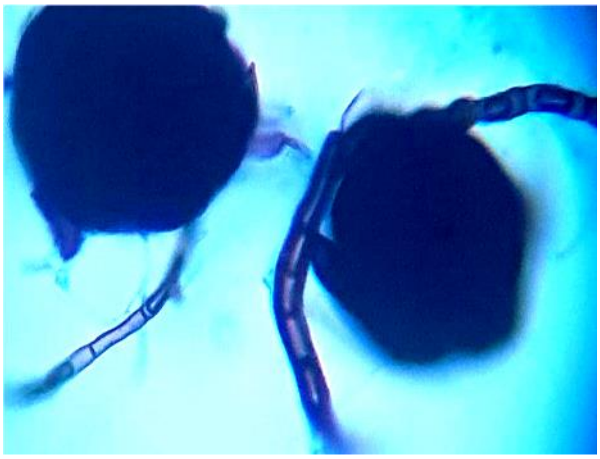


Photo 7. Microsclerotia of *Macrophomina phaseolina* sampled from diseased sunflower plant (microscope photo x40).
Source: Original photo by Cotuna O. (2021) [20].

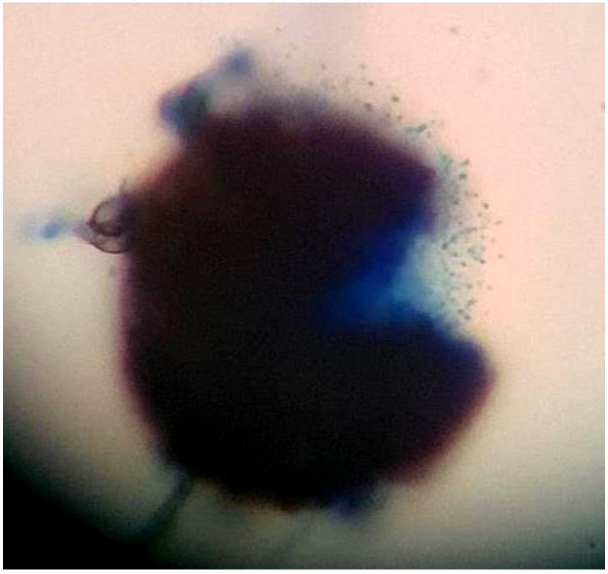


Photo 8. *Macrophomina phaseolina* pycnidia sampled from diseased sunflower stems from western Romania (Timiș County) (microscope photo x40).
Source: Original photo by Cotuna O. (2021) [20].

The fungus pycnidia are forming rarely in nature, they have greater sizes compared with the microsclerotia and have the colour from brown to black (Photo 8). According with Lakhran *et al.* [41] the pycnidia can be globulous or with irregular shape and are presenting an ostiole. The pycniospores can be oval - prolonged or cylindrical, colourless and unicellular. The size of the pycniospores is comprised between 14 - 32 x 5 - 11 μm [27].

The importance of the pycnidia in the epidemiology of the fungus *M. phaseolina* depends in a great measure by the host plant, but also depends by the fungal isolate [2][6].

The fungus attacks plants mostly during the drought periods associated with high temperatures. The temperature, air humidity and the available water are very important for the realisation of the infection with charcoal rot fungus. The microsclerotia are germinating at temperatures comprised between 30 – 35 ºC [43]. The attack of the fungus is mainly influenced by the soil temperatures that has to be greater than 28 ºC and by the rainfalls [28].

In the first development stages of the plants the fungus has the capacity to infest the host in 24 – 48 hours in conditions of low temperature and high humidity. In this phenophase usually the symptoms aren't visible and the fungus is developing slow in the attacked plants until to the development of the achenes. The symptoms characteristic for the disease are becoming visible when the humidity is low and the temperature is high during the seed formation stage [5][38].

In recent studies is shown that the fungus *Macrophomina phaseolina* produces high amounts of toxins and enzymes that are destroying the cell walls. With the help of the hydrolytic enzymes the fungus degrades the polyzaharides from the cell walls and the lignin. The pathogen produces enzymes for the hydrolysis of the cellulose (exocellobiohydrolases, endoglucanases and β-glucosidases) and for the lignin degradation (lignin peroxidases, laccases, galactose oxidases, chloroperoxidases, haloperoxidases and heme peroxidases). The toxins and enzymes produced by the pathogen are

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favouring the infections leading to the appearance of the first symptoms and finally to the host death [37][43].

According with Popescu [46] the fungus usually infects the plants with physiological disorders, when the growth of the main root is stopped and the secondary roots start to grow old. In such plants the root system will be colonized by *Fusarium sp*. and by other fungi that are preparing in this way the root tissues for the infection with *Macrophomina phaseolina*. The mechanical lesions, high plants density and pests attack are factors that are favouring the infection with the charcoal rot [51][4].

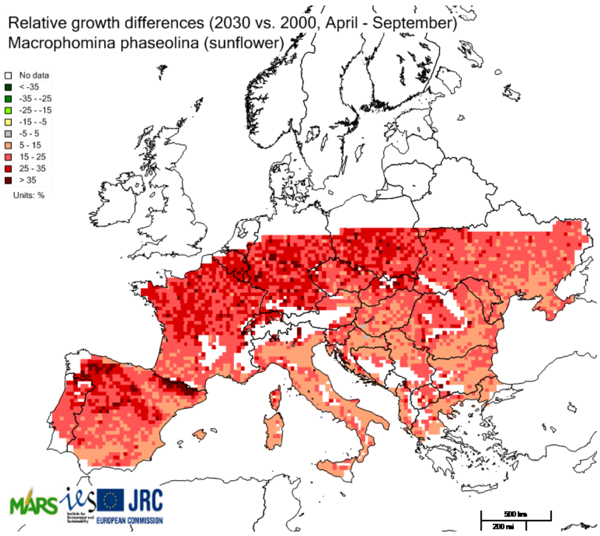
Influence of the climate changes on the fungus Macrophomina phaseolina

Macrophomina phaseolina is a pathogen that is developing in conditions of hot and dry climate. Temperature growth in the temperate regions accompanied by the absence of the rainfalls could create great problems in the sunflower crops [24]. High temperatures and the deficit of water in soil affects the sunflower plants development making them vulnerable to the attack of the pathogen *M. phaseolina* that due to the microsclerotia that are forming in the plant tissues (in roots and stems) succeeds to survive in unfavourable conditions [18][50].

The question that arises is:"*Exist there climatic scenarios in Europe for the future evolution of the pathogens that survive in soil in the case of major climate changes*". The answer is "*Yes. Exist.*"

Regarding the fungus *Macrophomina phaseolina* there was realised a study on the potential answer in the cases of climate changes predicted for Europe. There were realised several experiments simulating conditions of high temperature (demanded by this pathogen). The simulation was realised in Europe with the help of IPCC A1B emission scenario as an achievement of the global climatic model Hadley - CM3. There have been obtained more many simulations of daily weather data for a period of 30 years. The scenarios focused on the year 2030 were compared with those from the year 2000. The response of the fungus *M. phaseolina* has leaded to the conclusion that it will grow and develop better in areas from Central Europe and in Southern Europe. The analysed fungi in the framework of this climatic scenario have shown a general trend to adapt in the cooler areas from Europe [42].

According with the results of this climatic scenario, there is believing that the fungus *M. phaseolina* will register increases of the incidence in countries from the continental Mediterranean areas as is Italy, France, Spain, but also it will enter in countries from Central Europe with relatively cooler climate. The spreading trend of this pathogen specific for warmer areas to the temperate areas was highlighted during the last years, there being reported more often great damages in the crops from these areas where the pathogen was appearing only occasionally only in the year with favourable weather conditions [42] [50] (Map 1).



Map 1. The relative growth differences of the spread of *M. phaseolina* in sunflower in the climatic scenario 2030 vs. 2000.
Source: Manici *et al*. (2012) [42].

In the year 2021 this fungus has destroyed several sunflower crops from Western Romania (Banat Plain, part of the Pannonian Plain) [20].

Can we control this pathogen?

Charcoal rot pathogen is very difficult to control, mainly due to its extraordinary capacity of survival of the microsclerotia in soil. From this point of view the chemical control of the disease is extremely difficult and inefficient from economic point of view.

In this way, the prevention measures represent the fair approach for the control of this pathogen [31]. There is recommended the use of resistant hybrids, irrigation of the crops in drought conditions accompanied by high temperatures, destruction of the infected litter, cultivation of the crops in conditions of proper soil texture, crop rotation *etc*. Regarding the crop rotation there are mentions assuming that it isn't always effective due to the fungus polyphagia (it can infect over than 500 species of cultivated and wild plants) [30][28][46]. Docea *et* Severin [27] recommends the use for seeding of the seed free of microsclerotia, superior quality soil works, crop hygiene and crop rotation.

The chemical control of the fungus *Macrophomina phaseolina* is extremely difficult because there aren't existing fungicides to control the pathogen at the root level. Nowadays are ongoing numerous researches in this topic [16][41][43]. In laboratory research developed by Csüllög *et* Tarcali [23] they show that fungicides aren't efficient against this fungus. They have tested several fungicides: azoxystrobin, cyproconazole, prochloraz and pyraclostrobin. From those only prochloraz has stopped the growth of the hyphae and microsclerotia. The conclusion of the research is that only the genetic resistance could have efficient results in the control of charcoal rot. In the infected soils can be applied fumigations with allowed substances. This methos is quite costly and pollutant, from this reason being used at small spatial scale [46].

Practically the fumigation substances are forbidden, but bio-fumigation could be an alternative for the management of the charcoal rot pathogen in sunflower. Bio-fumigation consists in cultivation of a cover crop from the family *Brassicaceae* and its incorporation in soil with the purpose to produce biocide substances in soil. Very recent researches show the biocide effects of the isothiocyanates on the pathogenic fungi from soil [1]. The efficiency of the bio-fumigation is variable being influenced by many factors shows Motisi *et al.* [44]. The same author brings in attention growths of the attack intensities of some pathogens after the application of bio-fumigation [44]. Thus, there are necessary more researches to attest if bio-fumigation is effective in the control of the pathogens from the sunflower crops and to highlight the potential disadvantages of this method [1].

A non-pollutant method that can be used for charcoal rot control is solarization of the infested fields. This methos is also difficult to be applied on big surfaces. At the application of this method the land cannot be cultivated.

There is interest also in the biological control by using antagonists (fungi and bacteria) and mycorrhizae. In this way are ongoing numerous tests in laboratory regarding their efficiency in the control of charcoal rot.

There is known from long time that the arbuscular mycorrhizae have positive effects on plants, favouring the absorption of the nutrients and protecting the plants against the attack of some pathogens and pests [43]. In the case of sunflower crop there was noticed that the symbiosis with arbuscular mycorrhizae cannot stop the infection with *M. phaseolina* [43].

In the integrated pathogens control systems of the sunflower crop the biological agents (fungi, bacteria and viruses) can replace some chemical treatments. The antagonist fungi *Trichoderma viride* and *Trichoderma harzianum* proved to be effective for the control of the fungus *M. phaseolina* [7]. In general, the fungi from the genus *Trichoderma* proved to be effective biological control agents in the management of this sunflower pathogen [34]. From the *Trichoderma* genus, *T. longibrachiatum* has reduced the mycelium growths of the charcoal rot fungus by modifying its structure, there being implied the direct inhibition and the microbial organic volatile compounds (antibiosis) [34].

Very good efficiency was registered in the case of the combinations between the fungus *Trichoderma harzianum* and the bacteria *Pseudomonas fluorescens* that have reduced the germination of the charcoal rot sclerotia in a rate of 60 % in conditions of natural infection [53].

Bacterial biological agents from the rhizosphere area are now more tested for the biological control of the fungus *M.*

phaseolina. Some rhizobacteria have proved their capacity to inhibit the growth of this fungus. Thus, *Bacillus amyloliquefaciens* and *Bacillus siamensis* have proved a very good fungistatic effect on the charcoal rot sclerotia [56][33]. The results obtained by Torres *et al.* [56] show that the rhizobacteria *Pseudomonas fluorescens* and *Bacillus subtilis* can inhibit *M. phaseolina* according with *in vitro* and *in vivo* tests. A recent study shows that *B. contaminans* could stop the development of *M. phaseolina* by reducing its pathogeny [59]. In the management of the charcoal rot of the sunflower crops is essential the use of a control strategy that includes preventive measures, biological measures and less the chemical measures, last ones being mostly inefficient. Only in this way it can be avoided the harvest loses that the pathogen is able to produce, thus being important to diminish the impact of the pesticides on the environment by limiting their excessive use [58].

CONCLUSIONS

Charcoal rot of sunflower roots and stems is a very dangerous plant disease mostly in the tropical and subtropical areas. In the last years it was noticed the expansion of the disease in new areas, mostly due to the climatic change. The pathogen *Macrophomina phaseolin* is expanding slowly and surely in the areas with relatively cooler climate on the background of temperature increase and the water deficit in soil. In temperate areas the presence of this pathogen is more often reported, not sporadically as it was in the past.

The invasive feature of the pathogen *M. phaseolina* was highlighted in many studies. Thus, the great number of host plants, the distribution at global level and climate changes show that this fungus has potentially great importance for the future of sunflower crop.

The analysed literature from this work shows that this fungus is difficult to be managed due to the survival mode as sclerotia into the soil. The inefficiency of the chemical control methods was highlighted in most of the published articles until in 2021. The approach of the prophylactic and biological control methods is essential in present. The new directions in charcoal rot control research from nowadays with emphasis on the biological control agents are encouraging, even there are needed more numerous tests in field natural conditions.

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