PLANT PESTS AND DISEASES IN SOME ORGANIC GREENHOUSES FROM MUNTENIA REGION

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Abstract: Organic greenhouse production has expanded during the past ten years. However, plant pests and diseases are the major impediment in obtaining good quality produce and yield. The purpose of this study was to highlight the phytosanitary problems faced by farmers in the organic vegetable production system from Muntenia region.-The list of arthropods fauna comprises 15 species, most of them specific to the studied area and analyzed cultures. Our survey highlights the presence of three invasive species: *Tuta absoluta*, *Nezara viridula* and *Halyomorpha halys* and their potential to become important pests of greenhouses tomatoes. Ten phytopathogenic agents were also present in the studied organic greenhouses. Among these, five were phytopatogenic fungi of Alternaria spp., *Cercospora beticola*, *Passalora fulva*, Septoria spp. and Erysiphae spp., and three were bacterial pathogens of *Pseudomonas syringae*, *Ps. lachrymans* and Pectobacterium spp. Two viral diseases were also detected based on plant disease symptoms, TSWV and CMV. On the other hand, the spectrum of natural enemies was very well represented in the organic production system. The best ways to reduce the infection are considered to be prevention and agro-technical measurements.

Key words: *organic greenhouse, plant pests and diseases*

INTRODUCTION

Organic greenhouse production has expanded during the past ten years. In Romania, the Ministry of Agriculture and Rural Development reported an increase in organic agriculture lands of 23.87% from 2010 to 2016. This is encouraged mainly by consumer interest for fresh and good quality food, as well as customer availability to pay a higher price for healthier groceries. Nevertheless, pests and diseases attack can diminish and contaminate organic production at a higher rate. There is, however, a greater advantage in the organic production system compared to conventional and intensive ones. A natural balance is achieved, for the benefit of growers, due to pest's natural enemies and microbial competitors of plant pathogens. These natural settings inspire biological control strategies in order to find appropriate solution in diminishing the attack with minimum costs. By analysing these ecological events and reproducing some of its regulating systems we could improve the plant's health.

The aim of this study is to reveal the major pathogens and pests found in two organic greenhouses from Muntenia region, and to analyse the ecological systems that contribute to the plant's health and phytosanitary balance.

MATERIALS AND METHODS

Plant pests and diseases, from two organic greenhouses of Muntenia region, were evaluated during the vegetation season of 2016. These greenhouses were located in Joiţa (Giurgiu County) and Pasărea (Ilfov County).

Vegetable plants from different botanical families, like Solanaceae, Cucurbitaceae, Fabaceae, Chenopodiaceae, Apiaceae, were analyzed *in situ* and laboratory.

Various sampling techniques were used to quantify pest's populations: periodically visual surveys, in situ counts and trapping with pheromone and yellow sticky traps (Figure 1). Plants were also visually evaluated *in situ* for disease symptoms and pathogen attack.

Figure 1. Yellow sticky trap
used for capturing and monitoring mobile insects

For laboratory analysis, biological samples were collected. These samples consisted of/in leaves colonized by aphids, beneficial insects, vegetal material with phyto-pathogenic infection, and soil samples. The laboratory examination was performed according to each sample type. The insect traps were examined under binocular stereoscope. Plant pathogenic fungi and bacteria were first studied under the microscope using different vital or complex staining methods. Some of the pathogens were isolated in pure cultures and tested for different biochemical reactions on special culture media (Severin & Cornea, 2009). Regarding obligate fungal parasites, if the cultivation was not possible on artificial media, we maintained the infected vegetal material in humid chambers, until the pathogen developed its characteristic growth.

RESULTS AND DISCUSSIONS

The spectrum of identified pests included arthropod species commonly found in Muntenia region, mostly insects from the orders Hemiptera, Thysanoptera, Coleoptera, Lepidoptera and Diptera (Table 1) as well as alien invasive species for our country.

The commonly found species include *Tetranychus urticae, Frankliniella occidentalis, Helicoverpa armigera* (Figure 2) and several Hemipterae species. Most of these species are polyphagous, known to have a large distribution in our country, considered as agricultural pests but also present on ornamental plants and urban green areas (Ciceoi et al., 2017).

Figure 2. The polyphagous pest *Helicoverpa armigera* on tomatoes (Pasărea, Ilfov County)

Table 1. Pests incidence in solanaceous vegetables

Taxonomic group	Specie	Pest incidence in			
		Tomatoes		Pepper	
		Joiţa	Pasărea	Joiţa	Pasărea
Acari Tetranychidae	*Tetranychus urticae*	+	+	+ +	+
Thysanoptera Thripidae	*Frankliniella occidentalis*	+ +	+	+	+ +
Diptera Agromyzidae	*Liriomyza* spp.	+	+	0	0
Lepidoptera Gelechiidae	*Tuta absoluta*	+ + +	+	0	0
Lepidoptera Noctuidae	*Helicoverpa armigera*	+ +	+ +	+ +	+
Hemiptera Aleyrodidae	*Trialeurodes vaporariorum*	+ + +	+ +	+ + +	+ +
	Bemisia tabaci	+ + +	+ +	+ + +	+ +
Hemiptera Aphididae	*Aulacorthum solani* *Macrosiphum euphorbiae* *Myzus persicae* *Aphis* spp.	+ + +	+ +	+ + + +	+ + + +
Hemiptera Pentatomidae	*Nezara viridula*	+ +	+	+ +	+
	Halyomorpha halys	+	0	+	0
Coleoptera Chrysomelidae	*Leptinotarsa decemlineata*	+	+	0	0

Where: (0) no incidence; (+) reduced incidence; (+ +) moderate incidence; (+ + +) high incidence; (+ + + +) significant incidence.

Among the alien invasive bugs we point out *Nezara viridula* (Figure 3). The pest is also known as the southern green stink bugs. Both larvae and adults cause damages. It is highly polyphagous on many crops, and a cosmopolitan species. *N. viridula* originates from equatorial region (Panizzi, 2008). First occurrence in Europe was reported in 1998, in Italy (CABI/EPPO, 1998). However, its presence in Romania was not mentioned until 2010, when it was accidentally observed in Timişoara (Timiş County) on tomato plants (Grozea et al., 2012). This stinky bug is causing both quantitatively and qualitatively losses, by injuring plants, inducing spotted aspects of fruits and altering fruits flavor. In 2016 survey, we found

the pest in both locations, on tomatoes and pepper with moderate occurrence in Joita and at lower rate in Pasarea greenhouses.

Figura 3. *Nezara viridula* larvae on tomato fruits

The tomato leaf miner moth (*Tuta absoluta*) is another alien invasive species present now in Muntenia Region. It was first mentioned in Romania in 2009 in Botoşani County (Leaotă, 2009). The insect has rapidly spread all over the country, after one year being mentioned in various regions: Maramureş, Bihor, Arad, Ilfov, Mureş, Cluj, Covasna, Vaslui (Cean & Dobrin, 2009). The caterpillar feed with the mesophyll tissues of leaves, can burrow into the stem and attack the fruits causing significant damage for tomato production. The main hosts are tomatoes but it has also been reported on other crops, mostly Solanaceae. In 2016 study, we found this pest on tomatoes in both locations, with a higher incidence at Joita greenhouse.

In the ecological conditions from Joița, mostly on pepper plants, it was observed that the aphid populations were naturally decreased by some natural enemies of these pests: *Aphidoletes aphidimyza,* some lady beetles, lacewings, syrphids, spiders and parasitoids. We also have identified entomopathogenic fungi from *Entomophthorales* order (Figure 4) that considerately contributed in reducing pest populations from a high to moderate incidence.

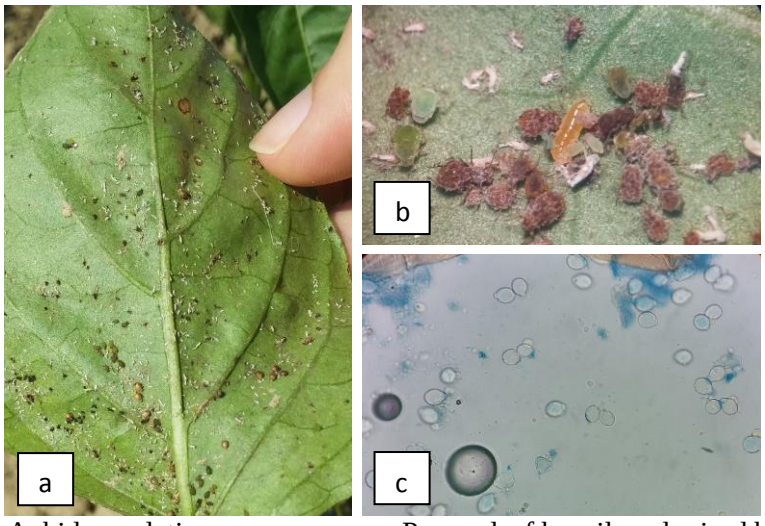

Figure 4. Aphid populations on pepper: a - Pepper leaf heavily colonised by aphids; b - Aphids population infected with *Entomophthora* sp. fungi, along with *Aphidoletes aphidimyza* larva not susceptible to infection; c - *Entomophthora* sp. conidia.

Although we found entomopatogenic infections only on aphid populations, it has been demonstrated that *Entomophthora* sp. are also mite-infecting fungi (Carner, 1976).

Regarding plant diseases, a wide spectrum of phytopathogens was identified during the vegetation season of 2016. Mycoses were identified infecting Solanaceae, Cucurbitaceae, Apiaceae and Chenopodiaceae plants; several bacterioses were found on tomato, peppers, bean and cucumber plants (Table 2). Some viral infections were also noticed in peppers and *Echinacea* plants.

Table 2. Incidence of plant diseases

Plant pathogens		Solanaceae		Cucurbitaceae	Fabaceae	Chenopodiaceae	Apiaceae		
		Tomatoes	Peppers	Cucumber	Bean	Mangold	Celery	Carrot	Parsley
Viral diseases	CMV	—	+ +	0	—	—	—	—	—
	TSWV	0	+	—	—	—	—	—	—
Bacterioses	*Pseudomonas syringae*	—	—	—	+	0	0	0	0
	Ps. Lachrymans	—	—	+ +	—	—	—	—	—
	Pectobacterium sp.	+ +	+	0	—	0	0	0	0
Mycoses	*Erysiphales*	0	0	+ +	0	0	0	0	0
	Septoria spp.	0	0	—	0	0	+	+ +	+
	Cercospora beticola	—	—	—	—	+ +	—	—	—
	Passalora fulva	+ + +	—	—	—	—	—	—	—
	Alternaria spp.	+	+	0	0	0	0	0	0

Where: (-) the pathogen is not specific for that crop; (0) no incidence; (+) reduced incidence; (+ +) moderate incidence; (+ + +) high incidence; (+ + + +) significant incidence.

The most damaging plant diseases of pepper were viroses, which were associated with thrips and aphid attack. Based on plant symptoms we detected TSWV (Tomato Spotted Wilt Virus on Pepper) and CMV (Cucumber Mosaic Virus on Pepper) infections.

Young pepper plants infected with TSWV displayed severe stunting, yellowing and chlorotic flecking (figure 5). Later, infected plants presented yellow spots with concentric rings and necrotic streaks on ripe fruits. The primary means transmitting this viral disease are *Frankliniella occidentalis* thrips.

Figure 5. Viral infections of pepper plants with Tomato Spotted Wilt Virus

Cucumber Mosaic Virus on Pepper induced dull light green foliage (figure 6) with a leathery appearance but not distinctive foliar markings. Infected plants presented low productivity. CMV can be mechanically transmitted but in the studied case, aphids were the main vectors.

Figure 6. CMV symptoms on pepper

Sporadic infections with *Alternaria* spp. causing fruit rot on pepper and tomatoes were also noticed (figure 7 a, b). It is considered that this disease was established as opportunistic on physiological stressed plants. The pathogen was detected occasionally on fruits damaged by sunscald, calcium deficiency, insect injury, or heat. The lesions were covered with a black mold. The pathogen was identified based on conidia morphology, grenade shaped, with vertical and transverse septa (figure 7c).

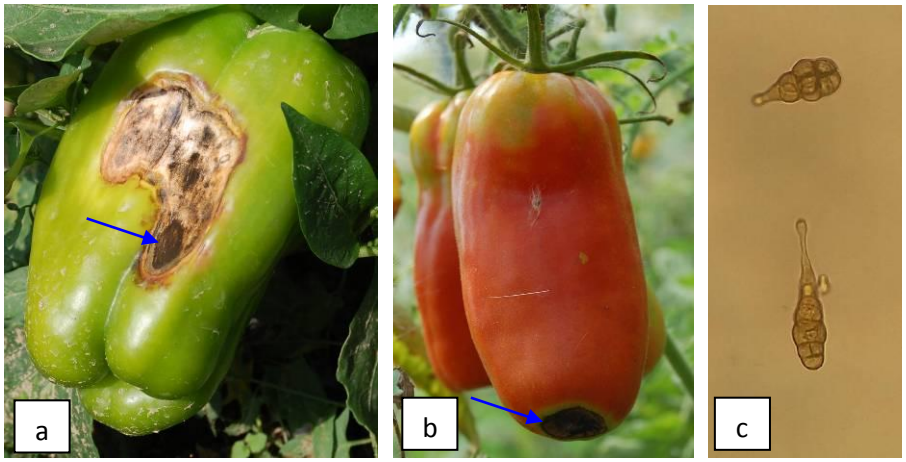

Figure 7. *Alternaria* spp.: a. sunscald injured pepper fruit with Alternaria mold, b - calcium deficiency damaged fruit with *Alternaria* spp. over infection, c- brown multiseptate conidia

A high disease incidence on tomato plants was caused by *Passalora fulva* (previously called *Fulvia fulva* or *Cladosporium fulvum*). Infections were seen mostly on leaves, only a few fruits were infected (figure 8). The pathogen was identified through microscopic analysis, correlated with plant symptoms.

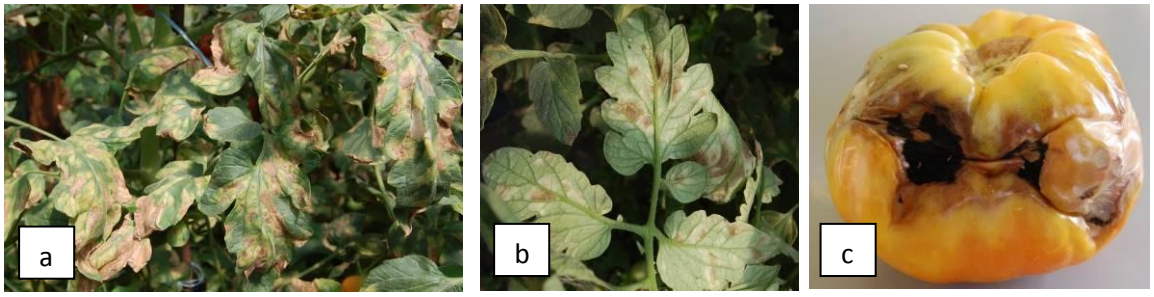

Figure 8. Symptoms of *Passalora fulva* infection: a - tomato leaf mould (severe attack), b - sporulated mycelia on the underside of a tomato leaflets, c - infected fruit

Bacterial diseases were also observed in tomato and pepper. Due to the soft rot (figure 9) and bad smelling infected tissue, we believed the causing agent is *Pectobacterium* spp. (previously known as *Erwinia* spp.).

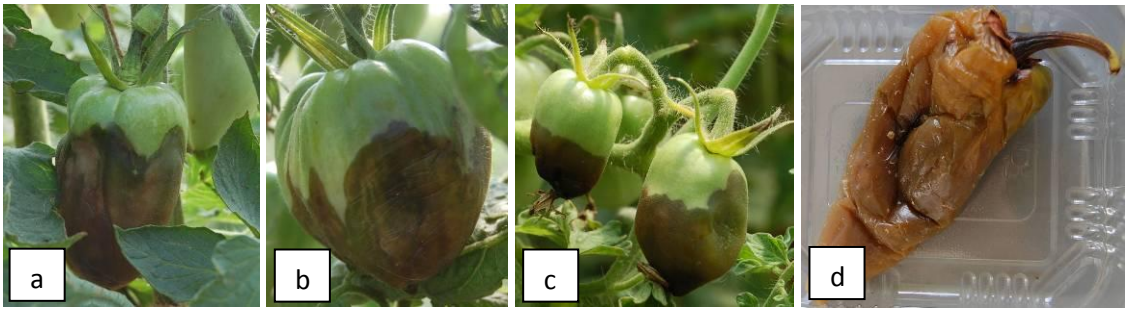

Figure 9. Bacterial soft rot of tomatoes (a, b, c) and pepper (d) fruits

Bacterial infection was confirmed by pathogen identification. The isolation was carried out from symptomatic tissue. Infected fruits were gently washed and disinfected, with 4% sodium hypochlorite, to avoid contaminating saprophytes. Macerated tissue was suspended and diluted in sterile phosphate buffer and a loopful was streaked on crystal violet pectate medium. Characteristic deep cavities were modelled by the pathogenic bacterial colonies. Pathogenicity assay was carried out on freshly cut potato slices. Infectious bacterial cultures induced the bad smelling soft rot of healthy potato slices, within 24 to 48h of incubation.

There are several authors suggesting that plants grown in high-calcium conditions have shown resistance to soft rot (McGuire & Kelman, 1984; Carpita & Gibeaut, 1993; Ngadze, 2012). As we already mentioned symptoms of calcium deficiency on the plants grown in Joița town, we could consider this physiological problem has increased tomato suscebility to various infectious diseases, like bacterial soft rot and Alternaria over infection of fruits tip.

Among bacterial diseases, angular leaf spot was detected on cucumber. The infection was caused by *Pseudomonas syringae* pv. *lachrymans* (sin. *Ps. lachrymans*). The pathogen was first recognised due to its specific symptomatology (figure 10). The pathogen was isolated from infected leaves on King B medium and identified based on cells and culture morphology, and LOPAT tests according to Olczak-Woltman et al. (2006). However, the pathogenicity test was performed on *Pelargonium zonale* leaves as we did not disposed of tobacco plants. KOH test indicated the bacterium was gram negative. Positive reactions were

observed for levan and catalase. Soft rot test, arginine dehydrolase and Kovac's oxidase, were negative.

Figure 10. Angular leaf spot of cucumber caused by *P. syringae* pv. *lachrymans* (different infection stages of the leaves)

Another disease detected on cucumber plants is powdery mildew, produced by microscopic fungi of *Erysiphales* order. The causing agents are obligated biotrophic pathogens, wich cannot be grown on artificial media. There are to major pathogen infecting cucumber plants, *Podosphaera xanthii* (previously known as *Sphaerotheca fuliginea*) and *Erysiphe cichoracearum*. In order to identify the disease causing agent we studied the biologic samples by optical microscopy. However, we captured only images of the mycelia and asexually produced spores (the conidia). We could not see any cleistothecia (the sexual fruiting bodies), which are the only microscopic criteria for differentiating this two pathogens.

CONCLUSIONS

The identified pest's spectrum comprises over 15 species of arthropods, most of them specific to the studied areas and analyzed cultures. However, organic farming system is particularly favorable to natural biological control, since for example the aphid populations were significantly limited by natural enemies like predatory and naturally occurring entomopathogenic fungi, from Entomophthorales order. In contrast, the invasive species *Tuta absoluta, Nezara viridula* and *Halyomorpha halys*, challenges research scientists to develop new approaches to monitor and manage these serious pests of Solanaceae crops.

Ten phytopathogenic agents were also present in the studied organic greenhouses. Among these, five were phytopatogenic fungi of Alternaria spp., *Cercospora beticola*, *Passalora fulva*, Septoria spp. and Erysiphae spp., and three were bacterial pathogens of *Pseudomonas syringae, Ps. lachrymans* and Pectobacterium spp. Two viral diseases were also detected based on plant disease symptoms, TSWV and CMV. These viral infections were associated with thrips and aphid attack. It is also assumed that some of the fungal and bacterial diseases were associated with plant deficiency in calcium which decreased the structural cell wall integrity of plants.

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