THE INFLUENCE OF PESTICIDES AND BIOPESTICIDES ON SWEET POTATO FUSARIUM MOLD

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Abstract. Growing sweet potato on the sandy soils of southern Oltenia has become a current practice. Unfortunately, this increased the incidence of phytosanitary problems. Among the phytopathogenic infection, fusariosis is the most common disease. The attack symptoms include basal leaf yellowing, plants wilt, root and crown rot and tubers dry rot. In the present study, *Fusarium equiseti* was found responsible for sweet potato tuber rot on stored KSP1 variety. This fungal pathogen is known to have a broad spectrum of infection; therefore, it could be transmitted among cereals and vegetable plants. The aim of this study was to evaluate several plant protection products for their antifungal activity against this fungal pathogen. Three commercial pesticides (CP) and one biocontrol measure were tested *in vitro* against *F. equiseti*. As biocontrol, the tested bacterial strain *Bacillus amyloliquefaciens* BW revealed 67.5 ± 0.8 % inhibitory efficacy against mycelial growth. The synthetic pesticides were also tested, in two doses (usual and reduced). Best results for inhibiting fungal growth were obtained when using CP2-F pesticide, based on triadimenol 43 g/L, spiroxamine 250 g/L and tebuconazole 167 g/L. Both usual (0.07%) and reduced doses (0.05%) revealed a fungal inhibitory efficacy of 86.67 ± 0.88%, and 82.50 ± 0.88% respectively. Moreover, CP1-NP pesticide, based on trifloxystrobin 150 g/L and prothioconazole 175 g/L, was less effective against *F. equiseti* compared to the BW biological treatment.

Keywords: biocontrol, *Fusarium*, low pesticide dose, sweet potato.

Rezumat. Influența unor pesticide și biopesticide asupra putregaiului responsabil pentru fuzarioza cartofului dulce. Cultivarea cartofului dulce pe solurile nisipoase din sudul Olteniei a devenit o practică curentă. Din păcate, acest lucru a crescut incidența problemelor fitosanitare. În rândul infecțiilor fitopatogene, fuzarioza este cea mai întâlnită boală. Simptomele atacului presupun îngălbenirea frunzelor bazale, ofilirea plantelor, putrezirea rădăcinilor și a bazei tulpinilor sau putrezirea tuberculilor. În prezentul studiu, patogenul *Fusarium equiseti* a fost găsit responsabil pentru putrezirea tuberculilor de cartof dulce, soiul KSP1. Despre această specie de mucegai se știe că prezintă un spectru de gazdă variat, fiind capabil să infecteze atât cereale, cât și plante legumicole. Scopul acestui studiu a fost evaluarea activității antifungice a unor produse de protecție a plantelor împotriva acestui fitopatogen. Trei pesticide comerciale (CP) și o tulpină de biocontrol au fost testate *in vitro* împotriva *F. equiseti*. Ca măsură de biocontrol, a fost testată tulpina bacteriană *Bacillus amyloliquefaciens* BW, care a prezentat o eficacitate de inhibare a creșterii miceliene de 67.5 ± 0.8 %. Pesticidele sintetice au fost testate în două doze. Cele mai bune rezultate pentru inhibarea creșterii fungice au fost obținute la utilizarea CP2-F, pe bază de triadimenol 43 g/L, spiroxamină 250 g/L și tebuconazol 167 g/L. Acest fungicid a prezentat o eficacitate antifungică de 86.67 ± 0.88%, la utilizarea în doza uzuală, recomandată de producător (0,07%), respectiv 82.50 ± 0.88% eficacitate, la testarea în doză redusă (0,05%). Fungicidul CP1-NP, pe bază de trifloxistrobin 150 g/L și protioconazol 175 g/L, a fost mai puțin eficient împotriva *F. equiseti*, comparativ tratamentului biologic cu BW.

Cuvinte cheie: control biologic, *Fusarium*, pesticide în doză redusă, cartof dulce.

INTRODUCTION

In the past ten years, growing sweet potato on the sandy soils of southern Oltenia has become a current practice (DIACONU et al., 2018; CROITORU et al., 2019; COTEȚ et al., 2023). Enlarging the growth areas of sweet potato, in Romania, increased crop susceptibility to pests and diseases. The Romanian grown sweet potato is highly exposed to Fusarium attack (BOIU-SICUIA et al., 2017; COTEȚ et al., 2023). Plants and tubers can be infected by both legumes adapted *Fusarium* spp. pathogens, such as *F. oxysporum* and *F. solani*, as well as cereal fusariosis, produced by *F. acutatum*, *F. equiseti*, *F. graminearum* or *F. proliferatum* (CENDOYA et al., 2018; SCRUGGS & QUESADA-OCAMPO, 2016; BALLOIS, 2012). The attack symptoms on plants include basal leaf yellowing, plants wilt, root and crown rot, while tubers can develop dry rot (YANG et al., 2018; PAUL et al., 2020).

Several phytosanitary measures are recommended to prevent the fusariosis of sweet potato. The first step is to avoid the risk of pathogen transmission from the infected tubers. To reduce the infection risks during shoot production, the planting material must be healthy. Therefore, the tubers intended for propagation should be carefully selected, while those with open wounds or infection symptoms should be eliminated. For conventional growing systems, pesticide treatments could be applied on tubers intended for propagation, using effective fungicides, such as thiabendazole. Choosing uninfected plots for shoots production is recommended. When shoots are ready for harvest, the veins should be cut above the ground level, avoiding the shoot parts that have been in direct contact with the ground. Moreover, it is not recommended to perform more than 2-3 slip harvests from the same plot. The vine cuttings should be collected from healthy, vigorous plants. For 15 minutes before planting, the slips should be immersed in a systemic pesticide solution or a biocontrol suspension of an endophytic colonizing microorganism for plant protection. The attack of certain pests, such as nematodes and white worms, could contribute to soil-born phytopathogenic infections, including fusariosis. Therefore, pest control is also required during the vegetation season. As Fusarium tuber infection can occur during harvesting and tuber manipulation, the crop should be carefully handled before storage, to reduce tuber injury. A curing step is mandatory to be properly ensured before storage. This procedure allows the healing of the superficial wounds. During storage, proper

ventilation and 12-15°C temperature should be ensured so that saprophytic microorganisms do not become infectious and develop tuber rot (BOIU-SICUIA et al., 2018).

The aim of this study was to identify the causing agent of sweet potato tuber rot at the species level and to evaluate different biological and chemical plant protection products for their antifungal activity against this phytopathogen.

MATERIALS AND METHODS

Pathogen isolation and identification.

Infected tubers of sweet potato, produced and stored at the SCDCPN Dăbuleni, were transferred into the RDIPP laboratory. These tubers belong to KSP1 cultivar, an acclimatised variety derived from the Pumpkin cultivar, brought from South Korea, in 2015. Tubers were kept in a humid chamber for one week. Then, fungal isolation was performed on a Potato-Dextrose-Agar (PDA) medium.

Fresh mycelial biomass was collected from the purified culture, and DNA extraction was used. Genomic DNA was purified using ZR Fungal/Bacterial MiniPrep™ commercial kit (ZymoResearch, SUA). Polymerase Chain Reaction (PCR) was then performed, to amplify the ITS1-5.8S-ITS4 region. Within the PCR we mixed 1X Green Buffer with $MgCl_2$, 0.2 mM dNTPs mixture, 0.5 µM ITS1 primer (5'-TCCGTAGGTGAACCTGCGG-3'), 0.5 µM ITS4 primer (5'-TCCTCCGCTTATTGATATGC-3'), 0.2 U of DreamTaq DNA Polymerase and ~ 10 ng of template DNA in up to 50 µl of sterile MilliQ water per reaction. The amplification program was performed as presented in table 1.

Table 1. PCR conditions for ITS1-5.8S-ITS4 region amplification.

PCR step	No of cycles	Temperature	Duration
Initial denaturation	1	94 °C	4 minutes
Denaturation	35	94 °C	1 minute
Primers' annealing		45 °C	1 minute
Elongation		72 °C	2 minutes
Final elongation	1	72 °C	10 minutes

The amplification products, resulted from PCR, were revealed in 1% agarose gel electrophoresis in Tris-Borate-EDTA buffer, compared to a 1 Kb ladder. Ethidium bromide was used for DNA staining. The electrophoretic pattern was analysed using the BioDoc-It Imaging System after Ultra-Violet exposure. The PCR product of the fungal strain was then submitted for purification and paired-end Sanger dideoxy sequencing, to CeMIA, in Greece. The obtained partial sequences were aligned using the BioEdit program, and the assembled sequence was subjected to the online NBLAST software for taxonomic identification. Based on the sequences similarities found in the NCBI database, the sample was identified at specie level.

Biocontrol analysis

The biocontrol strain *Bacillus amyloliquefaciens* BW was tested against the sweet potato fungal pathogen, isolated in the current study, to evaluate antifungal efficacy. Tests were performed *in vitro*, on a PDA medium, using the double culture technique. Bacterial biomass was inoculated at 2.5 cm distance from the fungal inoculum (5 mm in diameter), placed in the center of the plate. Fungal control plates were also prepared on PDA. Tests were performed in triplicate and incubated at 26°C. Periodically, plates were analysed and compared to the control. Antifungal activity was evaluated after 10 days of incubation.

***In vitro* pesticide analysis**

Three commercial pesticides (CP), available on the Romanian market, were used in this study (Table 2). The fungicides were encoded as CP1-NP, CP2-F and CP3-FP. These were tested *in vitro* against the sweet potato fungal pathogen, isolated in the current study. The fungicides were tested in two doses. One of the tested doses (U – usual) was in accordance with the product label, recommended to control cereal fusariosis. The second tested dose was reduced (R) by 25% or 28.57%, compared to the usual recommended dose.

Table 2. Commercial fungicides.

Commercial pesticide	Active ingredients	Tested dose (%)	
CP1 – NP	Trifloxystrobin 150 g/L; Prothioconazole 175 g/L	Usually recommended (U)	0.07
		Reduced (R)	0.05
CP2 – F	Triadimenol 43 g/L; Spiroxamine 250 g/L; Tebuconazole 167 g/L	Usually recommended (U)	0.07
		Reduced (R)	0.05
CP3 – FP	Prothioconazole 53 g/L; Spiroxamine 224 g/L; Tebuconazole 148 g/L	Usually recommended (U)	0.08
		Reduced (R)	0.06

The antifungal activity of these pesticides was tested *in vitro* on Potato Dextrose Agar. The fungicides were incorporated within the medium, while the fungal pathogen was inoculated on top of the agar layer, in the centre of the Petri plates. Fungal inoculum was calibrated as mycelial plugs of 5 mm diameter. Control plates with the fungal pathogen were prepared, on PDA medium without pesticides. Plates were incubated at 26°C. Biometric measurements of the fungal growth were taken after 10 days from inoculation. The fungal inhibition efficacy of the pesticides was calculated based on LAHLALI & HIRJI (2010) algorithm.

RESULTS AND DISCUSSIONS

Sweet potato pathogen identification

Infected tubers stored in humid chamber developed white cottony mycelia on top of the brown spots (Fig. 1a). From these fungal growths, the pathogen was purified on PDA (Fig. 1b). Isolated strain was subjected to molecular identification based on the ITS1-5.8S-ITS2 region. The resulting amplification product (Fig. 1c) was subjected to Sanger dideoxy sequencing.

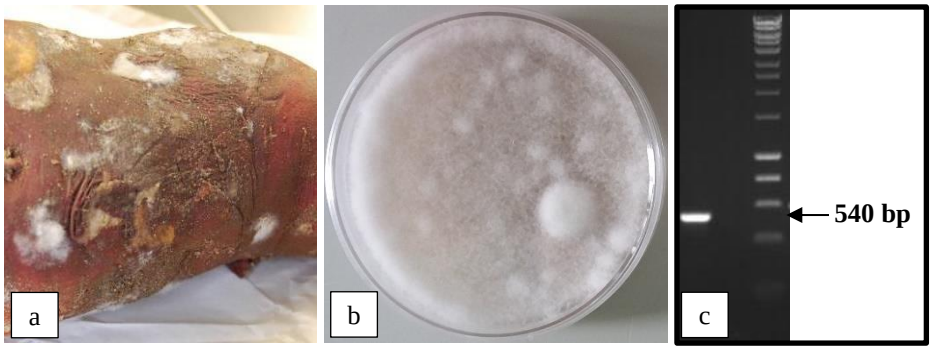

Figure 1. *Fusarium equiseti* pathogen from isolation to molecular identification
a. infected sweet potato tuber; b. pure culture on PDA; c. amplification product of the ITS1-5.8-ITS2 region.

After assembling the paired-end sequencing results, a partial sequence of the ITS1-5.8S-ITS2 region was obtained. Based on these 521 bp long sequence, the fungal pathogen was identified at species level, by analysing the identity percent (100%) and its query cover (99%) with other similar species from NCBI database.

Biopesticide influence on fusarium mold

Antifungal activity of *Bacillus amyloliquefaciens* BW was evaluated in vitro against newly isolated sweet potato pathogen. After 10 days of co-cultivation, clear inhibition zones, of 5.8 ± 0.7 mm, were seen against *F. equiseti* (Fig. 2). This biometric parameter reveals the potential biocontrol activity of the beneficial BW strain and the bacterial ability to release into the substrate various diffusible compounds with antifungal action. Various antifungal metabolites are known to be produced by *Bacillus amyloliquefaciens* biocontrol strains. The *B. amyloliquefaciens* FZB42 strain, known to be found as active ingredient in various commercial biopesticides, is mainly producing fengicin, as antifungal compound against *Fusarium* sp (HANIF et al., 2019). GONG et al., (2015) revealed iturin A and plipartatin A as the most abundant cyclic lipopeptides (CLPs) in biocontrol strain *B. amyloliquefaciens* S76-3 strain, that reveal antifungal activity against *Fusarium graminearum*. Many other CLPs were detected by MALDI-TOF mass spectrometry in *B. amyloliquefaciens* strains. The DHA6 strain, expressing antifungal activity against *F. oxysporum*, produces five families of CLPs: bacillomycin, iturins, pumilacidin, surfactins, and syringfactin (AL-MUTAR et al., 2023).

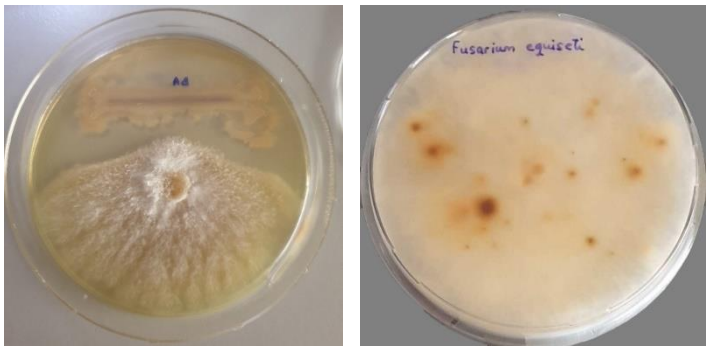

Figure 2. Antifungal activity of *Bacillus amyloliquefaciens* BW vs. *Fusarium equiseti*
a. Test plate; b. control plate.

Other antifungal mechanisms involved in microbial antagonism are not excluded. Biocontrol bacteria are also known for their antifungal volatile compounds, lytic enzyme production, competition and direct inhibition of fungal pathogens (SICUIA et al., 2015; BOIU-SICUIA et al., 2023).

Regarding antifungal efficacy, the biocontrol strain *B. amyloliquefaciens* BW revealed 67.5 ±0.8 % inhibitory activity against the sweet potato pathogen *F. equiseti*. Compared to other Bacillus sp. strains, tested against sweet potato *Fusarium* pathogens, the BW strain revealed higher antifungal potential (BOIU-SICUIA et al., 2022).

Chemical pesticide influence on fusarium mould

The mycelial growth of *F. equiseti* was measured after 10 days of incubation on PDA, and PDA supplemented with different commercial pesticides (CP) at the recommended and reduced application dose. The biometric measurements made on the mycelial growth were used to calculate pesticide efficacy to inhibit fungal growth. According to our data, the CP1-NP pesticide, containing trifloxystrobin 150 g/L and prothioconazole 175 g/L, was less effective in fungal inhibition compared to the other two products, CP2-F and CP3-FP (Fig. 3). Even when tested in a reduced dose, CP2-F and CP3-FP pesticides were significantly more effective in reducing *F. equiseti* fungal growth (Table 3).

Table 3. Fungal inhibition efficacy against *Fusarium equiseti*.

Commercial pesticide dose	CP1-NP	CP2-F	CP3-FP
Usual recommended dose (U)	44.58 ± 0.88 c	**86.67 ± 0.88 a**	80.83 ± 0.01 b
Reduced dose (R)	38.33 ± 4.42 d	82.50 ± 0.88 b	80.00 ± 3.54 b
The data are presented as average values ± standard deviation. Different letters indicate a significant difference between the experimental variants, regarding their fungal inhibition efficacy			

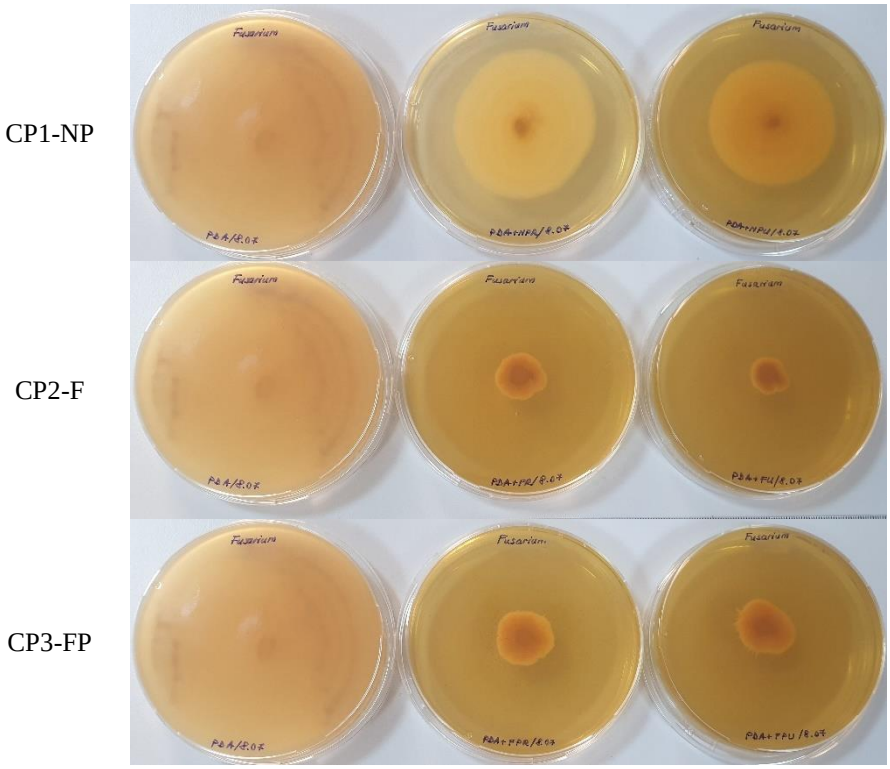

Figure 3. Commercial pesticides inhibiting the growth of *Fusarium equiseti*
isolated from sweet potato. Control (left plates), Reduced fungicide treatment (centre plates),
Fungicide treatment in usual dose, recommended by the producer (right plates).

Best *in vitro* results were obtained with the usual dose of CF2-F fungicide, recommended by the producer to be used in the field to control fusariosis attack. This treatment dose revealed 86.67 ± 0.88% fungal inhibition efficacy. No statistically different results were obtained when using CP3-FP in U and R doses compared with CP2-F in R dose (Table 3). Among the tested pesticides, CP1-NP revealed the weakest fungal inhibition efficacy. Even in the usually recommended dose, fungal inhibition was less effective, not only compared to the other tested commercial fungicides, but also compared to the biocontrol strain.

MÜLLENBORN et al., (2008) studied the inhibitory effect of two triazole type fungicides (prothioconazole 250 g/L and tebuconazole 251,2 g/L) and other two strobilurin class substances (azoxystrobin 250 g/l and fluoxastrobin 480 g/l) against various pathogenic fungi, including *Fusarium* spp. The inhibitory effect of fungicides against these pathogens was tested *in vitro* by growing the fusaria on PDA supplemented with various concentrations of commercial pesticides, of up to

100 mg/l. Out of the tested fungicides, prothioconazole was among the most effective active substance in reducing *Fusarium* spp. mycelial growth (MÜLLENBORN et al., 2008).

CONCLUSIONS

The sweet potato KSP1 variety, adapted to be grown in sandy soils from Southern Oltenia, is susceptible to fusariosis attack. *F. equiseti* was identified as a pathogen, able to infect tubers before the harvest and continue to produce damages in storage conditions. This pathogen could also be encountered in cereals; therefore, it could be transmitted while crop rotation.

In vitro testing, for controlling *F. equiseti* growth, revealed a wide difference between some commercial pesticides, recommended to prevent fusariosis attack in cereals. Best results were revealed by the CP2-F pesticide, based on triadimenol 43 g/L, spiroxamine 250 g/L and tebuconazole 167 g/L mixture, when used in the recommended dose (0.07%). Although the biocontrol treatment based on *B. amyloliquefaciens* BW strain revealed reduced fungal inhibition efficacy (67.5 ±0.8%) compared to CP2-F (86.67 ± 0.88% in U dose, and 82.50 ± 0.88% in R dose) or CP3-FP (86.67 ± 0.88% in U dose, and 82.50 ± 0.88% in R dose), it is more efficient in reducing *F. equiseti* growth than the CP1-NP fungicide (44.58 ± 0.88% in U dose).

Reducing pesticide dose for some commercial fungicides could be feasible in sweet potato crop. This approach, as well as using biocontrol measures, could be considered for the integrated pest management of sweet potato.

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