Romanian Journal of Ecology & Environmental Chemistry, 3(2), 2021
https://doi.org/10.21698/rjeec.2021.205

Proceedings of the 24th International Symposium "The Environment and the Industry" (E-SIMI 2021), 24 September 2021, online event

In vitro effect of salinity and pH on *Fusarium* sp., the causal agent of sweet-potato root rot

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Received:	*Accepted:*	*Published:*
10.08.2021	*03.11.2021*	*17.12.2021*

Abstract
Fusarium root rot in a common pathogen of sweet potato, with a wide range of host plants. In the current study six new isolates of Fusarium sp., collected from infected sweet potato plants, along with a reference strain of Fusarium oxysporum, had their growth behavior studied in various pH and saline conditions. In vitro studies showed that salinity higher than 6% NaCl in the PDA substrate significantly reduces the fungal growth. At 12% NaCl, four of seven strains revealed complete mycelia inhibition. However, for the other two isolates, and for the reference strain, 12% salinity only reduced the growth with 77.4%. Regarding the fungal growth at different pH values, it was noticed that tested fusaria were not perturbed at up to 8.5 alkalinity. However, at a pH of 4.5, the growth rate was reduced, although the growth differences were diminished during prolonged incubation time. Considering the in vitro results, saline water should be tested as preventive immersion treatment on the sweet potato sprouts, before their planting, in order to reduce the incidence of Fusarium infection.

Keywords: *Fusarium, pH, salinity*

INTRODUCTION

Sweet potato (*Ipomoea batatas* (L) Lam.) is an important crop worldwide due to its nutritional values and productivity [1-3]. In Romania, the culture is finding appropriate growth conditions in the south area of the country, especially on sandy soils, but not only [4-6]. This vegetable increased crop diversification in our country and ensures a good productivity under the current climate change conditions [7, 8].

As all crops, sweet potato can be attacked by various pests and diseases [9]. Fusariosis is one of the common diseases of sweet potato, and can be found in a wide range of host plants [10-12]. *Fusarium* sp. is an important soil, field and postharvest pathogen, with a high frequency in Romania. On infested soil, *F.oxysporum* and related species cause foot and root rot as well as plant wilt [13, 14]. Chlorotic leaf distortions can also be seen in the field [15]. Such symptoms can be also seen during *F.lateritium* infections [16, 17]. During storage, species such as *F.solani* and *F.javanicum* can cause sweet potato tuber rot [18, 19]. Various other *Fusarium* species were reported on sweet potato, many of them being pathogens also to other agricultural crops [20].

The aim of the present work was to characterize fungal behavior of several fungal pathogens belonging to *Fusarium* genus, when exposed to abiotic stress conditions. In the current study the fungal growth was evaluated under various pH and saline conditions in order to determine if

pathogens can enlarge their spreading area and colonize lands with such abiotic conditions.

MATERIALS AND METHODS

Fungal strains

Six strains of *Fusarium* sp. isolated from sweet potato, and a reference strain of *F. oxysporum* f.sp. *radicis lycopersici* ZUM 2407, were used in the experiments (Table 1).

Table 1. Fungal strains used in the study

***Fusarium* sp. strains**	**Source**
2TChV2	Isolated from sweet potato DK 19/1 line conventionally grown
TCh+BioV2	Isolated from sweet potato DK 19/1 line conservative grown
6TChV6	Isolated from sweet potato DK 19/5 line conventionally grown
Fusarium sp. isolate 8	Isolated from sweet potato ROK1 variety conventionally grown
9TCh+BioV2	Isolated from sweet potato DK 19/1 line conservative grown
P10	Isolated from sweet potato Juhwangmi variety conventionally grown
FORL	*Fusarium oxysporum* f.sp. *radicis lycopersici* ZUM 2407 reference strain from Research Institute for Plant Protection (IPO-DLO), Wageningen, The Netherlands

The fungi were long stored under mineral oil at 4°C. Fresh cultures were prepared on Potato Dextrose Agar (PDA) and incubated at 27°C. During each experimental trial the fungal cultures were maintained on PDA at 4°C.

In vitro assays for *Fusarium* behavior to saline conditions

Three sets of Petri dishes containing 20 ml of PDA with 7 different sodium chloride concentrations (0% NaCl - as control, and 1%, 2.5%, 4%, 6%, 8%, 12% NaCl – as test variants) were used for each fungal strain. Plates were inoculated in their center, with a 5 mm diameter plug cut from 7 days old pure cultures, and incubated at 27°C ± 1ºC in darkness. After inoculation, the radius of the colonies was measured, in three random directions, at 3, 4, 5, 6 and 9 days of incubation. Cultures were stored at room temperature, for an additional week, to detect possible changes in the growth rate of the colonies.

In vitro assays for *Fusarium* behavior to various pH conditions

Five different pH levels ranging from 4.5 to 8.5, with a difference of 1.0, were tested to assess *Fusarium* radial growth to various pH conditions. The control cultures were considered at the pH value of 5.5. The culture media were prepared by adding either 0.2N HCl or NaOH in the PDA before autoclaving. The media were sterilized at 121°C for 20 minutes. Three sets of Petri dishes containing 20 ml test or control media were used for each fungal strain. The inoculation was done with 5 mm diameter mycelia plugs cut from 10 days old pure cultures. Plates were then incubated at 27°C ± 1ºC in darkness. After inoculation, the radius of the colonies was measured in the similar manner as mentioned above.

Fungal growth inhibition

The inhibition rate was expressed as the percentage reduction of fungal growth compared to the control plates, were each fungal strain was grown on Potato Dextrose Agar (pH 5.5). The percentage of reduction was calculated according to Pacheco Marino et al. [21], using the following formula:

$$Inhibition\ \% = 100 \times \left[1 - \left(\frac{Fungal\ Radius\ in\ Test\ plates}{Fungal\ Radius\ in\ Control\ plates}\right)\right]$$

Data were summarized as average, range, and standard deviation to cover statistical concepts such as correlation and estimation and modeling. The statistical analysis performed will be considered significant when $P < 0.05$.

RESULTS AND DISCUSSION

The effect of NaCl on the growth of tested strains of *Fusarium* sp. is shown in Figure 1. The fungal growth inhibition increased proportionally with the salt concentration. Therefore, at 12% NaCl, four of seven strains (TCh+BioV2, 6TChV6, 9TCh+BioV2, and P10) revealed complete mycelia inhibition. No fungal growth was seen to these strains neither after nine days of incubation at 27°C in complete darkness, neither after another week of maintenance at room temperature. However, for the other two isolates from sweet potato, *Fusarium* sp. isolate 8 and 2TChV2, and for the FORL reference strain, 12% salinity only reduced the growth with 72.5 to 74.7%, and 85% respectively, after 9 of incubation at 27°C.

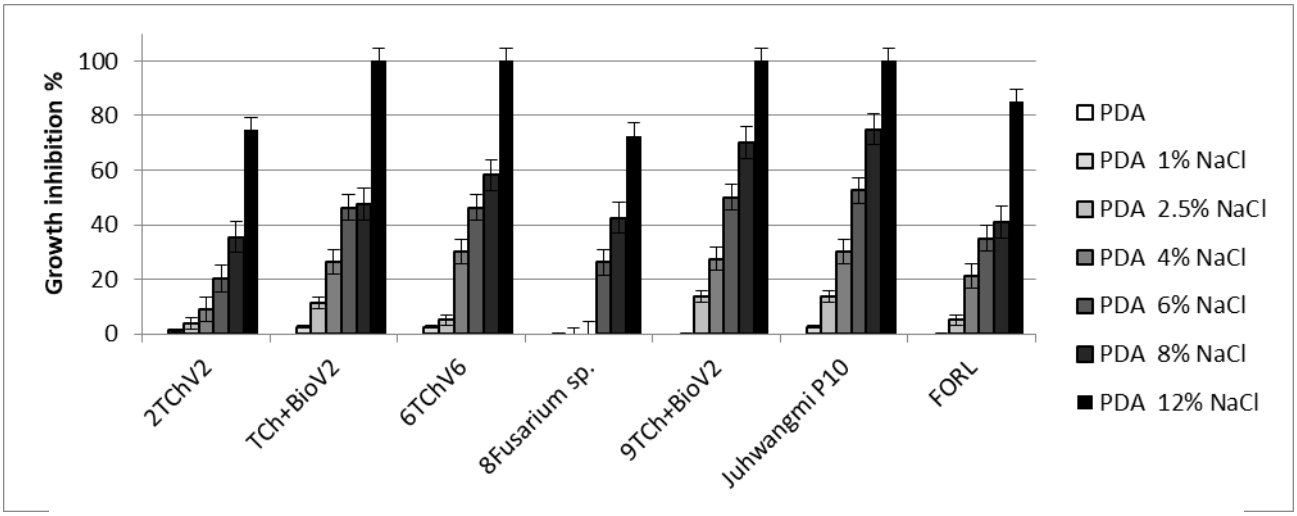


Fig. 1. Fungal growth inhibition due to the abiotic stress caused by NaCl concentration in the culture substrate

In vitro studies showed that salinity higher than 6% NaCl in the PDA substrate significantly reduces the fungal growth with 20.3 to 52.5%, depending on the fungal strain tolerance to salt conditions (Figure 2).

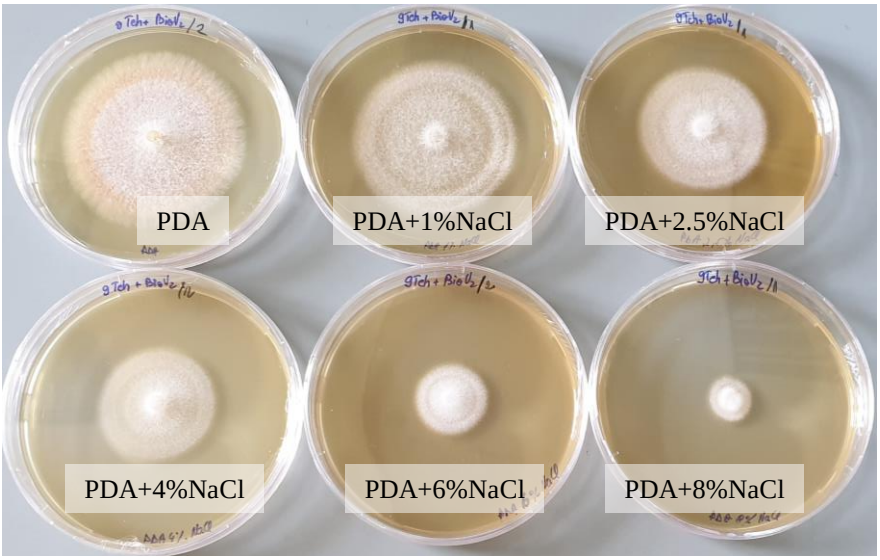
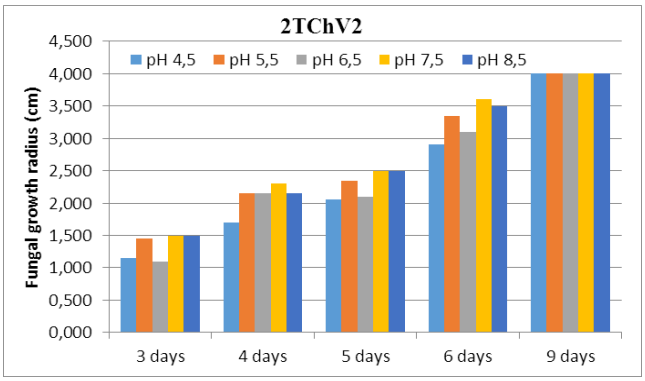


Fig. 2. Colonies of *Fusarium* sp. isolate 9TCg+BioV2 at various NaCl concentrations after 6 days of incubation at 37°C

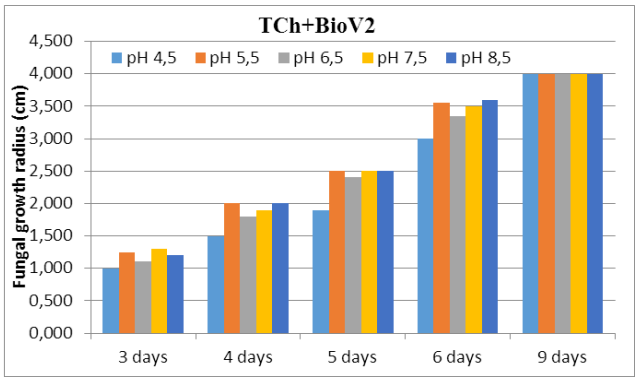
Among *Fusarium* sp. strains isolated from sweet potato, the most vulnerable to NaCl were TCh+BiolV2, 6TchV6, 9TCh+BioV2, and P10.

Regarding the fungal growth at different pH values, it was noticed that tested Fusaria were not significantly perturbed by the pH of the substrate (Figure 3). In less than 10 days of incubation at 27°C, al strains, including the reference, were able to completely colonize the surface of the Petri plates.

As fungi prefer a slight acidic pH, of 5.5 to 6.5, it was expected a growth inhibition on alkaline media. Therefore, the obtained results are attributed to the organic acid production by all studied strains, which could have change the pH of the media, thus conferring them tolerance to such conditions.



a)



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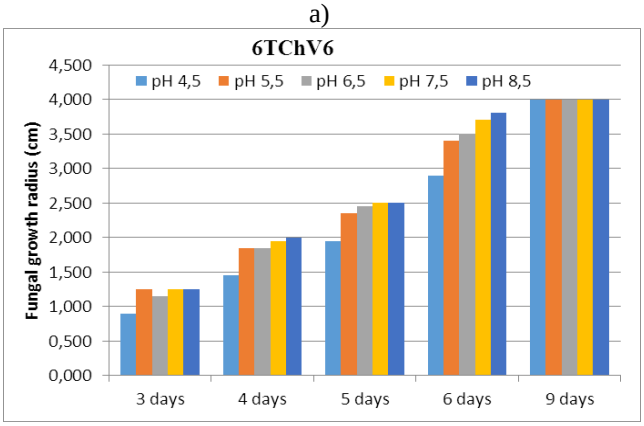

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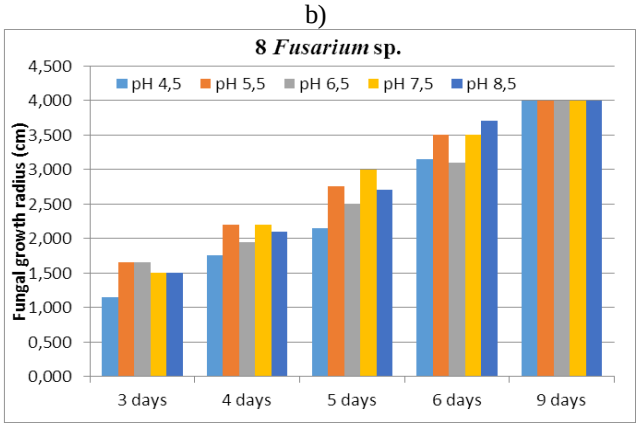
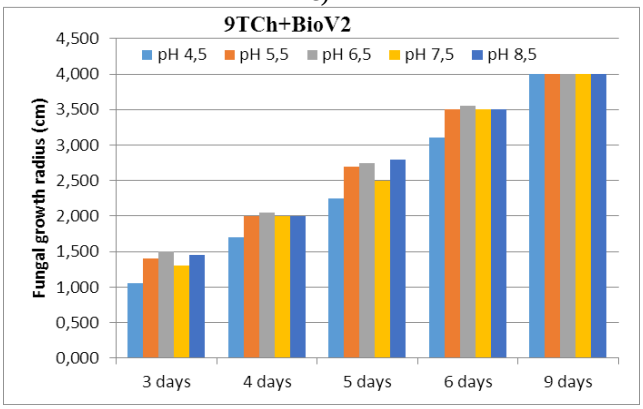


d)



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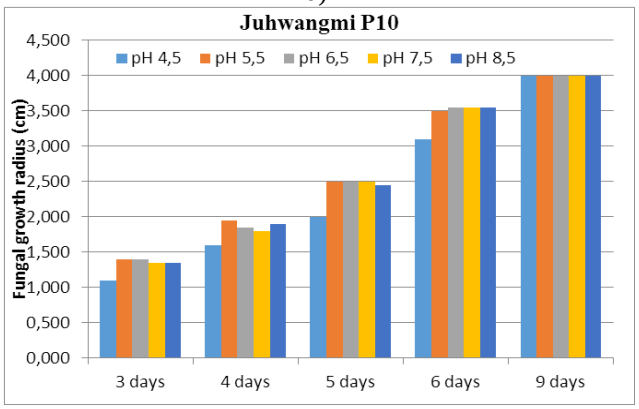

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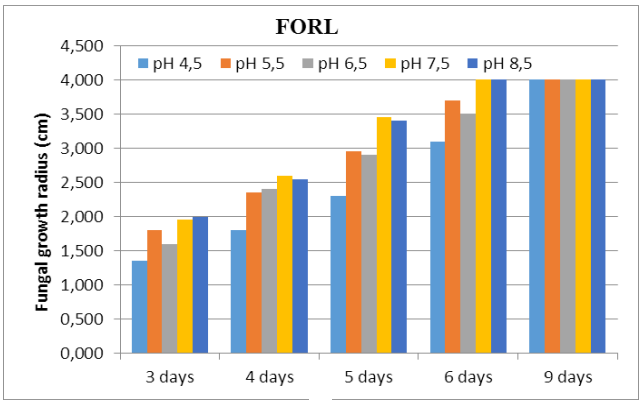

g)

Fig. 3. Growth dynamics of the fungal strains on PDA with different pH values: a) 2TChV2 strain; b) TCh+BioV2; c) 6TChV6; d) 8 Fusarium sp.; e) 9TCh+BioV2; f) Juhwangmi P10; g) FORL.

Studies on *Fusarium oxysporum* have shown that the ability of fusaria to grow at 8.5 pH is depending on the strain or isolate. Related to this, studies on 14 isolates of *F.oxysporum* from soybean revealed no growth at the pH of 8.0 [22], however *F.oxysporum* isolates from tomato infected lands revealed only a slight reduction of the mycelia biomass with 19% compared to the control (pH 5.5) when cultured at pH of 7.5 [23].

CONCLUSIONS

As the *Fusarium* sp. strains isolated form sweet potato showed high inhibition rates in saline environment, saline water (6% NaCl) should be considered to be evaluated as immersion treatment on the sweet potato sprouts, before their use as planting material. This measurement, if effective and not deleterious the future plant growth, could replace the classic overnight immersion in water of the shoots harvested form the greenhouse before their planting in the field.

ACKNOWLEDGEMENTS

The authors would like to acknowledge PhD. Florica Constantinescu for providing the ZUM 2407 reference strain. This study was supported by the sectorial project ADER 7.3.4./2019 "Research on in vitro selection in order to identify, multiply and promote sweet potato genotypes with tolerance to thermohydric stress" financed by the Ministry of Agriculture and Rural Development.

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Citation: Barbu, L.D.-N., Boiu-Sicula, O.-A., Pintilie, B., In vitro effect of salinity and pH on Fusarium sp., the causal agent of sweet-potato root rot, *Rom. J. Ecol. Environ. Chem*., **2021**, 3, no.2, pp. 42-47.