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FUNGAL BIODIVERSITY AND CLIMATE CHANGE ON CORN: A KEY TOOL IN BUILDING AN INNOVATIVE AND SUSTAINABLE AGRICULTURE ON DOBROGEA AREA

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Abstract

Elaboration of biocontrol strategies to mycotoxins producing fungi and integration of beneficial microorganisms in protection systems of corn crops, involves in the initial phase, the development of methods for sampling and analysis of soil and plant debris samples and later, the analysis of cob corn for the purpose of differentiating toxigenic and atoxigenic fungi of Aspergillus and Fusarium group.
Risk factors on corn production specific for Dobrogea agricultural area are: the attack of fungal pathogens from primary inoculum that survive in the soil and on the plant debris: Aspergillus flavus (aspergillus or yellow mould); Giberella zeae/anamorph Fusarium graminearum (gibberella ear rot) and Giberella fujikuroi/anamorph F. verticilloides (fusarium ear rot); wrong conception of crop rotation (wheat – corn) and drought causing water stress in corn, as an indicator of climate change.
To bring together relevant knowledge and experience for Dobrogea farmers about improvements in fungi biodiversity, the research are focus on developing new methodologies for assessing toxigenic-pathogenic and beneficial fungi, leading to a quantitative risk-benefit assessment strategy.

Key words: *toxigenic-pathogenic fungi, beneficial fungi, climate change, drought.*

INTRODUCTION

In Romanian agricultural conditions, one of the most common rotation is wheat-corn. Plant debris left on the ground, favors the development of some microorganisms pest agents, including mycotoxin producing fungi of *Aspergillus* and *Fusarium* genus.
Conservation agriculture is being increasingly promoted as constituting a set of principles and practices that can make a contribution of sustainable production because it addresses to missing components in the intensive tillage based standardized seed-fertilizer-pesticide approach to agriculture intensification.
Suppressing soil-borne diseases with residue management and organic amendments is a relatively recent approach in agricultural practice to reduce the primary inoculum level of pathogens (Bailey and Lazarovits, 2003).
In Romanian conditions, the attack of *Giberella zeae (Fusarium graminearum)* on the corn cob produce 7-15% losses (Nagy et al., 2006; Zaberca and Borcean, 2010).
Reducing primary inoculum of *Fusarium graminearum*/*Giberella zeae* by using conservative technologies of biofumigation in the succession of wheat/corn crops, is so beneficial for corn, too.
The influence of *Fusarium* spp ear infection on production and mycotoxin content in maize is much more than that reported for Fusarium Head Blight in wheat, in case of favorable climatic conditions (Nagy et al., 2009).
Keeping pest and crop management records over time will allow farmers to evaluate the economics and environmental impact of pest control and determine the feasibility of using certain pest management strategies or growing particular crops.

MATERIALS AND METHODS

New methodologies to simulate *in vivo,* as initial stage in the drafting a method of sampling adapted to corn grower, offers the advantage that it processes for the identification of the toxigenic fungi in the soil and on plant debris, allow for an analysis of a large volume of data, which correspond to real-field, with a low cost and suggest the risk of corn

contamination with mycotoxins in the following year of cultivation.

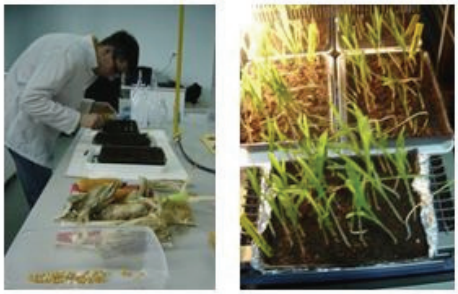

Figure 1. Research techniques and methods of artificial soil contamination by fungi spores

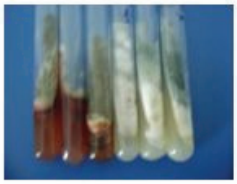

Figure 2. Selective culture medium of *Aspergillus*, *Fusarium* and *Trichoderma fungi* used in soil artificial inoculation

Research techniques and selective culture medium with dimethyl-β-cyclodextrin were used to studying the influence of soil contamination level on germination and emergence corn stage (Figure 1 and 2). The seeds were sowed in heat-treated soil and artificially infected with a concentration spores of $2.5x10^6$ spores/ml of *Fusarium* sp. and *Aspergillus flavus*, respectively $7,5x10^6$ spores/ml of *Trichoderma harzianum* (Tdh al12).

After 24 hours of inoculation with fungi, corn was sown, every 5 rows in each tray contains 10 seeds/row and incubated in a growth chamber for 2 weeks at 22^0 C.

The antagonism between pathogenic and beneficial fungi was assesed by using double culture method.

Experimental variants analyzed:

V_1 -untreated corn in heat-treated soil;

V_2-untreated corn in soil infected with *F. graminearum+F. oxysporum* and *Aspergillus flavus*;

V_3-untreated corn in soil infected with *F. graminearum+F. oxysporum*;

V_4-untreated corn in soil infected with *Aspergillus flavus*;

V_5-untreated corn in soil infected with *Trichoderma harzianum* (Tdh al12 strain);

V_6-untreated corn in soil infected with *A. flavus*, *F. graminearum+F. oxysporum* and *T. harzianum* (Tdh al12 strain);

V_7-corn treated with *Trichoderma pseudokoningii* (Td85 PTS-2 kg/t) in infected soil *A. flavus* and *F. graminearum+F. oxysporum*;

V_8- corn treated with Thiram (3 kg/t) in infected soil with *A. flavus* and *F. graminearum+ F. oxysporum*;

V_9-corn treated with Thiram (3 kg/t) in infected soil with *A. flavus*;

Biological material sampling were collected from agricultural Dobrogea crop with different meteorological and soil conditions (Figure 3). Location sampling points by GPS coordonates: Amzacea (N4358108; E02825026); Agigea Black Sea (N4404953; E02838050); Agigea Danube Canal (N4405113; E02832968); Oltina (N4407982; E02740820); Harsova (N 4442261; E02756012); Fantanele (N4438534; E02832161). The period analyzed in this study was 2011-2013.

Figure 3. Corn samples collected from Dobrogea agricultural area, 2012

RESULTS AND DISCUSSIONS

The objective of this research is to developing strategic solutions to reduce soil contamination by mycotoxin producing fungi (*Fusarium* and *Aspergillus* genus) and to predict risk level associated to corn crops in Dobrogea agricultural areas.

The use of novel methodologies based on research techniques and selective culture medium with dimethyl-β-cyclodextrin will allow to describe the contamination level of different crops and predict its value by simulate *in vivo* corn conditions (Based on our results published in Patent No 123355).

Climate change effects and Dobrogea agriculture

Risk factors for *A. flavus* are plant debris and contaminated soil, insect attack, hot and semi-arid regions. Corn plant affected by water stress are more susceptible to attack fusarium ear rot *Giberella fujikuroi/Fusarium verticilloides*.

The development of *Aspergillus* and *Fusarium* fungus on corn infection is associated with mycotoxin contamination. The causes of yellow mold and Giberella ear rot including: natural evolution of *Aspergillus* and *Fusarium* populations, climate change, conservative extension technologies.

Drought causing water stress in corn, as an indicator of climate change is characterized by frequency, duration and intensity increased especially in Dobrogea agricultural area. The effect of drought causing significant losses on corn cob by limiting growth potential of plants expressing, leaf wilting and drying, high frequency sterile plants and partial coverage of the ear with grains.

Climate change effects on Dobrogea agriculture will affect farmers by reducing the acreage in corn due to water stress caused by prolonged drought.

The novel methodologies using in this research are selective culture medium for differentiation between toxigenic and non-toxigenic species of *Aspergillus* and *Fusarium*. The results for culture medium for differentiation of aflatoxigenic and non-aflatoxigenic fungi are published in Patent no. RO 125071 and culture medium for differentiation of *Fusarium* toxigenic fungi are published in Patent no. RO 123 355. The research techniques of artificial soil contamination by fungi spores allow us to describe the contamination level of corn crops simulate in *vivo* conditions.

Culture medium for differentiating toxigenic and non-toxigenic fungi shows most differentiation capacity for *Aspergillus* and *Fusarium* non-toxigenic with 2.8-3.2 parts of dimethyl-ß-cyclodextrin. (Figure 4 and 5).

Culture medium for differentiation between toxigenic and non-toxigenic species.

The technical problem solved by selective culture medium with 2.8-3.2 parts of dimethyl-ß-cyclodextrin is rapid isolation and differentiation of phytopathogenic and toxigenic fungi of the genus Aspergillus and Fusarium.

Figure 4. Selective culture medium for differentiation between toxigenic and non-toxigenic species; corn samplies, Oltina, 2011

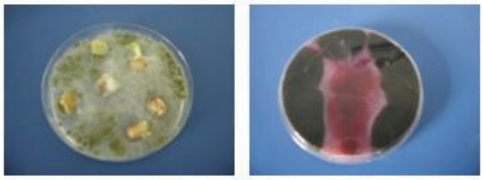

Figure 5. Differentiation between *Aspergillus* and *Fusarium* pathogenic-toxigenic species on selectives culture medium; corn seeds, Oltina, 2011

Figure 6. Influence of *Aspergillus flavus* (on left) and *Trichoderma harzianum* Tdh al12 (on right) isolates on corn seed germination and emergence

Giberella zeae (Fusarium graminearum) on corn crop, cause disease in all stages of development, the seedlings, the stem and the seeds. Attacked seedlings rotting (Figure 7).

Figure 7. Pathogenicity of *Fusarium graminearum* fungus in germination–emergence stages

The level of soil contamination with mycotoxin producing fungi (*Aspergillus flavus; Fusarium oxysporum* and *F. graminearum*) has influenced *in vivo* conditions on germination–emergence corn stage (Figures 8 and 9).

Seeds corn treated with *Trichoderma pseudokoningii* (Td85 PTS-2 kg/t) in infected soil with *A. flavus* and *Fusarium graminearum+F. oxysporum* (V_7-variant) has presented the best influence of biological activity of *Trichoderma pseudokoningii* applied as bioproduct Td85, followed by variant 6. The results are compared with those obtained in V_1 (natural control) that there were only seven plants grown at both 10 days and 14 days.

Untreated corn in soil infected with *F. graminearum+F. oxysporum* and *Aspergillus flavus* (V_2) influenced negatively the germination and emergence of corn plants, followed by untreated corn in soil infected with *Aspergillus flavus* (V_4).

After 14 days of sowing, there were 19 plants emergence delayed (V_2), respectively, 15 plants springing delayed (V_4), the results were compared with untreated corn in soil infected with *Trichoderma harzianum* strain Tdh al12 (V_5) in the number of plants springing delayed was reduced to half (8 plants).

Number of plants with emergence delayed (8 plants) obtained in untreated corn in soil infected with *A. flavus, F. graminearum + F. oxysporum and T. harzianum*-Tdh strain al12 (V_6) was similar to that obtained in untreated corn variant in soil inoculated with Tdh strain al12 (V_5).

Untreated corn in soil infected with *F. graminearum+F. oxysporum* (V_3) did not affect the corn plants grown but was observed the pathogenicity of *Fusarium sp*.on corn seed (Figure 7).

The large number of plants with delayed emergence (11 plants) was obtained in corn treated with Thiram (3 kg/t) in soil infected with *A. flavus* (V9), after 14 days from sowing. This results is due to the inaction of fungicide against *Aspergillus flavus* and of the influence of the fungus on emerged corn plants (Figure 6).

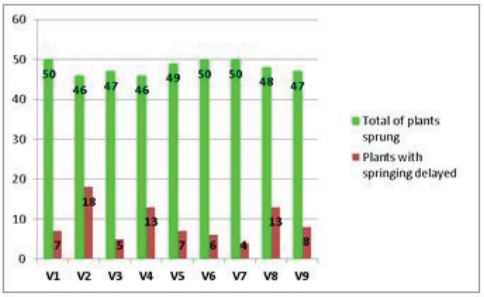

Figure 8. The influence of contamination level on germination–emergence corn stages after 10 days from sowing

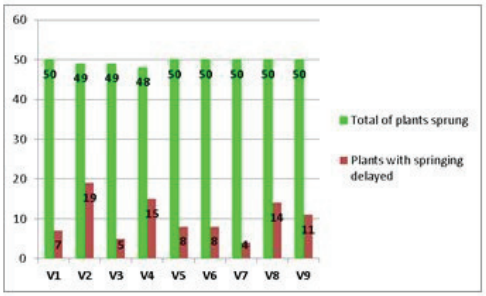

Figure 9. The influence of contamination level on germination-emergence corn stages after 14 days from sowing

In variant control untreated corn, sown in heat-treated soil (V_1), there were 50 plants sprung and a total of 7 plants springing delayed so after 10 and 14 days after sowing (Figure 8 and 9).

Number of plants springing delayed obtained in V7 (4 plants) was exceeded in control V1 (7 plants).

Comparative results has obtained in V7 and V8. *Trichoderma harzianum* Tdh al12 and *Trichoderma pseudokoningii Td 85* strains have the ability to colonize the roots of corn plants and to inhibit pathogens of *Aspergillus* and *Fusarium genus.*

Sustainable agriculture on Dobrogea corn crop by local beneficial microorganisms

New opportunities for Dobrogea agriculture had been identified. Beneficial microorganisms of *Trichoderma* genus, *Trichoderma harzianum* Tdh al12, isolated from corn seeds substrate, Dobrogea agricultural area, 2012 (Figure: 10). Tdh al12 strain could be identified to species level based on morphological and physiological characteristics, assigned to the species *Trichoderma harzianum.*

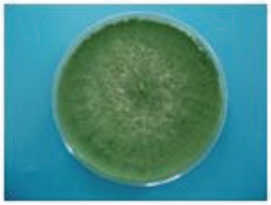

Figure 10. Pure culture of beneficial strain *Trichoderma harzianum* Tdh al12, isolated from corn seeds; Dobrogea samples, 2012

Trichoderma harzianum Tdh al12 strain has different action against microbiological agents showing strong antagonism *in vitro* against *Fusarium oxysporum* and *F. graminearum* pathogens and limiting development of toxigenic *Fusarium* fungi (Figure 11); using Tdh al12 strain as biocontrol agent to reduce mycotoxin producing fungi (*Aspergillus flavus*) (Figure 12) and to stimulate.

In vitro antagonism of *Fusarium oxysporum* assesed by double culture method mathematical coefficient were: x = 0.19 to 0.33 (4 days), x = 0.34 to 0.38 (8 days) and of *Fusarium graminearum* x = 0,30-0,37 (4 days); x = 0,32-0,44 (8 days). x < 1 antagonism (A) the stronger (PA) as the values are closer to the value 0.

Antagonistic strains of the genus *Trichoderma ssp* are able to produce various secondary metabolites that may play a role in the mechanism of action of their biological activity. *Trichoderma harzianum* strain 1295-22 (commercial product T-22 / TRIANUM-G) has currently the greatest ability to colonize plant roots and to inhibit pathogens like: *Pythium, Rhizoctonia, Fusarium*.

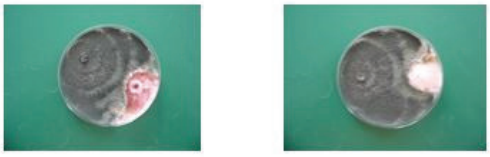

Figure 11. Tdh al12 strain antagonism against pathogens fungus *Fusarium graminearum* (on left) and F. oxysporum (on right)

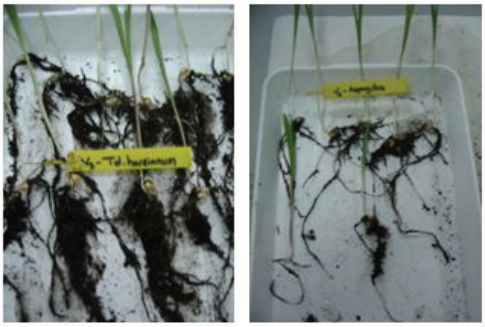

Figure 12. Biocontrol agent Tdh al12 to reduce *Aspergillus flavus* toxigenic fungi in corn

Figure 13. Stimulating corn root system development by Tdh al12 beneficial strain

CONCLUSIONS

Developing new methodologies for assessing toxigenic-pathogenic fungi of *Aspergillus and Fusarium* genus using culture medium with 2.8-3.2 parts of dimethyl-β-cyclodextrin and beneficial fungi (*Trichoderma pseudokoningii* Td 85 and *T. harzianum* al12) leading to a quantitative risk-benefit assessment strategy and bring together relevant knowledge and experience about improvements in Dobrogea agricultural area.

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