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IN VITRO ANTIFUNGAL ACTIVITY OF SOME BIOPESTICIDE PROTOTYPES ON THE FUNGUS *Fusarium* spp.

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Abstract

Our research was to evaluate the antifungal activity of a prototype biofungicide with antifungal and fertilizing activity, in the variants, combinations of plant extracts of skinduf and tagetes, steroidal glycoalkaloids from tomato, camelina oil and protein hydrolyzate, on the mycelial growth of the fungus Fusarium spp. The research followed in vitro mycelial growth at 3, 6, 9, 12 days by measuring the diameter of the colonies and determining the average value. The effectiveness of the variants of the prototype was calculated and it was found that the highest value of the effectiveness, over 50%, was recorded on vegetative growth up to 9 days of observation, for both variants. After 12 days of observation, the effectiveness decreased, reaching 19.55% in variants with tagetes extract.

Key words*: antifungal activity, micelial growth, eficacy.*

INTRODUCTION

Fusarium spp. are a complex of pathogens of the genus *Fusarium* that colonize plant organs, being associated in particular with the attack on seeds (de la Pena et al., 1999; Dudoiu et al., 2016; Cîrstea et al., 2022; Gheorgieş et al., 1996; Iacomi and Gheorghies, 2008; Cristea et al., 2024). The attack of *Fusarium* induces characteristic symptoms of rot and tracheomycosis, and in the case of seeds, it causes their qualitative deterioration and decreases in the harvest (Hasan, 1999). The severity of the attack depends on the presence of the inoculum, environmental conditions and plant resistance (Bunta et al., 2020; Ittu et al., 1978.) Also, *Fusarium graminearum* induces the appearance of mycotoxins in the affected seeds with an effect in the food trophic chain (Hasan H.A.H., 1999; Zaharia et al., 2022; Radoi et al., 2011; Zhu et al., 2019; Tamba-Berehoiu et al., 2010; Placinta et al., 1999; Jurado et al., 2006; Jedidi et al., 2018; Atoui et al., 2011). The control of *Fusarium* micromycetes with fungicides is not always efficient enough, in favorable climatic conditions, the presence of the infection and the sensitivity of the varieties (Mesterhazy et al., 2011). In the concerns of supporting agriculture, the use of simple plant extracts or in combination with different sources constitutes measures not only friendly to the environment but also possibilities to obtain ecological products that meet the requirements of the population to eat healthy (Elwaziri et al., 2023). Research on the antifungal effect of some products was carried out on some plant pathogens both during the vegetation period and in post-harvest conditions, knowing the effect of losses caused by pathogens that produce severe attacks such as rots (Nunes et al., 2001). Also, the risk of developing strains

resistant to many fungicides (Spotts and Cervantes, 1986) impels the taking of measures to ensure the protection of plants and the environment through alternative control methods in vegetation and post-harvest (Wisniewski and Wilson, 1992) and, therefore, research is focused on biological control (Grzegorczyk et al., 2017). An alternative for the control of pathogenic fungi in plants is the use of plant extracts with antifungal activity, extracts that can be sources of bioproducts or protection formulations against pathogens (Calvo et al., 2011; Ichim et al., 2017). Plants belonging to the *Solanum* genus were used to investigate the pathogenicity and virulence of some phytopathogens (Ichim et al., 2017). Steroidal glycoalkaloids, secondary metabolites extracted from *Solanum* species have antimicrobial properties (Iijima et al., 2013; Itkin et al., 2011; Milner et al., 2011). The literature cites numerous materials that have shown potential in the control of plant diseases such as silicon (Belanger et al., 1995), calcium (Conwayet al., 1982), carbonate and bicarbonate salts (Smilanick et al., 1999), essential oils (Thomson, 1989).

MATERIALS AND METHODS

Our research aimed to test *in vitro* the antifungal action of some prototypes combined of plant and protein extracts on the growth of *Fusarium* spp. Formulation studies at the laboratory level between plant extracts and protein extracts led to the configuration of some prototypes, between which also the Glycam-Stim Combo prototype (with antifungal and fertilizing activity). The purpose of our research was to evaluate *in vitro* the antifungal activity of a prototype, in the variants, AI GLY T - (glycoalkaloids from baby tomato) + camelina oil + skinduf butylene glycol extract + protein hydrolyzate CHC3B and AII GLY T - (glycoalkaloids from baby tomato) + camelina oil+ butylene glycol extract of tagetes + protein hydrolyzate CHC3B, in 1% concentration on the mycelial growth of the fungus *Fusarium* spp. isolated from corn seed. 10-day-old fungus *Fusarium* spp. grown in 55 mm Petri dishes and the PDA culture medium (potato-dextrose-agar) were used. The method of including the tested product in the culture medium was used (Schmitz, 1930). Each variant was placed in three repetitions and one control (variant control). The average diameter of the fungus colonies was measured at 3, 6, 9, and 12 days after incubation. And the effectiveness (E%) was calculated as percentage of colony inhibition. The efficacy of the product was determined after 3,6,9,12 days of observation, as the rate of inhibition of mycelial growth from the test variants compared to the control variant, according to the formula: I%= [(Dc-Dt)/Dc]x100, where, I % is mycelian growth inhibition (the efficacy), Dc is average mycelian growth diameters of fungus colony in control, Dt is average mycelian growth diameters of fungus colony in treatment (Pandey et al., 1982).

RESULTS AND DISCUSSIONS

In *in vitro* conditions, the influence of biofungicide prototypes on the vegetative growth of *Fusarium* spp. fungus colonies was evaluated.

Table 1. The antifungal activity of the prototype AI GLY T and AII GLY T (1%)

Pathogen	Variants/ AI GLY T/ AII GLY T	Average Diameter of the mycelial colony (mm) / days			
		3 days	6 days	9 days	12 days
Fusarium spp.	AI GLY T test	3.33	5.83	18.33	32.00
	control	7.33	12.5	40.66	44.66
	AII GLY T test	1.66	7.00	19.83	35.66
	control	4.33	15.16	41.16	44.33

The data in Table 1 show that in the test variant AI GLY T the colony of *Fusarium* fungi registered 3.33 mm average value of the diameter of the colonies after 3 days, and 5.83 mm after 6 days, showing a lower speed of colonial growth in the first 6 days. At 9 days

the average diameter of the colony had the value of 18.33 mm and after 12 days the mycelial colony developed to the average value of 32.00 mm, finding that the growth rate of the fungi increased between 6 and 12 days. In the control variant, the *Fusarium* fungi developed faster starting from 7.33 mm after 3 days and reaching 40.66 mm after 9 days. After 12 days of incubation, the fungus invaded the culture plate reaching a value of 44.66 mm, the average value of the two values of the diameters of the colonies (Figure 1).

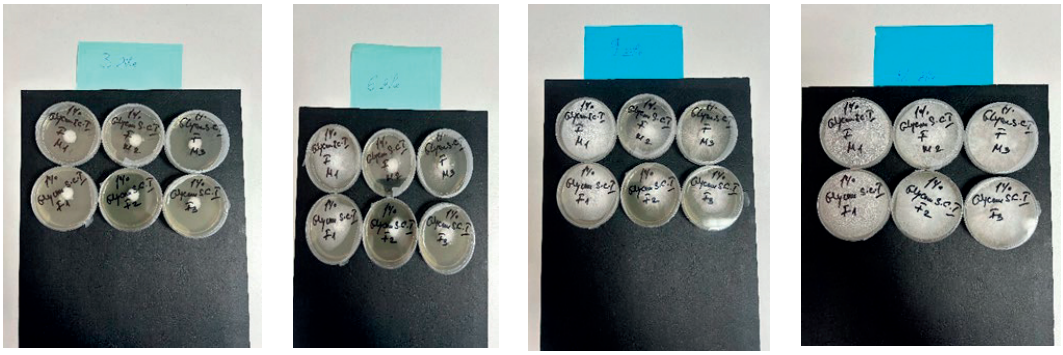

Figure 1. Mycelial growth of *Fusarium* spp. fungi at 3, 6, 9, 12 days - AI GLY T variant

In the case of the AII GLY T test variant regarding the influence of the tested prototype on the growth of the colony of *Fusarium* spp. fungi, the data from the same table show that in the test variant the values were reduced compared to the control variant, so that after 3 days the average diameter was 1.66 mm in the test variant of 4.33 mm in the control variant and after 6 days the diameter of the colony reached 7.00 mm in the test variant compared to 14.00 mm in the control version. After 9 days, the diameter of the colony was 19.83 mm and 35.66 mm after 12 days in the test variant compared to the control variant where the diameter reached over 41 mm after 9 days and over 44.33 mm after 12 days (Figure 2).

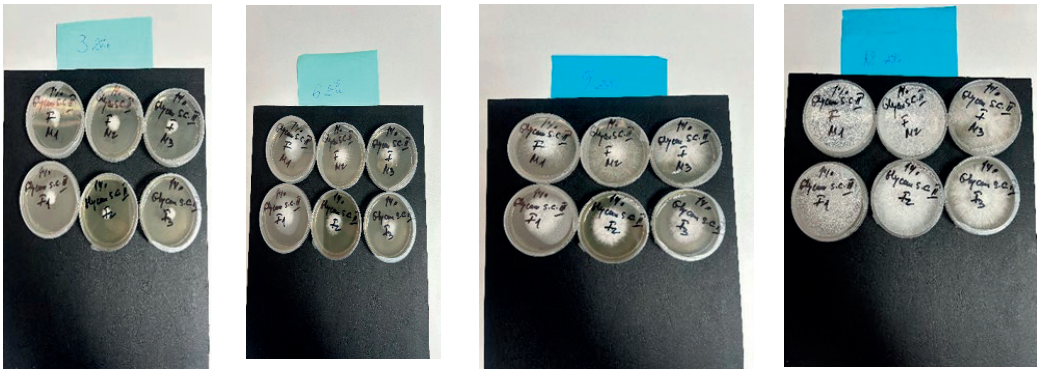

Figure 2. Mycelial growth of *Fusarium* spp. fungi at 3, 6, 9, 12 days - AII GLY T variant

The effectiveness or the percentage of inhibition of the mycelial growth of the fungus was also calculated and it was found that in the AI GLY T variant the highest values of the percentage of inhibition were recorded during the growth period from 3 days to 9 days with values over 50% respectively 54.7% at 3 days, 53.36% at 6 days and 54.91% at 9 days. After 9 days of incubation, the vegetative mass developed so that the percentage of inhibition decreased to 28.34% (Figure 3). Regarding the effectiveness of the AII GLY T variant, the highest percentage of inhibition was determined in the first 3 days after incubation

of 61.66%, and in the growth interval 6-9 days the effectiveness was 53.82% at 6 days and 51.82% at 9 days after incubation. A decreasing trend of the inhibition percentage was observed, which after 12 days was 19.55% (Figure 3). Research on the antifungal activity of some glycoalkaloid steroids extracted from species belonging to *Sopanum* genus was carried out by Cristea et al. (2017), Perişoara et al. (2022) also studied the development of a phytostimulant based on *Tagetes esrecta* and *rhizobacteria* to increase the activity antifungal against some phytopathogens, including *Fusarium* spp. (Perişoara et al., 2022).

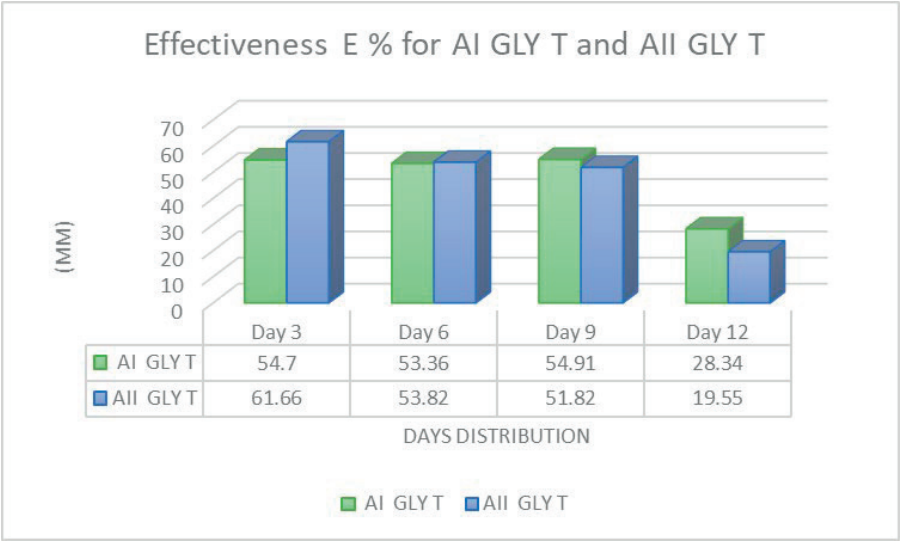

Figure 3. The efficacy of AI GLY T and AII GLY T 1% on *Fusarium* spp.

CONCLUSIONS

The two variants of the prototype Glycam-Stim Combo tested on the vegetative growth of the pathogen *Fusarium* spp. had an efficacy of over 50% in the interval of 3-9 days. After 12 hours of observation, the effectiveness of the tested products was reduced to 28.34% for AI GLY T and 19.55% for the AII GLY T variant. It was found that in the AI GLY T variant, during the period 3-9 days, the effectiveness had a slight tendency to increase, and in the AII GLY T variant, a slight tendency to decrease in the dynamics of the effectiveness value was observed. In the AII GLY T variant, the effectiveness was exceeded 60% in the first 3 days of observation

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REFERENCES

Atoui, A., El Khoury, A., Kallassy, M., Lebrihi, A. (2011). Quantification of *Fusarium graminearum* and *Fusarium culmorum* by real-time PCR system and zearalenone assessment in maize. *Int. J. of Food Microbiol.*, 154(1-2): 59-65. ISSN 0168-1605.

Belanger, R.R., Bowen, P.A., Ehrent, D.L., Menzies, J.G. (1995). Soluble silicon: its role in crop and disease management of greenhouse crops. *Plant Dis* 79:329-336.

Bunta, G., Bucurean, E., Cosma, C. (2020). Results regarding the *Fusarium* head blight attacck on wheat in Western Romania. *Annals of the University of Oradea, Fascicle: environamental Protection,* vol. XXXV:19-30.

Calvo, M.A., Arosemena, E.L., Shiva, C., Adelantado, C. (2011). Antimicrobial activity of plant natural extracts and essential oils. In: Science against microbial pathogens: communicating current research and technological advances A. Méndez-Vilas (Ed.) Formatex. *Microbiology Book Series Number* 3: 1179-1185.

Cîrstea, G. V., Ivan, E.S., Cristea, S. (2022). Research on micoflora associated with alfalfa seeds (in vitro). *Scientific Papers -Series A- Agronomy,* vol. 65 (1):245-249.

Conway, W.S. (1982). Effect of postharvest calcium treatment on decayGoldenDelicious apples. *Plant Disease,* 74:134-137.

Cristea ,S, Manole, M.S., Zala, C., Jurcoane, Ş., Dănăilă-Guidea, S., Matei, F., Dumitriu, B., Temocico, G., Popa, A.L., Călinescu, M., Olariu, L. (2017). In vitro antifungal activity of some steroidal glycoalkaloids on *Monilinia* spp. *Romanian Biotechnological Letters*, Vol. 22, 5.

Cristea, S., Popescu, S-E., Joita-Pacureanu, M., Vlad, A.I. (2024). Mycoflora associated with *black point* on two –row and six-row barley- First report. *Romanian Agricultural Research.,* Vol 41: 307-313.

De la Pena, R.C., Smith, K.P., Capettini, F. (1999). Quantitative trait loci associated with resitance to *Fusarium* head blight and kernel discoloration in barley. *Theor. Appl. Genet*., 99:561-569.

Dudoiu, R., Cristea, S., Lupu, C., Popa, D., Oprea, M. (2016). Micoflora asociata with maize grains during storage period. *Agrolife scientific Journal*, vol. 5 (1): 63-68.

Elwaziri, E., Ismail, H., El-Khairl, E-S., Al-Qahtani, M. S., Al-Harbi, A. N., El-Gawad, A.B.D.G.H., Omar, A. W., Abdelaal, K., Osman, A. (2023). Biostimulant application of whey protein hydrolysates and potassium fertilization enhances the productivity and fertilization and tuber quality of sweet potato. *Notulae Botanicae Horti Agrobotanici* Cluj-Napoca Vol 51, Issue 2, Article number 13122.

Gheorghieş, C., Cristea, S., Iacomi, B., Groza, O. (2004). Research on wheat kernel black-pont and involved pathogens. *Lucrări Ştiintifice USAMV, Bucuresti, Seria* A., Vol. XLVII, 280:287.

Grzegorczyk, M., Zarowska, B., Restuccia, C., Cirvilleri, G. (2017). Postharvest biocontrol ability of killer yeasts against *Monilinia fructigena* and *Monilinia fructicola* on stone fruit. *Food Microbiology,* vol. 61, 93-101.

Hasan, H.A.H. (1999) Phytotoxicity of pathogenic fungi and their mycotoxins to cereal sedling viability. *Mycopathologia,* 148: 149-155.

Iacomi, B., Gheorghies, C. (2008). Studies on interrelationship between microorganisms associated with wheat black point micoflora. *Lucrări Stiintifice USAMVB.,* Seria A, vol LI, 821-830.

Ichim, E., Maruntescu, L., Popa, M., Cristea, S. (2017). Antimicrobial efficacy of some plant extracts on bacterial ring rot pathogen, *Clavibacter michiganense* ssp. sepedonicus. *The Eurobiotech Journal*, vol 1 Issue 1, 93-96.

Iijima, Y., Watanab, E.B., Sasaki, R., Takenaka, M., Ono, H., Sakurai, N., Umemoto, N., Suzuki, H., Shibata, D., Aoki, K. (2013). Steroidal glycoalkaloid profiling and structures of glycoalkaloids in wild tomato fruit. *Phytochemistry*, Vol. 95, 145-157.

Itkim, M., Rogachev, I., Alkan, N., Rosenberg, T., Malitsky, S., Masini, L., Meir, S., Iijima, Y., Aoki, K., deVos, R., Prusky, D., Burdman, S., Beekwider, J., Aharoni, A. (2011). Glycoalkaloid Metabolism1 Is Required for Steroidal Alkaloid Glycosylation and Prevention of Phytotoxicity in Tomato. *The Plant Cell*, Vol. 23: 4507–4525.

Ittu, M., Saulescu, N.N., Ittu, Gh., Moldovan, M. (1979). Aspecte genetice ale relatiilor de tip gazda-parazit in cadrul genului *Fusarium*. *Probl.Genet.Teor. Apl*., XI (3): 193-211.

Jedidi, I., Soldevilla, C., Lahouar, A., Marín, P., González-Jaén, M.T., Said, S. (2018). Mycoflora isolation and molecular characterization of *Aspergillus* and *Fusarium* species in Tunisian cereals. *Saudi Journal of Biological Sciences*, 25: 868-874.

Jurado, M., Vázquez, C., Marín, S., Sanchi, V., González-Jaén, M.T. (2006). PCR-based strategy to detect contamination with mycotoxigenic *Fusarium* species in maize. *Syst. Appl. Microbiol*., 29: 681-689.

Masterhazy, A., Toth, B., Varga, M., Bartok, T., Szabo – Hever, A., Farady, l., Lehoczki-Krsjak, S. (2011). Role of fungicides application of nozzle types and the resistence level of wheat varieties in the control of *Fusarium* head blight and deoxynivelanol. *Toxins* 3(11): 1453-1483.

Milner, E.S., Brunton, P.N., Jones, W.P., O'Brien M.N., Collins, G.S., Maguire, A.R. (2011). Bioactivities of glycoalkaloids and their aglycones from *Solanum* species. *Journal of Agricultural and Food Chemistry*, 59(8), 3454- 3484.

Nunes, C., Usall, J., Teixido, N., Ochoa de Eribe, X., Vinas, I. (2001). Control of post –harvest decay of apples by preharhest and postharvest application of ammonium molybdate. *Pest Management Science*: 57: 1093-1099.

Pandey, D.K., Tripathi, N.N., Tripathi, R.D., Dixit S.N. (1982). Fungitoxic and pfytotoxic of essential oil of Hyptis suaveolens. Z. *Planzenk,* vol. 89, 344-349.

Perişoara, A., Marinaş, I.C., Geana, E.I., Constantin, M., Angheloiu, M., Pirvu, L., Cristea, S. (2022). Phytostimulation and synergic antipathogenic effect of *Tagetes erecta* extract in presence of *Rhizobacteria*. 2022. *Horticulturae* 8 (9), 779.

Placinta, C.M., D'Mello, C.P.F., MacDonald, A.M.C. (1999). A review of worldwide contamination of cereal grains and animal feed with *Fusarium* mycotoxins. Anim. *Feed Sci. Technol*., 78: 21-37

Radoi, F., Israel, R.,F., Cristea, S., Smeu, I., Radu, A. (2011). Quantitavie study of Deoxynivalenol and ochratoxin accumulation in synthetic media. *Romanian Biotechnological Letters,* vol 16(1):33- 39, Suplement S.

Schmitz, H. (1930). Poisoned food technique. Industrial and Engineering Chemistry. *Analyst.* 2:361.

Smilanick, J.L., Margosan, D.A., Mlikota, F., Usall, J., Michael, I. (1999). Control of citrus green mold by carbonates and bicarbonates salts and the influence of commercial postharvest practices on their efficacy. *Plant Dis*. 83 ;139-145.

Spotts, R.A., Cervantes, L.A. (1986). Populations, pathogenicity and benomil-rezistance of *Botrytis* spp., *Penicillium* spp and Mucor piriformis in packinghouses. *Plant Dis*.70: 106-108.

Tamba- Berehoiu, R., Popa, C.N., Popescu, S., Cristea, S., Culea, R., Tamba- Berehoiu, S. (2010). Distribution of some toxic contaminants in the milling products, during the milling process. *Romanian Biotechnological Letters,* vol. 15(3): 5281-5286.

Thomson, D.P. (1989). Fungitoxic activity of essential oil components an food storage fungi. *Mycologia,* 81:151-153.

Wisniewski, M.E., Wilson, C.L. (1992). Biological control of postharvest diseases of fruits and vegetables: recent advances. *HortScience,* 27:94-98.

Zaharia, R., Petrisor, C., Cornea, P., Diguta, C., Cristea, S., Ştefan, S. (2022). Isolation and molecular identification of fungal isolates from stored cereals using PCR-RFLP method. *Romanian Agricultural Research,* vol. 39 :13-22.

Zhu, Z., Hao, Y., Mergoum, M., Bai, G., Humphreys, G., Cloutier, S., Xia, X., He, Z. (2019). Breeding wheat for resistance to *Fusarium* head blight in the global North China, USA and Canada. *The Crop Journal* 7(6): 730-738.