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PRELIMINARY STUDIES REGARDING THE POTENTIALLY EFFECT OF EXTRACTS FROM *Citrullus lanatus* PEELS ON SOME CEREAL`S AND FRUIT'S PATHOGENS

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Abstract

The attack of phytopathogens specific to the main cereal types must be limited because it can lead to increasing mycotoxins levels in the plants and grains, above the limit allowed by the legislation in force. In this regard, at the present, there are intense concerns regarding the use of specific product types for plant protection, in favor of natural (green technologies) products, to avoid the use of fungicidal products obtained by chemical synthesis. In this regard, the present study investigated the effect generated in vitro by some dried peel (ethanolic) extracts, obtained from different varieties of Citrullus lanatus, on pathogens specific to Triticum aestivum and Zea mays. The bioproducts obtained from these varieties were tested on the pathogenic microorganisms for cereals such as Fusarium culmorum, Fusarium verticillioides, Fusarium graminearum, and also on the pathogenic microorganisms for fruits such as Penicillium expansum, and/or Penicillium digitatum. The results performed in vitro revealed that the tested bioproducts exhibit local/moderate antifungal activities, recommending them as potential candidates for obtaining natural bioproducts with antifungal activities.

Key words: *Citrullus lanatus peels extracts, antifungal effect, cereals, fruits.*

INTRODUCTION

It is estimated that 25% of plant production globally and about 20% in the EU is contaminated with mycotoxins. Most are produced by fungi of the genus *Fusarium*, *Aspergillus*, *Penicillium,* and *Alternaria* (Kępińska-Pacelik, 2021). *Fusarium* species are among the most dangerous phytopathogenic microorganisms because they contaminate cereal grains with high toxicological potential mycotoxins (Mielniczuk & Skwarylo-Bednarz, 2020). Among the mycotoxins produced by *Fusarium*, deoxynivalenol (DON) - a mycotoxin from the trichothecene group is found most in cereal grains. Its action is manifested at the cellular level, where its presence induces the signal to start the process of programmed cell death for normal cells (Mielniczuk & Skwaryło-Bednarz, 2020). Fumonisins (FB) (produced by *Fusarium verticillioides*) are the most toxic mycotoxins produced by Fusarium strains, being considered neurotoxins, because they affect the nervous tissues and the brain. Zearalenone (ZEA), another toxic compound is produced by *Fusarium culmorum* and *Fusarium graminearum*; it can also be called estrogenic mycotoxin due to its similarity to the natural structure of estrogens. The presence of this compound can cause changes in the production of hormones and respectively in the normal functioning of the reproductive system. The compound named deoxynivalenol (vomitoxin), represents one of the most powerful toxins because it can cause digestive problems, hemotoxicity, leukocytes, or even death. Patulin and penicillic acid, although not considered strong toxins, can cause cytotoxicity, immunotoxicity and even cancer after repeated exposure. Most commonly mycotoxins found in cereals (wheat, corn) are deoxynivalenol, fumonisin B1 and B2, and zearalenone (Otero et al., 2020; Kępińska-Pacelik, 2021; Palou, 2014). Phytopathogenic species of the genus *Penicillium* and other produce patulin and citrinin, mycotoxins with moderate toxicity. They act especially on kidney cells and can cause the destruction of DNA molecules in the brain, liver, and/or kidneys of laboratory animals (Altomare et al., 2021; Radu et al., 2012). The strategy to reduce mycotoxins generated by the presence of phytopathogenic agents consists in the application of different treatments for the protection of plants during vegetation. Protection from the attack of the phytopathogens can be achieved by biological methods (natural pests or bioproducts obtained from microorganisms of the *Trichoderma* type), the use of synthetic fungicides (triazole, metaconazole, tebuconazole, cyproconazole) or the use of plant protection agents obtained from natural sources. From this point of view, polyphenolic compounds are important, which most of the time can be obtained from residues from the food industry - respectively cucubitaceae peels. The family Cucurbitaceae is a large group of plants, with over 800 known species worldwide. Vegetables in this family have been used for centuries, not only for consumption, but also for their medicinal value. The species belonging to this family are rich in carotenoids, terpenoids, saponins, and other phytochemical compounds (Rolnic et al., 2020). These are non-nutritive compounds and occur naturally in plants. There are numerous phytochemical compounds with antimicrobial activities such as polyphenolic compounds, alkaloids, anthocyanins, carotenoids, tannins, monoterpenes, triterpenes, and saponins (Harith et al., 2017; Badr et al., 2015), the main antimicrobial effects reported by specialized literature being presented in Table 1. An important class of triterpenoid compounds is represented by cucurbitacins, which have the role of defense and appear in the peels of different curcubitaceae species, including *Citrullus* sp. (Kim et al., 2018). In vitro research demonstrated the cucurbitacin compounds identified in *Citrullus lanatus* peels (structure presented in Figure 1), have the antitumor effect, and their mechanism of action is based on the activation of the p53 gene (tumor suppressor gene), arrest the cell cycle in the presynthetic stage (G1) and apoptosis (Duangmano et al., 2012; Ge et al., 2018; Mohammed et al., 2019).

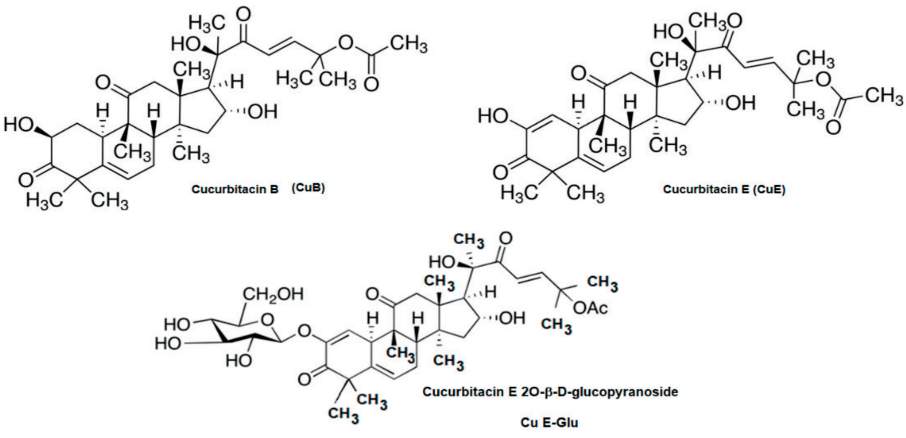

Figure 1. The general structure of compounds from the class of cucurbitacins highlighted in the peels of *Citrullus lanatus*

In recent times, more attention has been paid to the effects that occur after the use of synthetic pesticides, attention due to the associated environmental pollution, and due to the phenomena of resistance to them. The bioproducts with pesticidal effects obtained from natural sources (microorganisms, plants, or animals) are biodegradable and do not produce adverse effects. In organic but also in conventional agriculture, there is a fairly high demand for bioproducts with pesticidal action obtained from natural resources (natural extracts), especially from plants (Lakshmeesha et al., 2019). The mechanisms involved in the antimicrobial effects are not yet known, but studies have shown that the whole extract works better than a single isolated compound (Salehi et al., 2019).

The aim of the work was to test the antifungal effect of some ethanolic extracts obtained from the dried peels of indigenous varieties of *Citrullus lanatus* on *Fusarium culmorum, Fusarium verticillioides, Fusarium graminearum, Penicillium expansum,* and/or *Penicillium digitatum*.

Tabel 1. The antimicrobial and antifungal effect of different extracts of the *Citrullus lanatus* Thunb

Citrullus species	Extract type	Microorganisms	References
Citrullus lanatus (dried peel)	Alcoholic extract (methanol; ethanol)	*S. albus; S. aureus; E. faecalis; P. fluorescens; E. coli; B. subtilis; M. luteus; L.innocua; K. oxytoca; S. enterica; S. sonnei; S. thermophilus; S.typhi; E.coli; K. pneumoniae; P. aeruginosa; B. subtilis; C. albicans; A. niger; P.chrysogenum; T. bepelli.*	Neglo et al., 2021 ; Egbuonu, 2015 ; Mohammed et al., 2020
Citrullus lanatus, Citrullus vulgaris, Citrullus colocynthis (dried peel)	Alcoholic extract (ethanol)	*F.oxysporum; P.chrysogenum; A. niger.* % of inhibition growth (% IG) in the case of *F. oxysporum* can be determined the with relation: *% IG = 22,025 ln(x)-82,283* % of inhibition growth in the case of *A. niger* can be determined with relation: *% IG= 19,845 ln(x)-67,219* *x= extract concentration, μg/mL*	Elonsary et al., 2020; Thirunavukkarasu et al., 2010; Shah et al., 2022

MATERIALS AND METHODS

Sources of polyphenolic compounds

The peels from 3 varieties of *Citrullus lanatus* Thunb (Figure 2 a, b, c) acquired from a local producer in Fundulea, Călărași), were used as the sources of polyphenolic compounds, namely:
- peels from the fruits of *Citrullus lanatus* Tropical variety, a watermelon with dark green peel and yellow pulp (Figure 2 a);
- peels from the fruits of *Citrullus lanatus* Huelva variety, a watermelon with dark green peel and red pulp (Figure 2 b);
- peels from the fruits of *Citrullus lanatus* Lusiana variety, a watermelon with a red core, whose peel shows both dark green and light green stripes (Figure 2 c).

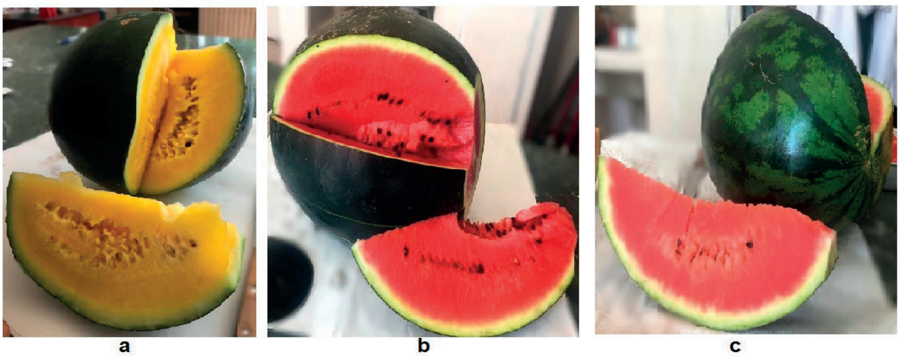

Figure 2. *Citrullus lanatus* varieties used for obtaining the polyphenolic products:
a) Tropical variety; b) Huelva variety; c) Lusiana variety

Strains of phytopathogenic fungi used in *in vitro* studies

The microorganisms used in the in vitro studies were the following: *Penicillium expansum* DSM 62841, *Penicillium digitatum* DSM 2731, *Fusarium verticillioides* DSM 62264, and three aggressive strains of *Fusarium*, isolated from the *Triticum aestivum* plants, respectively *Fusarium culmorum* 46 (a gift from Prof. Thomas Miedaner, University Hohenheim

Germany), *Fusarium graminearum* 96, *Fusarium culmorum* 1056 and *Fusarium culmorum* 1471 (Ittu et al., 2010).

The tested *Fusarium* species are aggressive and were previously characterized through molecular analysis (Cornea et al., 2013), confirming the genus and species of each one, after which were included in the NARDI Fundulea collection.

Obtaining polyphenolic extracts

The peels from three varieties of *Citrullus lanatus* were washed with distilled water, cut into small pieces, and dried at 40°C in a thermostated oven. After drying, the peels were soaked in ethanol 70% (dry plant material:solvent ratio = 1: 5), in dark glass containers, in the dark for 1 month, after which they were filtered and the resulting supernatant was kept in dark color containers. The alcoholic extracts obtained from the peels of the Huelva variety were called CL-100, the alcoholic extract from the Lusiana variety was called CL-101, and the extract obtained from the Tropical variety was named CL-103. All extracts obtained were standardized to 5 mg GAE/ml extract, by dilution with 70% ethanol.

Evaluation of the antifungal effect

Spores from each fungal species were inoculated with a sterile cotton swab on Petri plates with 4 compartments, with PDA (Potato Dextrose Agar) culture medium. After 30 minutes, on the surface of each inoculated Petri plate were put 3 cellulose discs with a diameter of 6 mm, previously soaked in the alcoholic extract. Additionally, were made an inoculation with a spot of 10 microliters of each extract, in a fourth compartment of each Petri plate. As a control sample, a 70% ethanol solution was used.

The effectiveness of the treatment was evaluated after incubation at 24°C for 48 h and measuring the inhibition diameter. The obtained results were presented in the form of the arithmetic mean of three determinations. The evaluation of the antifungal activity of each extract was carried out according to a random scale, established as follows:

- average *inhibition* diameter = 0 mm: extract without antifungal effect;
- 1 mm ≤ average diameter of inhibition ≤ 10 mm: extract with local antifungal effect;
- 10 mm < average diameter of inhibition ≤ 20 mm: extract with moderate antifungal effect
- average diameter of inhibition > 20 cm: extract with significant antifungal effect.

RESULTS AND DISCUSSION

The results obtained after processing the experimental data are presented in figures 3÷6 and reveal the following:

- In the case of CL-100 extract, the best results were obtained for *F. verticillioides* (average diameter of inhibition = 12.67 mm), *P.* expansum (average diameter of inhibition obtained = 11.33 mm), and *F. graminearum* 96 (inhibition diameter obtained = 10.33 mm), the antifungal effect obtained in the case of these micromycetes being a moderate one (Figure 3, Figure 6). For *P. digitatum*, *F. culmorum* 46 and *F. culmorum* 1056 obtained a local antifungal effect, much weaker in the case of the two strains of *F. culmorum* (Figure 3 and the Figures 6 a1, a5, a7).
- The extract CL -101 has a moderate antifungal effect in the case of the following fungal strains: *P. expansum* (inhibition diameter 11.7 mm), *F. graminearum* 96, and *F. verticillioides* (inhibition diameter achieved in the case of the two species of *Fusarium* = 11 mm) (Figure 4, Figure 6 a2, a3, a4). In the case of the other microorganisms studied, the antifungal effect of CL-101 extract is local, except the *F. culmorum* 46, where this extract has no effect.
- The results obtained at exposure of the studied microorganisms at the CL-103 (Figure 5, Figures 6, a6, a8) showed that an inhibition diameter of 15.3 mm is obtained in the case of *P. expansum*, the antifungal effect of the extract on this microorganism being a moderate one. Exposure of *F. graminearum* 96 and *P. digitatum* at the CL-103 has no effect. In the case of the other microorganisms studied, the antifungal effect is local, the diameters of inhibition obtained being less or equal to 10 mm. The results obtained in this study confirm the antifungal effect of the alcoholic extracts of *Citrullus sp.* and are similar to conclusions obtained by Shah and team (Shah and collab., 2022).

• Similar inhibition diameters were obtained for *A. niger* (20 mm) and *F. oxysporum* (13.5 mm), in experiments carried out by El ZAwi et al., by exposing several species of microorganisms to alcoholic extracts of C. *lanatus*. They highlighted a local antifungal activity for *F. oxysporum*, in the case of an alcoholic extract of *C. lanatus* that contained 20 mg crude extract/mL (El Zawai et al., 2015). Other authors (Harith et al., 2018) in their studies regarding the antimicrobial activity of methanolic extracts of *C. lanatus*, found that these extracts inhibit the development of dermatophytes fungi such as *T. mentagrophytes*, the results obtained being better than those obtained with nystatin. Analytical determinations made by Kim and collab. (Kim et al., 2018) using HPLC and LC-MS techniques, with three commercial standards of cucurbitacin (CuB; CuE, CuE-Glu) showed that the alcoholic extracts obtained from dry peels of *C. lanatus* or *C. colocynthis* contain up to 170 µg/g CuE; 50 µg/g CuE-Glu and respectively 7 µg/mL CuB. They found that the cucurbitacins concentration found in *C. colocynthis* is higher than in *C. lanatus*..

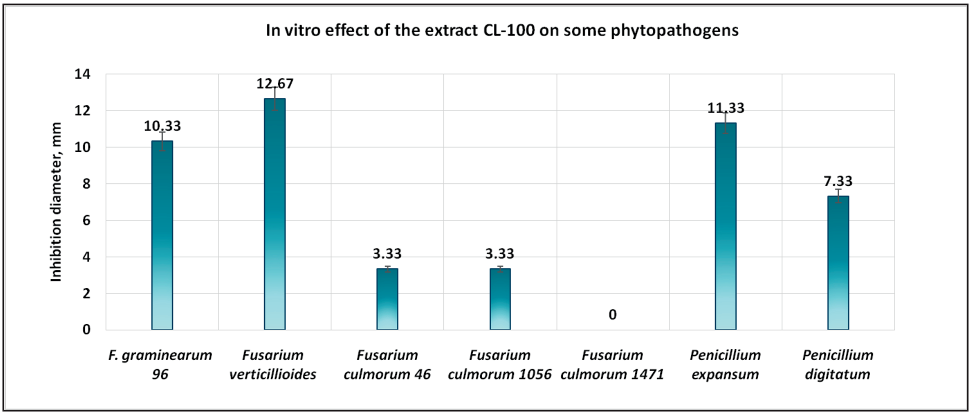

Figure 3. Antifungal effect of CL-100

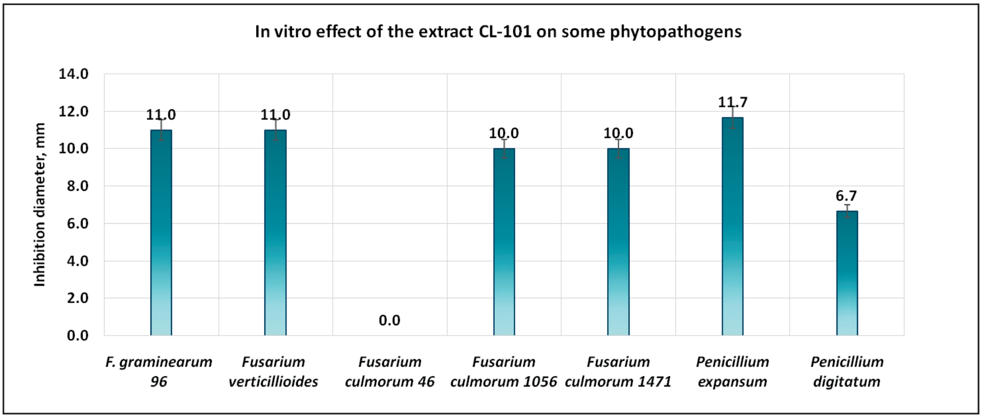

Figure 4. Antifungal effect of CL-101

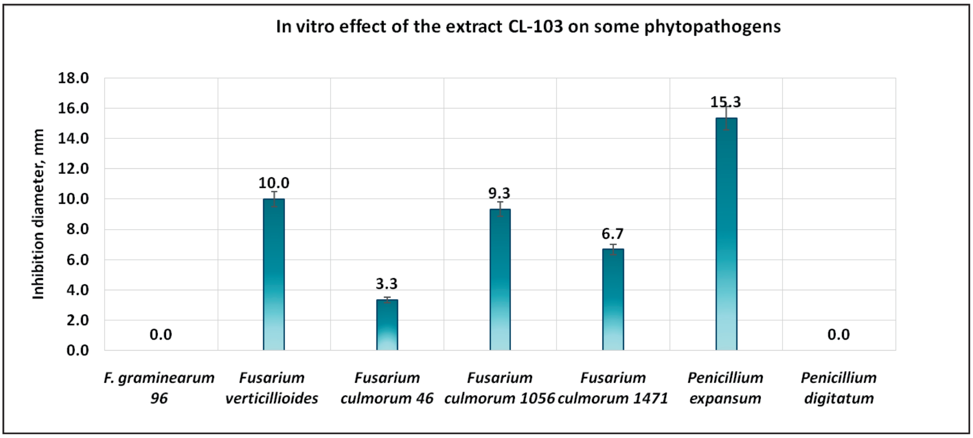

Figure 5. Antifungal effect of CL-103

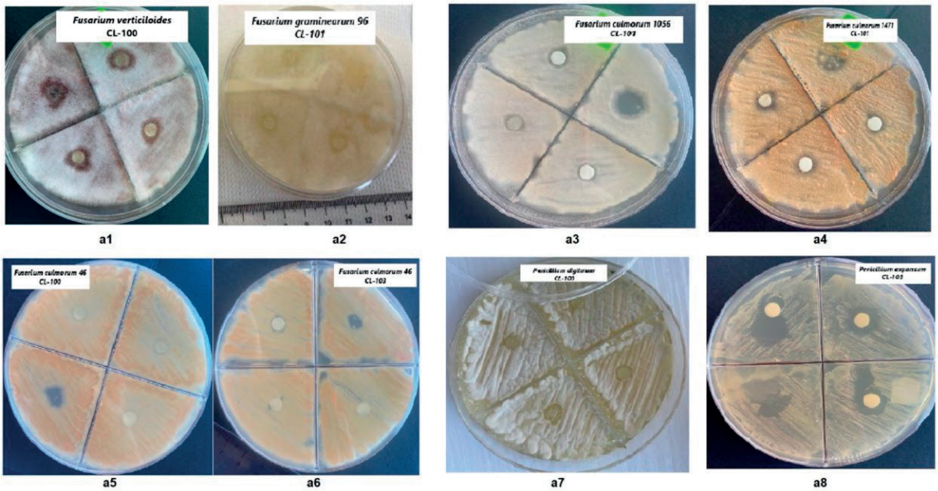

Figure 6. Susceptibility of tested phytopathogens to ethanolic extracts of *C. lanatus*

CONCLUSIONS

Following the carried-out studies, a moderate antifungal effect was highlighted for the peel ethanolic extracts obtained from different indigenous varieties of *Citrullus lanatus*. For the first time, it was highlighted the antifungal effect of *Citrullus lanatus* peel ethanolic extracts on *Fusarium graminearum* type micromycetes and *Fusarium verticiloides*, phytopathogens fungi that cause great damage of cereals production from *Zea mays* and *Triticum aestivum*. In addition, the performed tests highlighted a moderate antifungal effect against *Penicillium expansum*, a phytopathogen that generally causes damage to citrus fruits, and respective of the indigenous fruits of apple, pear or cherry. The results obtained from the in vitro studies have highlighted the antifungal effect of the alcoholic extracts obtained from the biomass of *Citrullus lanatus* (peels), making of this ethanolic peel extract a potential candidate in obtaining new types of natural biopreparations intended the protection of plants grown for cereals and respectively intended for the protection of stored fruits in closed spaces.

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