Comparative Cytotoxicity of Moniliformin and Patulin: Assessing the Impact of Binary Mycotoxin Combination on SH-SY5Y Cells

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Introduction

Mycotoxins are toxic compounds produced by filamentous fungi such as *Penicillium*, *Aspergillus*, *Fusarium*, and *Alternaria*. Moniliformin (MON) induces chromosomal aberrations and micronuclei formation, while patulin (PAT) exhibits cytotoxic, genotoxic, neuronal, hepatic, renal, and reproductive toxicity. Ingesting food contaminated by these mycotoxins can have a significant impact on the health of both humans and animals. However, their relevance lies mainly in the high frequency with which binary mixtures are found in food and agricultural contexts. This is due to multiple factors, including those related to environmental conditions and agricultural practices, which favor the simultaneous growth of fungi with similar metabolic characteristics [1,2].

Objective

This study aimed to assess the cytotoxic effects of MON, PAT, and their binary combination in SH-SY5Y cells, with a particular focus on comparing the impact of these mycotoxins when the cells were exposed to them individually versus when they were exposed to the combination.

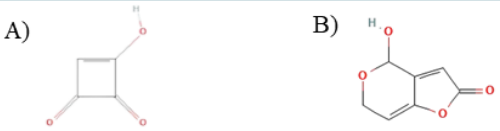

Figure 1. Chemical structures of the mycotoxins (A) moniliformin (MON) and (B) patulin (PAT).

Material and methods

The experimental study consisted of the evaluation of the cytotoxicity of mycotoxins MON, PAT, and their binary combination in SH-SY5Y cells, using the cytotoxicity assay based on 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) [3]. This method is based on a colorimetric reaction, characterised by the reduction of the MTT reagent and the obtaining of purple insoluble formazan crystals.

Table 1. Concentration range (µM) of mycotoxins studied individually and in binary combinations. The dilution ratios was 200:1 for MON + PAT.

Mycotoxin	Concentration Range (µM)
MON	0.78 - 200
PAT	0.01 - 3
MON + PAT	0.784 - 201

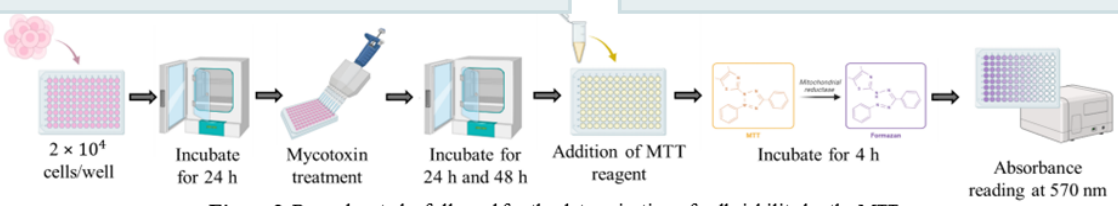

Figure 2. Procedure to be followed for the determination of cell viability by the MTT

Results

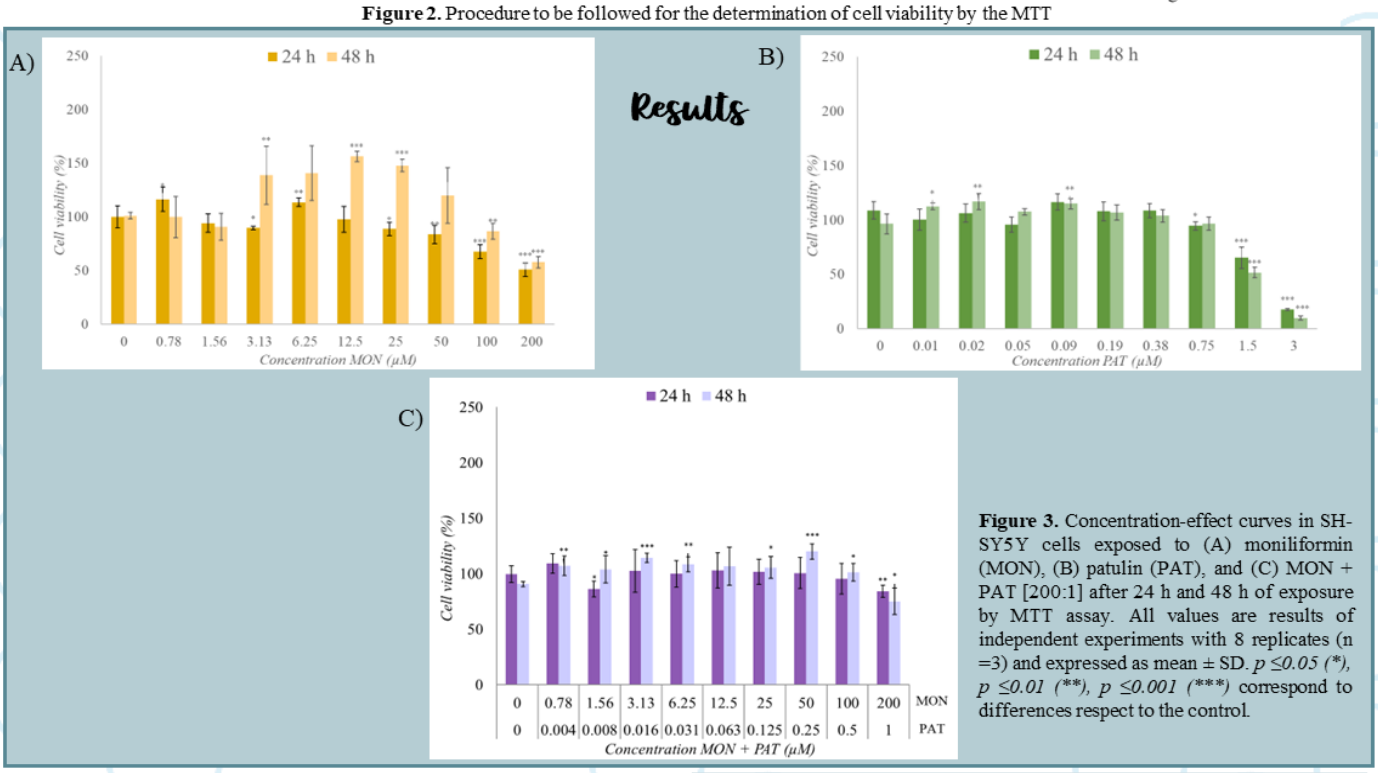

Figure 3. Concentration-effect curves in SH-SY5Y cells exposed to (A) moniliformin (MON), (B) patulin (PAT), and (C) MON + PAT [200:1] after 24 h and 48 h of exposure by MTT assay. All values are results of independent experiments with 8 replicates (n =3) and expressed as mean ± SD. *p ≤0.05 (*), p ≤0.01 (**), p ≤0.001 (***)* correspond to differences respect to the control.

Table 2. Medium inhibitory concentration (IC_{50} ± SD) of moniliformin (MON), patulin (PAT) and its binary combination for SH-SY5Y cells after 24 h and 48 h of exposure, determined by the MTT assay. Three independent experiments were performed with eight replicates each.

Mycotoxin	Exposure time (h)	IC_{50} (mean ± SD) (µM)
MON	24	(200 ± 6)
	48	N/A
PAT	24	(2 ± 1)
	48	(1.5 ± 4.5)
MON + PAT	24	N/A
	48	N/A

Conclusion

PAT demonstrated marked cytotoxicity, reducing cell viability to 17 % after 24 h of exposure and to 10 % after 48 h.

MON did not induce significant decreases in cell viability, indicating limited toxicity under the conditions evaluated.

The binary combination of PAT and MON did not show toxic synergism, evidenced by the difficulties in reaching IC_{50} values and the minimal reductions observed in cell viability.

These findings underscore the **importance of further research** to characterize the effects of binary combinations of mycotoxins and to improve the assessment of the risk associated with food contamination by these substances.

References

[1]. Malir, F.; Pickova, D.; Toman, J.; Grosse, Y.; Ostry, V. Mycotoxin Research 39:2, 39, 81–93 (2023). [2]. Ali, S.; Freire, L.G.D.; Rezende, V.T.; Noman, M.; Ullah, S.; Abdullah; Badshah, G.; Afridi, M.S.; Tonin, F.G.; de Oliveira, C.A.F. Foods, Vol. 12, Page 4314 2023, 12, 4314 -8 (2023). [3]. Juan-García, A.; Juan, C.; König, S.; Ruiz, M.J. Toxicol Lett 2015, 235, 8–16 (2015).

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