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Fate of ivermectin in soil and impact on micro-organisms

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INTRODUCTION

-Avermectins are a class of macrocyclic lactone drugs with insecticidal and anthelmintic properties, which have been developed for the protection of animals, humans and crops
-The intraruminal-sustained release bolus of ivermectin is considered an important advance in the control of endoparasitic nematodes in ruminants because of the long-term protection of animals
-High levels of faecal excretion of the drug could induce toxic effects on key dung-colonizing families of insects, and on soil or aquatic ecosystems inhabiting (micro)-organisms

→ We present here our results concerning the fate of ivermectin in soils, as a basis for exposure assessment, and its impact on bacteria and fungi.

EXCRETION OF IVERMECTIN

Faecal concentrations of ivermectin are obtained after the administration of a sustained-release bolus to calves (initial amount: 1.72 g per animal)

Time after the administration of the bolus (days)	Time of presence on pasture (days)	Faecal concentrations (mg/kg)
4	15	0,688
	30	1,617
	60	0,456
	90	0,181
14	15	1,123
	30	1,473
42	15	0,839
	30	1,184
70	15	1,056

→ 1 mg/kg untransformed drug is released in faeces during a 4-month period

BIOAVAILABILITY OF IVERMECTIN

Amount of ivermectin measured in the soil solution was 24 ± 14 ng
→ 0.2 % of initial amount in soil → $6 \mu\text{g/L} \rightarrow 10^{-5}$ M

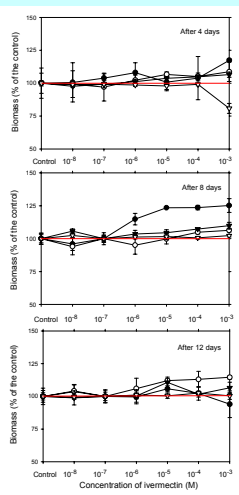
GLOBAL EFFECTS OF IVERMECTIN ON BACTERIA

No effect of ivermectin (10, 50 and 250 mg/kg) on the number of colony-forming organisms after 15 days on solid media
No effect of ivermectin (10, 50 and 250 mg/kg) on the microbial biomass after 15 days in the soil (carbon extracted after fumigation)

No effect of ivermectin (10 and 50 mg/kg) on the nitrification of NH_4^+ in the soil
No effect of ivermectin (10 mg/kg) on the mineralization of labeled carbofuran (insecticide)
No effect of ivermectin (10, 50 and 250 mg/kg) on the turn-over of microbial carbon (using labeled glucose) in the soil

No effect of ivermectin (10 mg/l) on the metabolic activities of soil isolates using Biolog plates

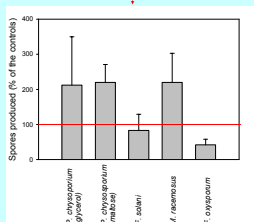
→ No effect of the drug was detected either on the size of the microbial biomass or functions, even at high concentrations



SHORT-TERM EFFECTS OF IVERMECTIN ON FUNGI

• Fungal strains, namely *Trametes versicolor* (●), *Fusarium solani* (○), *Mucor racemosus* (▼), and *Fusarium solani* (△) have been cultured in liquid media in the presence of ivermectin at various concentrations

• Fungal cultures, inoculated with spore suspensions, were allowed to grow in the presence of ivermectin (10^{-4} M). The spores produced by the new hyphae have been harvested and counted. (*T. versicolor*, unable to produce spores, was replaced by *P. chrysosporium*, another white-rot strain)



→ No negative impact of the drug has been evidenced on the fungal biomass production after 4, 8 and 12 days of growth

→ Ivermectin was also unable to inhibit the germination of fungal spores, even at high concentrations (10^{-3} M, data not shown)

→ The drug diversely affected fungal sporulation, according to the strains

INDUCTION OF ENZYMATIC ACTIVITIES BY IVERMECTIN

Fungal cultures have been grown in liquid media in the presence of 10^{-5} M ivermectin. Enzymatic activities were then measured using colorimetric assays

Laccases of *T. versicolor* after 3 days of treatment

Controls	
Water	0.16 ± 0.02 U/mL
Xylydine	5.69 ± 0.21
Ivermectin	0.32 ± 0.06
8,9-Z isomer	0.33 ± 0.06
10,11-Z isomer	0.49 ± 0.09

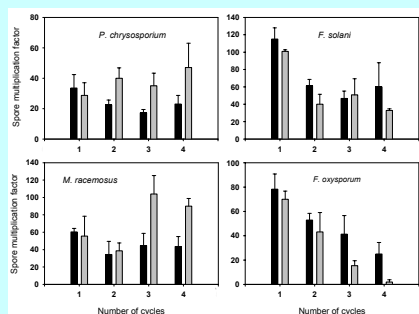
Lignin-peroxidases of *P. chrysosporium* after 4, 8 and 12 days

→ No effect

→ Ivermectin is not an inducer of fungal enzymatic activities

LONG-TERM EFFECTS OF IVERMECTIN ON FUNGI

Fungal cultures have been inoculated with spores, without ivermectin (●) or in the presence of the drug (◻) at 10^{-5} M. The culture were then allowed to grow and sporulate for 10 days. Spores were harvested and the experiments were still repeated 3-times. The spore multiplication factor reflected the ratio between the amounts of spores produced by a culture versus the amounts of spores used for inoculation.



→ Ivermectin diversely affected fungal sporulation, according to the strains
→ It was without effect on fungal growth (data not shown)

CONCLUSIONS

- 1 - Ivermectin can reach the soil in high amounts after the treatment of cattle with the intraruminal sustained release bolus
- 2 - In the soil, the drug undergoes minor transformation reactions, only due to photo-isomerization
- 3 - The bioavailability of ivermectin for soil micro-organisms is very reduced
- 4 - Ivermectin induces no negative effect on bacteria
- 5 - It only impacts fungal reproduction, with positive or negative effects according to the strains