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Removal of Pyrimethanil and Fenhexamid from *Saccharomyces cerevisiae* Liquid Cultures

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Summary

The capacity for the removal of pyrimethanil and fenhexamid, two fungicides commonly used for the control of *Botrytis cinerea* in vineyards, has been evaluated during an alcoholic fermentation process in batch system. Commercial and wild strains of *Saccharomyces cerevisiae* were used. Batch fermentations were carried out in yeast extract-malt extract medium (YM) with 18.0 % (by mass) glucose, and the fungicides were added separately at three concentrations: 0.1, 1.0 and 10.0 mg/L. The removal capacity of yeast strains was also examined in stationary phase cultures of *Saccharomyces cerevisiae*. Stationary assays were performed with yeast biomass harvested from the stationary phase of an anaerobic fermentation process, with separate additions of 0.1, 1.0 and 10.0 mg/L of both fungicides. Removal studies with stationary phase cells were performed with viable and non-viable cells inactivated with sodium azide. This study clearly shows that both *Saccharomyces cerevisiae* strains were able to remove fenhexamid and pyrimethanil in stationary and fermentative assays. The removal potential is shown to be strain dependent in stationary but not in fermentative assays. However, the removal potential is dependent on the type of fungicide in both stationary and fermentative assays. In stationary phase cultures no significant difference in fungicide removal potential between viable and non-viable cells was observed, indicating that both pesticides were not degraded by metabolically active cells. However, the presence of both pesticides influenced fermentation kinetics and only pyrimethanil at 10.0 mg/L increased the production of volatile acidity of both strains.

Key words: alcoholic fermentation, *S. cerevisiae*, pyrimethanil, fenhexamid, synthetic media

Introduction

Pesticides have proven beneficial effects on the preservation of crop yield. However, they are extensively used, and sometimes overused, posing serious human health concerns (*1*). In wine production *Botrytis cinerea* is a fungal pathogen of serious economic importance. Because of its increasing tolerance to the old generation of fungicides, the treatments have become more severe, and consequently new and more effective pesticides are being developed. These are characterized as being less toxic for human health and have a lesser ecological impact (*2–4*).

One of the most widely used botryticides is pyrimethanil. Pyrimethanil (*N*-(4,6-dimethylpyrimidin-2-yl)-aniline) is a colourless, crystalline substance, which is practically insoluble in water and belongs to the anilinopyrimidine class (*5*). With a lesser ecological impact, one of the new types is fenhexamid (*N*-(2,3-dichloro-4-hydroxyphenyl)-1-methylcyclohexanecarboxamide), which belongs to the chemical class of hydroxyanilides (*6*). This fungicide is less ecotoxic because it is readily degraded to non-toxic derivatives (*7–9*).

Stronger concentrations are used during the years when the conditions for *Botrytis* infection are extremely good, which can lead to the presence of pesticide resi-

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dues on grapes; sometimes higher than their maximum permitted levels (*2*).

Even though the solubility and stability of a pesticide can influence the concentrations of residues on grapes, residue levels are also influenced by the type of handling and processing (*10*,*11*). The fact that all wines have been found to have lower concentrations of residual pesticides than detected on the corresponding grapes confirms this observation (*12–17*).

Yeasts, during alcoholic fermentation, as well as other types of processing, have the ability to decrease pesticide residues. Studies concerning a large number of classes of pesticides showed that yeasts can decrease the amount of pesticides by degradation and/or adsorption, the latter of which was found to be the most effective and frequent (*1*). The removal of toxic pesticides during wine processing has been widely studied (*18*,*19*).

The main agent for adsorption is the yeast cell wall, which contains polysaccharides as basic building blocks. Therefore, it offers host functional groups capable of xenobiotic binding (*20*). Nuñez *et al.* (*21*) demonstrated that the main fraction of mannoproteins is released in the first week after the completion of alcoholic fermentation and that during this stage the predominant adsorptive action is observed, which determines the removal potential of the yeast. This mechanism was also confirmed for ochratoxin A (*22*).

During the alcoholic fermentation of grape juice, the technology of 'battonage' is frequently used at the end of the fermentation process (*23*). This involves mixing yeast lees in wine in order to obtain wines of higher quality. During this process, mannoproteins are released, and it is at this stage that the main adsorption of chemical contaminants is observed (*24*,*25*).

The adsorption activity of yeast lees is notably different among strains, and because of the cell wall structure it is clear that the physicochemical conditions, especially pH, affect the adsorption ratio (*18*,*24*,*25*). However, not only strain properties, but also differences in the binding affinity of pesticides, are important factors (*19*,*26*).

Removal of pesticides by degradation is a less common mechanism. Cabras and Angioni (*1*) showed that yeasts have the ability to degrade certain pesticides belonging to the pyrethroid class and certain insecticides belonging to the class of the thiophosphates. A mixed degradation/adsorption action has also been shown for some agrichemicals. Cabras *et al.* (*27*) showed that during fermentation yeasts partially degraded quinoxyfen and adsorbed it completely.

In addition to yeasts affecting the concentration of pesticides in the medium, pesticides can also affect microorganisms under certain conditions. For example fenhexamid did not affect alcoholic fermentation in studies performed by Cabras *et al.* (*28*), while a high concentration of pyrimethanil (10.0 mg/L) was found to significantly diminish the anaerobic growth of *Hanseniaspora uvarum* in YM medium (*29*). In some cases the presence of pesticides has been found to stimulate yeasts, particularly *Kloeckera apiculata*, which produced more alcohol (*30*). The presence of fungicides during alcoholic fermentation has also been demonstrated to affect secondary metabolite production, such as aroma compounds. Different pesticides have been shown to affect the aromatic profile of red and white wines (*2*,*31–34*).

The aim of this study is to elucidate the interactions of the fungicides pyrimethanil and fenhexamid with two *S. cerevisiae* wine yeast strains, one commercial and one isolated from a spontaneous fermentation, under stationary and fermentative conditions in synthetic media.

Materials and Methods

Yeast strains

The yeasts used in this study were a commercially available *Saccharomyces cerevisiae* Lalvin EC–1118 (Lallemand, Montreal, Canada), as active dry yeast, and the yeast strain *S. cerevisiae* ZIM 1927, previously isolated from a spontaneous fermentation of cv. Malvasia grape must in 2001 and obtained from the culture collection of industrial microorganisms, University of Ljubljana, Ljubljana, Slovenia. The first strain was rehydrated as described by the producer (Lallemand), and then cultured anaerobically in yeast extract-malt extract (YM) medium (0.3 % yeast extract, 0.3 % malt extract, 0.5 % peptone (by mass) obtained from Biolife, Italy) supplemented with 18 % (by mass) glucose (Merck, Darmstadt, Germany) at 28 °C for 24 h, with rotary shaking at 190 rpm. The *Saccharomyces cerevisiae* ZIM 1927 was a three-day-old culture, maintained on YPD agar (YPD Broth, Oxoid, Basingstoke, UK) and cultured in YM medium as described for Lalvin EC-1118.

Cultivation and medium

The fermentations were carried out under anaerobic conditions. The cultivations were performed in liquid YM with 18.0 % (by mass) glucose at pH=6.37, previously sterilized by membrane filtration (0.20 μm, Sartorius, Göttingen, Germany). A 300-mL fermentor contained the control medium of 279 mL of YM and 1 mL of dimethyl sulfoxide (DMSO, Sigma-Aldrich, St. Louis, MO, USA). Three stock solutions of pyrimethanil and fenhexamid (dissolved in DMSO) were prepared separately to reach three concentrations of the pesticide in 280-mL medium: 10.0, 1.0 and 0.1 mg/mL, and sterilized by membrane filtration (0.20 μm, Sartorius). The final pH of the medium was 6.37. The fermentations were performed in triplicate for each strain and concentration of both pesticides (0.0 mg/L (control), 0.1, 1.0 and 10.0 mg/L).

Culture conditions and sampling

The inocula of the yeasts were prepared as described above and added directly to the fermentors to give a concentration of 10^6 CFU/mL of the medium (cell count by haemocytometer). Fermentations were carried out at 20 °C and fermentation kinetics was followed by CO_2 release (mass loss). Cultures were shaken/mixed daily. The final sample was taken 7 days after the end of the fermentation process (less than 2 g/L of residual sugars) (*21*,*22*). A final sample was also collected for measurement of volatile acidity.

Biomass recovery in stationary assays

In stationary assays, the biomass produced during fermentation under anaerobic conditions in liquid YM, with 18.0 % (by mass) glucose at pH=6.37, was recovered by centrifugation (10 min, 11 200×*g*) and washed three times (10 min, 11 200×*g*) with sterile phosphate buffer (pH=6.37), previously sterilized by filtration (0.20 μm, Sartorius). Finally, the cells were resuspended in phosphate buffer to give a final concentration of 0.44 g/mL.

Stationary assays of pyrimethanil and fenhexamid removal by the biomass

The assays were performed in duplicate in tubes containing 5 mL of assay solution, consisting of 4.882 mL of phosphate buffer (pH=6.37) and 0.018 mL of stock solution of DMSO/pyrimethanil or fenhexamid (Sigma-Aldrich) to reach three respective concentrations of pesticide in the medium (10.0, 1.0 and 0.1 mg/L). To each tube, 0.1 mL of yeast biomass suspension (viable or non-viable cells) in phosphate buffer were added to give a final concentration of 0.0088 g/mL. After 7 days of contact between the biomass and the medium (*21,22,35–37*), the solution was centrifuged and the non-adsorbed amounts of pesticides in the medium were measured. Non-viable cells were prepared by the addition of sodium azide (Sigma-Aldrich) to the biomass suspended in phosphate buffer (0.025 % final volume fraction in the media) in order to exclude metabolic action and preserve the structural integrity of the cells (*21,38*).

Determination of fungicide residues

The extraction procedure and determination of pyrimethanil and fenhexamid residues in liquid yeast extract-malt extract (YM) medium was done using a gas chromatography-mass spectrometry system (GC–MS) and liquid chromatography-tandem mass spectrometry system (LC/MS/MS), respectively according to the methods described previously (*15,39*).

For the determination of pyrimethanil, in the GC-MS analysis HP-5MS capillary column (30 m, 0.25 mm i.d., 0.25 μm film thickness) was used. Injector temperature was 250 °C, ion source temperature was 230 °C, auxiliary temperature was 280 °C and quadrupole temperature was 150 °C. GC oven temperature was programmed from 55 °C (held for 2 min) to 130 °C at a rate of 25 °C/min (held for 1 min), then to 180 °C at rate 5 °C/min (held for 30 min), then to 230 °C at a rate of 20 °C/min (held for 16 min), then to 250 °C at a rate of 20 °C/min (held for 13 min), and finally to 280 °C at a rate of 20 °C/min (held for 20 min). The helium constant flow was 1.2 mL/min. The liner used was Agilent 5181-3316 (Agilent Technologies, Palo Alto, CA, USA).

The content of fenhexamid residues in methanol extract was analyzed using liquid chromatography (PE200, PerkinElmer, Waltham, MA, USA) coupled with triple quadrupole mass detector (3200 QTrap, Applied Biosystems MDS Sciex, Concorde, Canada). Turbo spray temperature was kept at 650 °C. The compounds were separated on a Gemini C18 column, 250×4 mm (Phenomenex, Torrance, CA, USA). Gradient elution was used for pesticide separation. Mobile phase A consisted of 75 % of 5 mM $HCOONH_4$ and 25 % methanol (by volume) with 0.1 % formic acid added and mobile phase B consisted of 5 % of 5 mM $HCOONH_4$ and 95 % methanol (by volume) with 0.1 % formic acid added. The initial conditions (100 % mobile phase A) were maintained for 5 min, then linear gradient was applied and, in 30 min, 100 % of mobile phase B was reached and maintained for 15 min. Conditioning of the column to the initial mobile phase A was carried out for 10 min. Data was collected in multiple reaction monitoring (MRM) mode (dwell time 5 ms) and for each compound two MRM transitions were monitored.

Determination of volatile acidity and fermentation dryness

After the cultivation was completed, the volatile acidity in the medium was determined according to the accredited methods in the Central Laboratory of the Agricultural Institute of Slovenia, Ljubljana, Slovenia. Volatile acidity was determined by titration of the distillate obtained by steam distillation. A total of 20 mL of sample, freed from carbon dioxide, was placed in the distillation flask and 0.5 g of tartaric acid was added. Distillation was done using steam distillation apparatus (Oenoextracteur Chenard, France). At least 250 mL of the distillate were collected. The distillate was titrated with 0.1 M sodium hydroxide solution, using phenolphthalein as an indicator. The volatile acidity expressed in grams of acetic acid per litre was calculated according to the volume of sodium hydroxide used in the titration (*40*).

Fermentations were considered to have reached dryness when the concentration of reducing sugars was lower than 2 g/L in the fermentation media. Fermentation dryness was monitored at the end of fermentation with Clinitest®, Bayer, Leverkusen, Germany.

Statistical analysis

The data were analyzed by one-way ANOVA (Microsoft Office Excel 2003, USA). The statistical level of significance was set at $p \leq 0.05$. The means were compared with the Tukey's test.

Results and Discussion

Interactions in stationary assays

Stationary phase assays were executed in phosphate buffer at the same pH as in the YM medium used in fermentation trials, *i.e.* pH=6.37. This medium was chosen to avoid any other external influence on the interaction between the yeast cells and the fungicides. We decided to use a contact time of 7 days (*21–25*). Viable and non-viable biomass were used, the latter being inactivated by sodium azide (*21,22,38*). The aims of the experiment were to determine the capacity of fresh biomass to remove the fungicides pyrimethanil and fenhexamid from synthetic media, the mechanism(s) of removal, (physicochemical or metabolic), and the effect of fungicide concentration on the interaction with two genetically different strains.

It can be seen in Table 1 that only 20 % of fenhexamid were removed by both strains when it was present at the initial concentration of 1.0 mg/L. Even at the initial concentration of 10.0 mg/L of fenhexamid, no difference

Table 1. Removal of pyrimethanil and fenhexamid in stationary assays by viable and non-viable biomass of *S. cerevisiae* strains ZIM 1927 and EC-1118

Initial concentration of fungicide in media/(mg/L)	Final concentration of fungicide in media/(mg/L)			
fenhexamid	ZIM 1927		EC-1118	
	viable cells	non-viable cells	viable cells	non-viable cells
0.1	0.08[a]	0.08[a]	0.08[a]	0.08[a]
1.0	0.83[a]	0.53[a]	0.57[a]	0.71[a]
10.0	5.76[a]	5.43[a]	7.08[a]	8.01[a]
pyrimethanil	ZIM 1927		EC-1118	
	viable cells	non-viable cells	viable cells	non-viable cells
0.1	0.05[a]	0.05[a]	0.05[a]	0.05[a]
1.0	0.43[a]	0.48[a]	0.41[a]	0.38[a]
10.0	5.10[a]	4.15[a]	3.12[a]	3.07[a]

All assays were performed in phosphate buffer, pH=6.37, at 20 °C for 7 days

Concentration of viable and non-viable yeast biomass added in tubes was 8.8 mg/mL

Non-viable cells: cells inactivated with 0.0025 % sodium azide

Mean values with the same superscript letter in the same line for the same strain do not differ significantly at $p \leq 0.05$

The data reported are average values of two independent replicates

between viable and non-viable cells of both strains was found. These results suggest that fenhexamid is removed from the media by adsorption and not by degradation since no significant difference between the removal potential of viable and non-viable cells could be observed. At the highest concentration of fenhexamid added (10.0 mg/L), viable and non-viable cells of EC-1118 removed 29.2 and 19.9 % of fenhexamid, respectively, whereas ZIM 1927 was able to remove 42.2 and 45.7 %, respectively. Other authors (*21,24,25,41*) have also observed a strain-dependent adsorption potential, as found in our assays.

The results obtained for pyrimethanil were similar to those for fenhexamid, as shown in Table 1. No significant differences in the removal potential between viable and non-viable cells suggest that the removal of pyrimethanil also involves adsorption. At the initial concentration of 0.1 mg/L, the removal was 50 % with both strains, whether viable or non-viable, and roughly similar results were obtained when the initial concentration was 1.0 mg/L. At the highest initial concentration studied (10.0 mg/L), viable and non-viable cells of ZIM 1927 removed 49.0 and 58.5 %, respectively, whereas EC-1118 had a higher potential for removal of up to 68.8 and 69.3 %, respectively.

Overall, it can be observed from these results that, independent of the strain and type of fungicide and its concentration, the removal of fungicide from synthetic media involves adsorption on yeast biomass and is not a consequence of metabolic degradation. This is similar to the previously obtained results (*42*). We observed that removal potential is strain dependent, but environmental conditions such as pH, temperature and the chemical composition of the media have also been shown to have a strong impact on binding capacities (*1,38*).

Fermentation kinetics in growth assays

The fermentation kinetics of the two strains varied in the YM media containing 18.0 % (by mass) of glucose at 20 °C. The duration of fermentations to dryness was 16 days for ZIM 1927 and 25 days for EC-1118 (Figs. 1 and 2). Fermentation duration did not affect the ability of each strain to achieve dryness.

The effect of both pesticides on the fermentation kinetics of strain EC-1118 can be seen in Figs. 1a and 2a. In the case of fenhexamid (Fig. 2a), its effect can be seen by the irregularity of fermentation curves when the pesticide was added; however, all spiked fermentations reached dryness. As the initial fungicide concentration increased, the fermentation performance decreased. Similarly, the addition of pyrimethanil (Fig. 1a) produced an irregularity of fermentation curves. When the initial concentration of spiked pyrimethanil was relatively low (0.1 or 1.0 mg/L), its effect on fermentation kinetics was not strong, but in the case of the addition of high amount (10.0 mg/L), the lag phase was longer and the logarithmic phase much slower in comparison with the control. However, in all cases dryness was reached.

In the case of ZIM 1927, for which the intensity of fermentation rate was higher, the effect of pesticides was found to be stronger. When fenhexamid was added at the two lower concentrations (0.1 and 1.0 mg/L) (Fig. 2b), no effects could be seen. On the other hand, at the concentration of 10.0 mg/L, longer lag phase and a strong reduction

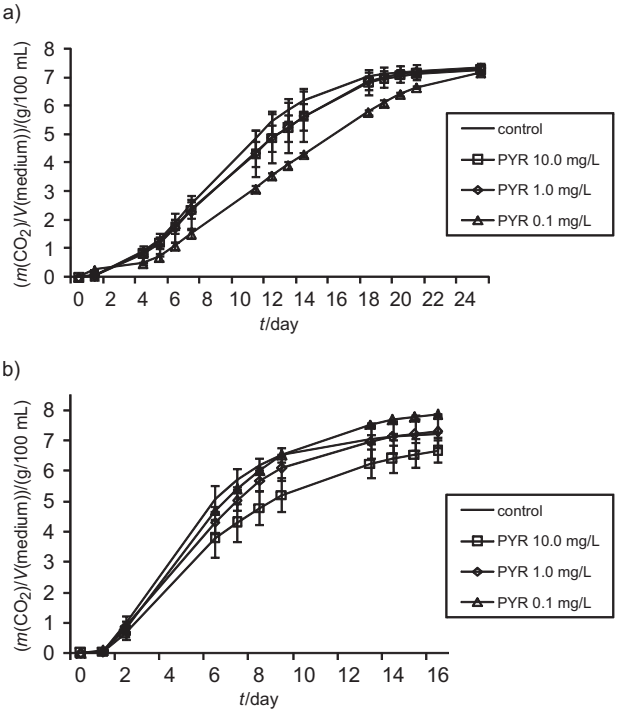

Fig. 1. Fermentation kinetics of *S. cerevisiae* strains: a) EC-1118 and b) ZIM 1927 in YM medium with 18.0 % glucose, pH=6.37, with the addition of pyrimethanil (PYR). The data reported are average values of three independent replicates

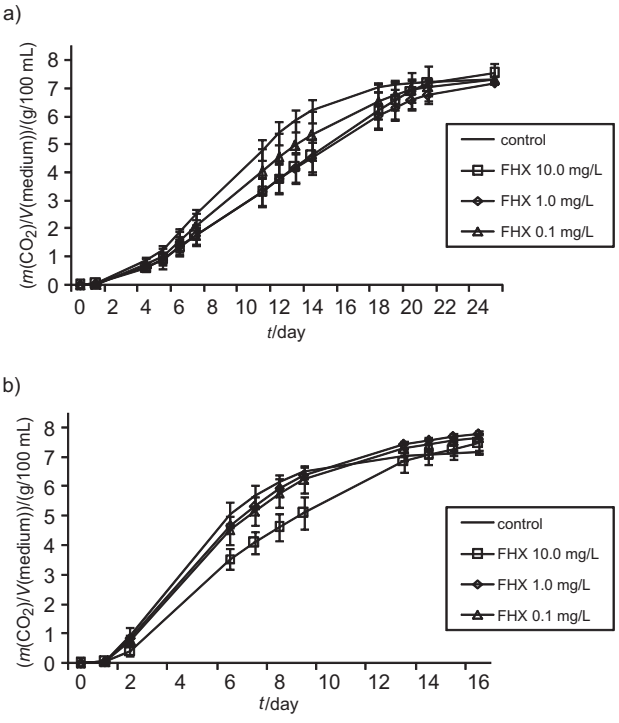

Fig. 2. Fermentation kinetics of *S. cerevisiae* strains: a) EC-1118 and b) ZIM 1927 in YM medium with 18.0 % glucose, pH=6.37, with the addition of fenhexamid (FHX). The data reported are average values of three independent replicates

in intensity of the logarithmic phase were observed. Despite this, in all cases, the fermentations reached dryness.

Similar trends can be seen in trials when pyrimethanil was added (Fig. 1b). Although no effect of the addition of pesticide at the two lower concentrations could be seen, a strong effect was present at the higher concentration of 10.0 mg/L. This is the only case when the fermentation did not reach dryness.

These results suggest that ZIM 1927 is more sensitive than EC-1118 to the effects of pesticides present in the media under the conditions tested. The intensity of fermentation rate could be the reason for higher sensitivity under stressful conditions. Additionally, irrespective of the strain, pyrimethanil had much stronger effect on the kinetics when compared to fenhexamid. These results are in agreement with previously published works on the effects of fenhexamid and pyrimethanil on yeast during fermentation (*28*,*29*). However, some pesticides such as tebuconazole were not found to have any effect on yeast kinetics during fermentation in synthetic media (*42*).

The effect of pyrimethanil and fenhexamid on the production of volatile acidity in growth assays

Volatile acidity production during alcoholic fermentation is an oenological parameter, and is a consequence of both genetic predisposition and environmental conditions. Stressful conditions in particular are known to induce the production of volatile acidity (*2*,*23*,*43*). The results in Table 2 show that, under our fermentation conditions, ZIM 1927 had significantly higher potential for volatile acidity production, which was 0.14 mg/L higher than for EC-1118 in the control fermentations. Fenhexamid was not found to have any effect on the volatile acidity production during alcoholic fermentation by either strain at any concentration studied. Likewise, pyrimethanil had no effect on volatile acidity production by either strain at the two lower concentrations (0.1 and 1.0 mg/L). However, significantly higher volatile acidity production was found with both strains at the highest concentration of pyrimethanil added, which means that pyrimethanil had a strong negative effect on both strains, as has also been shown in spontaneous fermentations (*29*). Although the production of volatile acidity significantly increased (by 21.6 %) in comparison with the control ZIM 1927, the strongest effect can be seen for EC-1118, where the increase in volatile acidity was 126.7 % higher than that of the control. These data show that the effect of pyrimethanil on volatile acidity production by EC-1118 was much greater than that of ZIM 1927.

Pyrimethanil and fenhexamid removal in growth assays

The results shown earlier in this paper suggest that the mechanism of removal of the fungicides pyrimethanil and fenhexamid by the yeast strains ZIM 1927 and EC-1118 does not involve metabolic degradation but rather is a consequence of physicochemical phenomena.

Extended contact with yeast lees following the completion of alcoholic fermentation was performed in order to determine the potential of the strains EC-1118 and ZIM 1927 to remove pyrimethanil and fenhexamid from the experimental media (*21*,*22*).

Table 2. Volatile acidity at the end of fermentation in YM with *S. cerevisiae* strains ZIM 1927 and EC-1118

Sample/strain	ZIM 1927	EC-1118	Sample/strain	ZIM 1927	EC-1118
control	$(0.74\pm0.01)^a$	$(0.60\pm0.02)^a$	control	$(0.74\pm0.01)^a$	$(0.60\pm0.02)^a$
PYR 0.1	$(0.69\pm0.01)^a$	$(0.63\pm0.03)^a$	FHX 0.1	$(0.70\pm0.05)^a$	$(0.54\pm0.07)^a$
PYR 1.0	$(0.69\pm0.00)^a$	$(0.66\pm0.07)^a$	FHX 1.0	$(0.67\pm0.02)^a$	$(0.57\pm0.04)^a$
PYR 0.0	$(0.90\pm0.08)^b$	$(1.36\pm0.05)^b$	FHX 10.0	$(0.70\pm0.07)^a$	$(0.65\pm0.09)^a$

control: fungicide not added
PYR: pyrimethanil added to the media at concentrations of 0.1, 1.0 and 10.0 mg/L
FHX: fenhexamid added to the media at concentrations of 0.1, 1.0 and 10.0 mg/L
Mean values with the same letter in the superscript in the same column do not differ significantly at $p\leq0.05$
The fermentation was carried out in YM containing 18.0 % glucose, pH=6.37, at 20 °C
The concentration of volatile acidity in media is expressed in mg/L of acetic acid
Data reported are average values of three independent replicates

In the case of pyrimethanil (Table 3), no difference between the two strains at any concentration could be seen, even at the highest initial concentration of 10.0 mg/L. Strain-related adsorption potential was not found in the growth assays, as was seen previously in the stationary assays. At all concentrations and for both strains there was roughly a 50 % removal. Similar results were observed for fenhexamid. At all initial concentrations studied, no significant differences between the two strains were found, and also in the case of fenhexamid, no strain-related adsorption potential was shown, as was found previously in the stationary tests.

Table 3. Removal of pyrimethanil and fenhexamid after the completion of alcoholic fermentation and prolonged contact for 7 days with yeast lees of *S. cerevisiae* strains ZIM 1927 and EC-1118

Initial concentration of fungicide/(mg/L)	Final concentration of fungicide in media/(mg/L)	
pyrimethanil	ZIM 1927	EC-1118
0.1	$(0.06\pm0.01)^a$	$(0.05\pm0.01)^a$
1.0	$(0.50\pm0.01)^a$	$(0.50\pm0.04)^a$
10.0	$(5.21\pm0.07)^a$	5.08^{a*}
fenhexamid	ZIM 1927	EC-1118
0.1	$(0.06\pm0.00)^a$	$(0.06\pm0.00)^a$
1.0	$(0.52\pm0.02)^a$	$(0.54\pm0.03)^a$
10.0	$(4.86\pm0.09)^a$	$(4.60\pm0.18)^a$

*average of two replicates
Mean values with the same letter in the superscript in the same column do not differ significantly at $p\leq0.05$
YM contained 18.0 % glucose, pH=6.37, at 20 °C
Data reported are average values of three independent replicates

The more negative effects were observed when the concentrations of added fungicides were the highest. Under these conditions fermentation performance was reduced to a greater extent and a greater increase in volatile acidity production was observed. The toxicity of fenhexamid was shown to be lower than pyrimethanil. Since the concentration of yeast cells influences the removal of chemical contaminants from media (*21*,*24*,*25*), lower toxicity of fenhexamid might be the reason for its significantly higher removal.

Conclusions

The aim of this work was to elucidate the interactions of the fungicides pyrimethanil and fenhexamid with two genetically different *Saccharomyces cerevisiae* strains; one commercially available, Lalvin EC-1118, and ZIM 1927, a wild strain isolated from a spontaneous fermentation. Both strains were found to have the ability to remove both fungicides from synthetic media in stationary assays, and the results strongly suggest that the removal is a consequence of adsorption only, and not of degradation by metabolic action, since no significant difference between the assays with viable and non-viable cells was found. Strain-dependent adsorption potential was only found in stationary assays when conducted at the highest concentrations of the added pesticide, *i.e.* 10.0 mg/L.

No significant differences in the removal potential between the two strains at the same concentration of both fungicides could be observed during growth assays. However, pyrimethanil inhibited the completion of fermentation when added at a high concentration of 10.0 mg/L in growth assays with ZIM 1927 but not with EC-1118. The results suggest that pyrimethanil is more toxic to yeast cells during alcoholic fermentations; however, significant effects were only seen at the highest additions of pyrimethanil in relation to fermentation kinetics and volatile acidity production. Fenhexamid was less toxic to yeasts, and while it did not affect volatile acidity production at any concentration, when either yeast was used, its presence in the media impaired their fermentation kinetics.

The lower toxicity of fenhexamid, studied also at the highest added concentration in the media, seems to be the main reason for its higher removal. The removal capacity of the strains in synthetic media was found to be affected by both the toxicity of the chemical contaminant (in this case the fungicide) and different environmental conditions when determined in growth assays but not in stationary tests, so the results of these assays cannot be directly related (*21*,*22*). Further work is needed to demonstrate that the yeast-fungicide interactions observed in synthetic media can be found in grape must.

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