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Volatile organic compounds from *Wickerhamomyces anomalus*, *Metschnikowia pulcherrima* and *Saccharomyces cerevisiae* inhibit growth of decay causing fungi and control postharvest diseases of strawberries

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Abstract

The effectiveness of *Wickerhamomyces anomalus*, *Metschnikowia pulcherrima* and *Saccharomyces cerevisiae* as biocontrol agents on postharvest decay of strawberry (*Fragaria* × *ananassa*, cv. 'Alba') fruit, and their inhibitory activities on some decay-causing fungi were evaluated. Volatile organic compounds from these yeasts decreased mycelial growth of *Botrytis cinerea* by 69%, and by less for *Monilinia fructicola*, *Alternaria alternata*, *Aspergillus carbonarius*, *Penicillium digitatum*, *Cladosporium* spp., and *Colletotrichum* spp. Strawberry fruit exposed to 6-day-old liquid cultures of *W. anomalus*, *M. pulcherrima* and *S. cerevisiae* for 48 h showed 89%, 40%, and 32% reductions, respectively, in grae~~a~~y mo~~u~~ld McKinney Index. Vapours of ethyl acetate, the main volatile organic compound of these yeasts, completely inhibited *B. cinerea* growth at 8.97 mg/mL, and suppressed gray mold ~~grey mould~~ on strawberry fruit at 0.718 mg/mL. The biocontrol activities of these yeasts can be ascribed to ethyl acetate, which can be used for control of postharvest grae~~a~~y mo~~u~~ld of strawberry fruit.

Keywords: *Botrytis cinerea*, ethyl acetate, *Fragaria* × *ananassa*, gray mold~~grey mould~~, yeast volatile organic compounds

Highlights

- Yeast volatile organic compounds can reduce *in vitro* growth of decay-causing fungi
- Strawberry exposure to yeast producing volatile organic compounds reduces gray mold ~~grey mould~~
- The main volatile organic compound of the three selected yeasts is ethyl acetate
- Ethyl acetate at 8.97 mg/mL completely inhibits *B. cinerea* growth in the *in vitro* trials
- Ethyl acetate at 0.717 mg/mL can control gray mold ~~grey mould~~ on strawberry fruit
- ~~Ethyl acetate at 8.97 mg/mL completely inhibits *B. cinerea* growth in the *in vitro* trials~~

1. Introduction

The use of yeasts as biocontrol agents for prevention of pre-harvest and postharvest diseases of fruit is an alternative strategy to the use ~~at~~ of synthetic fungicides (Spadaro and Droby, 2016). Various yeasts genera have been widely used for control of decay caused by pathogens such as *Botrytis* spp., *Aspergillus* spp., *Penicillium* spp., *Monilinia* spp. and *Colletotrichum* spp. (Janisiewicz and Korsten, 2002). In ~~a~~ previous trials, the three yeasts *Wickerhamomyces anomalus* (formerly *Hansenula anomala* and *Pichia anomala*), *Metschnikowia pulcherrima* and *Saccharomyces cerevisiae* were applied as pre-harvest treatments to tree canopies, and they were effective for reduction of sweet cherry postharvest decay that was mainly caused by the fungus *Monilinia laxa* (Oro et al., 2014).

~~Generally, t~~The biocontrol ability of these yeasts can be related to different mechanisms through which they interact with pathogens and fruit tissues including: competition with pathogens for space and nutrients (Bencheqroun et al., 2007); parasitism and production of extracellular proteins (Lima et al., 2013); killer activity; induction of resistance in fruit tissues through elicitors that are ~~either~~ secreted by the biocontrol agent (Spadaro and Droby, 2016). Moreover, several studies have demonstrated that the production of volatile organic compounds (VOCs) by yeasts, has a significant role in their antagonistic activities (Huang et al., 2012; Di Francesco et al., 2015~~; Huang et al., 2012~~). In particular, ethyl acetate, commonly found in food and beverages, has proven antimicrobial properties (Vimala et al., 2007) (https://www.nlm.nih.gov/). These antimicrobial properties change across species, and are affected by yeast growth stage, culture mode, temperature, medium composition, and other factors (Martins et al., 2010). The aim of the present study was to evaluate the effectiveness of

the VOCs from these three yeasts in *vitro* and ~~postharvest~~ controlling of the postharvest decay of strawberry fruit~~..~~.

2. Materials and methods

2.1. Yeast and strawberry

The antagonist yeasts *W. anomalus* Disva 2, *M. pulcherrima* Disva 267, and *S. cerevisiae* Disva599 from the collection of the Department of Life and Environmental Sciences (Polytechnic University of Marche, Ancona, Italy) were used for the *in vitro* and *in vivo* assays. Yeast strains were grown in yeast potato dextrose (YPD) medium (prepared with 10 g/L yeast extract, 10 g/L peptone, 20 g/L glucose). All of the strains were sub-cultured at 3-month intervals on YPD agar medium and maintained at 4 °C.

The *in vivo* trials were carried out on strawberry (*Fragaria* × *ananassa*, cv. 'Alba') fruit harvested from an organic farm in Ancona, central-eastern Italy, with the strawberry fruit selected for uniformity in size and colour, and absence of defects.

2.2. Profiles of yeast volatile organic compounds and higher alcohols, and their production over time

The yeast VOC compositions were quantitatively evaluated using head-space solid-phase microextraction with a gas chromatograph (GC-2014; Shimadzu, Kyoto, Japan). Analyses were performed after 1 day and 6 days of growth of *W. anomalus*, *M. pulcherrima* and *S. cerevisiae*. Five millilitres of each yeast culture were heated to 35 °C, during which time the headspace volatiles were collected with divinylbenzene/ carboxen/ polydimethylsiloxane fibre (Supelco,

Bellefonte, PA, USA) following the procedures of Ando et al. (2012). The chemical analyses were performed in triplicate, and are expressed as means ± standard deviation.

2.3. In vitro antimicrobial activity of yeast volatile organic compounds against decay-causing fungi

The experimental assays were based on a dual culture method to test antagonistic activities without contact between cultures, of the yeast VOCs against some postharvest pathogens of fruit. The yeast antimicrobial activities were targeted for seven decay-causing fungi from the culture collection of the Department of Life and Environmental Sciences (Polytechnic University of Marche, Ancona, Italy): *B. cinerea*, *Monilinia fructicola*, *Alternaria alternata*, *Aspergillus carbonarius*, *Penicillium digitatum*, *Cladosporium* spp. and *Colletotrichum* spp..

Yeast potato dextrose agar medium plates were seeded with each yeast, and after a 24-h incubation at 25 °C, the lids of the Petri dishes were replaced with plates of potato dextrose agar (PDA), with the plates inoculated with the fungal pathogen alone. These plates with the two colonies were sealed (Parafilm) and incubated at 25 °C for 7 days, after which the fungal growth was recorded. Per each fungus, the control treatment was represented by the Petri dishes that did not have the yeast cultures. The percentage of growth inhibition of the fungal mycelia by the yeasts was calculated as compared to the control. Three replicates were used for each yeast/ fungus combination.

2.4. In vitro antimicrobial activity of ethyl acetate

To determine the antimicrobial activity of ethyl acetate, a plug of the fungus *B. cinerea* was placed on the base of a Petri dish containing PDA, and a sterile absorbent paper disk that was soaked with different concentrations of ethyl acetate (from 0.897 to 17.94 mg/mL) was

placed in the lid of this Petri dish. The control Petri dishes were prepared with the same procedure, but the sterile absorbent paper disk was soaked with 1 mL water. For each ethyl acetate concentration, three Petri dish replicates were used. The Petri dishes were kept for 7 days at 25 ±1 °C, after which the fungal growth was recorded. The percentage of growth inhibition of the fungal mycelia was calculated as compared to the control.

2.5. Effects of yeast volatile organic compounds on postharvest decay of strawberry fruit

Wickerhamomyces anomalus, *M. pulcherrima* and *S. cerevisiae* strains were pre-grown separately on 30 mL YPD medium for 24 h at 25 °C, and then used to inoculate 1500 mL YPD medium in each trial, with incubations for 1 or 6 days at 25 °C. Petri dishes containing the liquid culture of each yeast were placed in plastic boxes (70.5 × 40 × 16.5 cm) together with the strawberry fruit. Each plastic box contained eight trays with six strawberries each (48 fruit per box), with 500 mL growing yeast culture placed in open 18-mm-diameter glass Petri dishes above the fruit. The plastic boxes were closed with a plastic lid and sealed immediately, then left under shaking at 100 rpm and 25 °C for 48 h. Plastic boxes with the same number of strawberries and with containers including water were used as the controls. After 48 h, the Petri dishes containing the growing yeast cultures were removed from the boxes, and the strawberries were kept at 25 ± 1 °C for 2 days. The percentages of decayed strawberry fruit were recorded daily, and disease severity was recorded according to an empirical scale with six ~~levels~~degrees: 0, healthy fruit; 1, 1% to 20% fruit surface infected; 2, 21% to 40% fruit surface infected; 3, 41% to 60% fruit surface infected; 4, 61% to 80% fruit surface infected; 5, ≥81% fruit surface infected ~~and showing sporulation~~. The infection index, or McKinney's Index, incorporates both the incidence and severity of the disease, and it ~~is~~ expressed ~~as~~ the weighted means of the

disease, as a percentage of the maximum possible level (McKinney, 1923). Specifically, it is calculated according to Equation (1):

$$I = [(d \times f)/(N \times D)] \times 100 \quad (1),$$

where *d* is the category of rot intensity scored on the strawberry, and *f* is its frequency, *N* is the total number of strawberry fruit examined (healthy and rotted), and *D* is the highest category of disease intensity that occurred on the empirical scale (Romanazzi et al., 2001). ~~Two replicates were carried out.~~

2.6. In vivo effects of ethyl acetate for the control of strawberry postharvest decay

The *in vivo* antifungal activity of ethyl acetate was studied for strawberry postharvest decay. Preliminary trials were carried out to evaluate the optimal concentration of ethyl acetate, which would be effective against postharvest decay and avoid phytotoxic effects on strawberry fruit. Forty-eight strawberry fruit were placed in the plastic boxes used previously in the *in vivo* VOC efficacy evaluation, testing different concentrations of ethyl acetate, as 0.0718, 0.718 and 7.18 mg/mL ~~(the higher concentrations tested in *in vitro* test caused damage to the fruit by changing the fruit's consistency)~~. Boxes containing strawberry fruit and water were used as the controls. Four replicates were used for each treatment. After 48 h, flasks containing ethyl acetate or water were removed and the boxes were kept for 3 d at 25 ±1 °C, after which the decay, severity and McKinney's Index were evaluated as previously reported. Since the highest concentrations tested in *in vitro* test (7.18 mg/mL) was phytotoxic on strawberries, a~~A~~ second trial was carried out according to the optimal concentration of ethyl acetate found in the preliminary test. Boxes containing thirty-four trays with 6 strawberries each were placed with the same arrangement with 0.718 mg/mL ethyl acetate for 48 h. ~~Same b~~Boxes containing strawberry fruit and water

were used as the controls. After 48 h, flasks containing ethyl acetate or water were removed and the boxes were kept for 3 d at 25 ±1 °C, ~~after which~~ then the number of infected fruit and disease severity were recorded. ~~strawberry decay, severity and McKinney's Index were evaluated.~~

2.7. Statistical analysis

The data were analysed using the Student *t*-test or one-way ANOVA, followed by Duncan tests or Fisher least significant difference (LSD) test (Statsoft, USA) at $p < 0.05$. The percentage data were arcsine transformed before analysis, to improve the homogeneity of variance when the range of percentages was >40. Actual values are shown. All of the trials were repeated at least twice, with at least three replicates. Data from two or more experiments were pooled, and the statistical analysis to determine the homogeneity of variances was tested using Levene's test.

3. Results

3.1. Solid-phase microextraction gas-chromatography analyses of the volatile organic compounds

In the 1-day-old and 6-day-old yeast liquid cultures, the most representative VOC was ethyl acetate. As expected, the 6-day-old yeast liquid cultures produced larger amounts of ethyl acetate and the other VOCs (Table 1) than seen for the 1-day-old yeast liquid cultures (data not shown), independent of the yeast species.

In these incubations with the 6-day-old cultures, *W. anomalus* and *M. pulcherrima* produced the highest levels of ethyl acetate, at 792.9 mg/L and 115.0 mg/L, respectively. In *W. anomalus*, isoamyl acetate was moderately abundant (26.5 mg/L), with low levels for *M. pulcherrima a*nd *S. cerevisiae* (1.2 and 5.0 mg/L, respectively). All three of the yeasts produced small amounts of the other VOCs, where ethyl butyrate, ethyl hexanoate, and phenylethyl acetate ranged from

0 mg/L to 5 mg/L. For the higher alcohols, *W. anomalus* produced greater amounts of 2-phenylethanol (10.0 mg/L) and isobutanol (12.2 mg/L) than the other two yeasts (Table 1).

3.2. In vitro antimicrobial activity of yeast volatile organic compounds against the main fungal pathogens of fruit

The data from the double plate assays showed that *W. anomalus* had the highest antimicrobial activity against *B. cinerea*, with 87% growth inhibition. Similar behaviours were observed for *M. pulcherrima* and *S. cerevisiae*, which reduced the growth of *B. cinerea* by 56% and 63%, respectively (Table 1). These three yeasts also reduced the development of the fungus *M. fructicola* with 55%, 42% and 57% growth inhibition by *W. anomalus*, *M. pulcherrima*, and *S. cerevisiae*, respectively. *W. anomalus* also inhibited the growth of *A. alternata* and *A. carbonarius* by 47% and 44%, respectively. *S. cerevisiae* also showed antimicrobial activity against *A. alternata*, with 35% growth inhibition. Little or no antimicrobial activity was observed for *W. anomalus*, *M. pulcherrima*, and *S. cerevisiae* against *P. digitatum*, *Cladosporium* spp. and *Colletotrichum* spp. (Table 2).

3.3. In vitro antimicrobial activities of ethyl acetate

Considering that ethyl acetate was the main representative VOC of the *W. anomalus* and *M. pulcherrima* cultures, synthetic ethyl acetate was also investigated to define a threshold of its antimicrobial effects.

In the in vitro trials for the full range of ethyl acetate concentrations, for 8.97 mg/mL ethyl acetate and above, there was total inhibition of the fungus *B. cinerea*. In the presence of lower concentrations of ethyl acetate, there was a slight reduction of 15% of mycelial growth, while the suppressive effect was completely ~~nullified~~ none with the least~~less~~ concentrations.

3.4. Effects of exposure to yeast liquid cultures on postharvest decay of strawberry fruit

The results of trials with the exposure of strawberry fruit to yeast liquid cultures of strawberry confirmed the *in vitro* data. The largest effects were seen for the exposure to liquid cultures of *W. anomalus*, with *M. pulcherrima* and *S. cerevisiae* also showing some inhibitory effects. When the strawberry fruits were incubated with the 1-day-old liquid yeast cultures, there were no significant effects seen compared to the control (data not shown). However, following the incubation with the 6-day-old liquid yeast cultures, the strawberry fruit showed significant reduction of greay mould, the main cause of postharvest decay (Fig. 1). Here, while only *W. anomalus* significantly reduced the strawberry fruit decay, the McKinney's Index was reduced by 89%, 40% and 32% by *W. anomalus*, *M. pulcherrima* and *S. cerevisiae*, respectively, as compared to the control.

3.5. In vivo effects of ethyl acetate for the control of strawberry postharvest decay

The main cause for strawberry postharvest decay was ~~grey~~ gray mould. From the preliminary trials, the 0.718 mg/mL ethyl acetate concentration showed significant reductions of the strawberry disease parameters while it did not result in any ~~matrix degeneration of the strawberry fruit~~phytotoxic effect~~, which maintained the integrity of their structure~~. In contrast, the 10-fold greater concentration of 7.18 mg/mL ethyl acetate ~~was toxic for these~~damaged strawberry fruit~~, as they showed deliquescence with vigorous fluid loss~~. No inhibitory effects were seen for the lowest concentration ~~of (~~0.0718 mg/mL) of ethyl acetate as compared to the~~;~~ ~~indeed, in this case, the development of the postharvest fungi on the strawberry fruit paralleled that of the~~ control (data not shown). Whereas,

~~Differently,~~ the vapours of 0.718 mg/mL ethyl acetate decreased ~~the strawberry disease, as compared to the control~~gray mold (Table 3). In particular, strawberry fruit treated with ethyl

acetate had ~~decay~~ disease incidence, ~~disease~~ severity and McKinney's Index~~,~~ of 36%, 52%, and 52% lower than the control, respectively.

4. Discussion

Volatile organic compounds are carbon-based, gas-phase products, and approximately 250 different VOCs have been defined as being produced by fungi. These are produced as mixtures of simple hydrocarbons, heterocyclics, aldehydes, ketones, alcohols, phenols, thioalcohols and thioesters, and/or their derivatives (Korpi et al., 2009; Ortiz-Castro et al., 2009). Due to the small sizes of these molecules and their diffusion through the atmosphere and soil, numerous studies have been carried out on the potential applications of VOCs in agriculture, industry and pharmacology, particularly as biocontrol factors (Liu et al., 2008; Arrebola et al., 2010; Morath et al., 2012; Giorgio et al., 2015~~; Liu et al., 2008; Morath et al., 2012~~).

In a recent study, Nally et al. (2015) showed that antifungal VOCs produced by *Saccharomyces* isolates can inhibit fungal mycelial growth of *B. cinerea*, *Aspergillus versicolor*, *Aspergillus caelatus* and *F. oxysporum*, while the VOCs produced by non-*Saccharomyces* strains, such as *Candida sake* and *Candida versatilis*, inhibited *Penicillium commune*, *Aspergillus terreus* and *A. carbonarius.* Di Francesco ~~and coworkers~~et al. (2015) identified a group of different alcohol VOCs that are produced by *Aureobasidium pullulans* that can protect apples from ~~grey~~ gray mo~~u~~ld. Parafati et al. (2015) ~~also~~ demonstrated inhibitory effects of the yeast *W. anomalus*, *M. pulcherrima* and *S. cerevisiae* against *B. cinerea in vitro* and *in vivo* on table grapes. They justified these yeast antimicrobial activities through multiple modes of action, including the production of VOCs.

In the present study, the effects of these three yeast, *W. anomalus*, *M. pulcherrima* and *S. cerevisiae*, previously characterised as ~~biological~~ biocontrol agents ~~for~~ on sweet cherries (Oro

et al., 2014), were tested on strawberry fruit, that is highly perishable, ~~that~~ and after harvest they can easily undergo fungal spoilage (Feliziani et al., 2016).

Our preliminary *in vitro* tests, ~~-~~carried out without contact between yeast/mo~~u~~ld cultures, showed an effectively reduction on the growth of *B. cinerea* and other fungi. ~~-~~This behaviour excluded killer activity, competition for nutrients or ~~-~~other modes of action involving cell to cell contact, and we focused our attention on VOCs that are toxic to these fungal pathogens, which would thus represent their probable mode of action. ~~These~~ This hypothesis ~~were~~ was confirmed by the *in vivo* tests, where the strawberry fruit exposed to the synthetic VOCs from these yeasts showed a reduction of ~~ed McKinney's Index for~~ strawberry ~~grey~~ gray mo~~u~~ld~~,~~ ~~compared to the control fruit~~. The VOCs naturally produced by *W. anomalus* were more effective ~~for~~ on~~the reduction of~~ postharvest decay of ~~these~~ strawberry fruit as~~,~~ compared to those of *M. pulcherrima* and *S. cerevisiae*. Indeed, *W. anomalus* produced the highest quantities of VOCs that remain confined in the boxes. The yeast with the highest production of antimicrobial VOCs, *W. anomalus*, ~~-~~was the one that ~~best~~ better controlled the fungal pathogen.

When the strawberry fruit were exposed to the yeast VOCs, reduction of the ~~grey~~ gray mo~~u~~ld was only seen for the 6-day-old yeast cultures since the VOCs produced after 1-day were not ~~sufficient to control the grey mould~~effective.

In the analysis of the VOC profiles of these yeasts, ethyl acetate was the major compound produced in terms of the quantity and *W. anomalus* showed the highest production if compared to *S. cerevisiae* and *M. pulcherrima.* In previous studies, the biocontrol activity of *W. anomalus* has been attributed to the production of ethyl acetate in terms of grain storage mo~~u~~lds, such as *Penicillium* spp. (Druvefors et al., 2005), and of 2-phenyl ethanol in terms of *Aspergillus flavus* (Hua et al., 2014). This would indicate that the active ingredient of *W. anomalus* VOCs against the fungus *B. cinerea* is ethyl acetate even if other volatile molecules may have an antimicrobial effect. ~~-~~Indeed, in the present study, ethyl acetate was effective for

suppression of *B. cinerea* on strawberry fruit, where the exposure for 48 h to 0.718 mg/mL ethyl acetate almost halved the McKinney's Index and severity of grea~~e~~y mo~~u~~ld. However, further investigations are needed to assess the potential additive or synergistic effect among the different VOCs produced by each yeast.

Nowadays, biocontrol appears to provide sustainable and reliable alternatives to chemical fungicides, which have raised serious concerns because of food contamination and environmental pollution. Here, we have demonstrated that the production of VOCs can have essential roles in the control of postharvest gra~~e~~y mo~~u~~ld of strawberry fruit. However, ~~more~~ further investigations are necessary to understand how the VOCs produced by *W. anomalus*, or indeed ethyl acetate, might be integrated into current practice, and if these are also effective for the control of postharvest diseases of other fruit.

Conflict of interest

The authors declare no conflicts of interest.

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Table 1. VOCs and higher alcohols produced by 6-day-old cultures of *Wickerhamomyces anomalus*, *Metschnikowia pulcherrima* and *Saccharomyces cerevisiae*.

Yeast ~~VOC~~ ~~source~~	VOCs and higher alcohols produced (mg/L)									
	Acetaldehyde	**Ethyl acetate**	**Ethyl butyrate**	**Isoamyl acetate**	**Ethyl hexanoate**	**Phenylethyl acetate**	**2-Phenyl-ethanol**	**Isobutanol**	**Amylic alcohol**	**Isoamyl alcohol**
Wickerhamomyces anomalus	**0 ±0^{b}**	**792.85 ±22.60^{a}**	**0.730 ±0.028^{a}**	**26.45 ±0.04^{a}**	**0.970 ±0.049^{a}**	**4.80 ±0.19^{a}**	**10.02 ±0.70^{a}**	**12.21 ±0.74^{a}**	**27.02 ±2.16^{a}**	**18.41 ±0.25^{b}**
Metschnikowia pulcherrima	**0.82 ±0.03^{a}**	**115.0 ±5.1^{b}**	**0.060 ±0.014^{b}**	**1.170 ±0.014^{c}**	**0.500 ±0.028^{b}**	**0 ±0^{b}**	**4.75 ±0.18^{b}**	**3.03 ±0.42^{c}**	**18.22 ±0.31^{b}**	**24.96 ±2.76^{b}**
Saccharomyces cerevisiae	**0 ±0^{b}**	**6.07 ±1.48^{c}**	**0.045 ±0.007^{b}**	**5.000 ±0.014^{b}**	**0 ±0^{c}**	**0.27 ±0.03^{b}**	**3.56 ±0.05^{a}**	**5.43 ±0.82^{a}**	**24.05 ±5.30^{a}**	**34.71 ±2.16^{a}**

Different letters show significant differences according to Duncan's tests ($p < 0.05$).

Table 2. *In vitro* antagonistic activity of yeast VOCs against the fungi, expressed as percentages of inhibition of the fungal mycelia, using the dual culture method.

Yeast	Fungal growth inhibition by yeast VOC (%)						
	Botrytis cinerea	***Monilinia fructicola***	***Alternaria alternata***	***Aspergillus carbonarius***	***Penicillium digitatum***	***Cladosporium* spp.**	***Colletotrichum* spp.**
Wickerhamomyces anomalus	87.0 ±3.6 [a]	55.0 ±11.8 [b]	47.0 ±8.2 [bc]	44.0 ±6.9 [c]	1.5 ±0.5 [d]	6.0 ±3.5 [d]	0.5 ±0.5 [d]
Metschnikowia pulcherrima	56.0 ±10.1 [a]	42.1 ±3.1 [b]	7.5 ±2.2 [c]	6.5 ±0.9 [cd]	0.0 ±0.0 [d]	4.5 ±1.0 [cd]	1.0 ±0.0 [d]
Saccharomyces cerevisiae	63.0 ±4.0 [a]	57.0 ±4.4 [a]	35.0 ±5.0 [b]	5.0 ±2.0 [c]	1.5 ±0.5 [c]	3.0 ±1.7 [c]	2.0 ±2.0 [c]

Different letters show significant differences of three yeast VOCs related to different fungal growth inhibitions (Fisher LSD tests; $p < 0.05$).

Table 2. *In vitro* antagonistic activity of yeast VOCs against the fungi, expressed as percentages of inhibition of the fungal mycelia, using the dual culture method.

Yeast VOC source	Fungal growth inhibition by yeast VOC (%)						
	Botrytis cinerea	*Monilinia fructicola*	*Alternaria alternata*	*Aspergillus carbonarius*	*Penicillium digitatum*	*Cladosporium* spp.	*Colletotrichum* spp.
Wickerhamomyces anomalus	87.0 ±3.6 ^{a}	55.0 ±11.8 ^{b}	47.0 ±8.2 ^{bc}	44.0 ±6.9 ^{c}	1.5 ±0.5 ^{d}	6.0 ±3.5 ^{d}	0.5 ±0.5 ^{d}
Metschnikowia pulcherrima	56.0 ±10.1 ^{a}	42.1 ±3.1 ^{b}	7.5 ±2.2 ^{e}	6.5 ±0.9 ^{cd}	0.0 ±0.0 ^{d}	4.5 ±1.0 ^{cd}	1.0 ±0.0 ^{d}
Saccharomyces cerevisiae	63.0 ±4.0 ^{a}	57.0 ±4.4 ^{a}	35.0 ±5.0 ^{b}	5.0 ±2.0 ^{e}	1.5 ±0.5 ^{e}	3.0 ±1.7 ^{e}	2.0 ±2.0 ^{e}

Different letters show significant differences of three yeast VOCs related to different fungal growth inhibitions (Fisher LSD tests; $p < 0.05$).

Table 3. Decay, severity and McKinney's Index of postharvest decay, mainly grae~~u~~y mo~~u~~ld, of strawberry fruit exposed for 48 h to 0.8 µL/mL ethyl acetate, and then kept for 3 d at 25 ± 1 °C. Different letters indicate significant differences within columns according to the Student's t-test ($p < 0.05$).

Treatment	Decay (%)	Severity (1-5)	McKinney's Index (%)
Control	25.0[a]	2.5[a]	15.1[a]
Ethyl acetate	16.7[b]	1.2[b]	7.3[b]

Figure caption

Figure 1. Decay and McKinney's Index of graey mould on strawberry fruit exposed to VOCs of 6-d old cultures of *W. anomalus*, *M. pulcherrima* and *S. cerevisiae*. Strawberries were kept at 25 °C for 48 h with the yeast, followed by 2 d of shelf life at 20 ±1 °C, when fruit decay was evaluated. Different letters (capital letters to Decay and small letters to McKinney's Index) show significant differences according to Fisher LSD test ($p < 0.05$).