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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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1	Volatile organic compounds from Wickerhamomyces anomalus,
2	Metschnikowia pulcherrima and Saccharomyces cerevisiae inhibit growth of
3	decay causing fungi and control postharvest diseases of strawberries
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15 Abstract

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17 The effectiveness of Wickerhamomyces anomalus, Metschnikowia pulcherrima and Saccharomyces cerevisiae as biocontrol agents on postharvest decay of strawberry (Fragaria \times 18 ananassa, cv. 'Alba') fruit, and their inhibitory activities on some decay-causing fungi were 19 evaluated. Volatile organic compounds from these yeasts decreased mycelial growth of Botrytis 20 21 cinerea by 69%, and by less for Monilinia fructicola, Alternaria alternata, Aspergillus 22 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 23 24 48 h showed 89%, 40%, and 32% reductions, respectively, in graey mould McKinney Index. Vapours of ethyl acetate, the main volatile organic compound of these yeasts, completely 25 inhibited B. cinerea growth at 8.97 mg/mL, and suppressed gray mold grey mould on 26 27 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 graey mould of strawberry fruit. 28

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Keywords: *Botrytis cinerea*, ethyl acetate, *Fragaria × ananassa*, <u>gray moldgrey mould</u>, yeast
volatile organic compounds

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35	Highlights
36	Yeast volatile organic compounds can reduce in vitro growth of decay-causing fungi
37	Strawberry exposure to yeast producing volatile organic compounds reduces gray mold grey
38	mould
39	The main volatile organic compound of the three selected yeasts is ethyl acetate
40	Ethyl acetate at 8.97 mg/mL completely inhibits B. cinerea growth in the in vitro trials
41	Ethyl acetate at 0.717 mg/mL can control gray mold grey mould on strawberry fruit
42	Ethyl acetate at 8.97 mg/mL completely inhibits B. cinerea growth in the in vitro trials
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- 63
- 64 **1. Introduction**
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The use of yeasts as biocontrol agents for prevention of pre-harvest and postharvest diseases of 66 67 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 68 69 Botrytis spp., Aspergillus spp., Penicillium spp., Monilinia spp. and Colletotrichum spp. (Janisiewicz and Korsten, 2002). In a previous trials, the three yeasts Wickerhamomyces 70 71 anomalus (formerly Hansenula anomala and Pichia anomala), Metschnikowia pulcherrima 72 and *Saccharomyces cerevisiae* were applied as pre-harvest treatments to tree canopies, and they 73 were effective for reduction of sweet cherry postharvest decay that was mainly caused by the 74 fungus Monilinia laxa (Oro et al., 2014).

75 Generally, tThe biocontrol ability of these yeasts can be related to different mechanisms 76 through which they interact with pathogens and fruit tissues including: competition with 77 pathogens for space and nutrients (Benchegroun et al., 2007); parasitism and production of extracellular proteins (Lima et al., 2013); killer activity; induction of resistance in fruit tissues 78 79 through elicitors that are either secreted by the biocontrol agent (-Spadaro and Droby, 2016). 80 Moreover, several studies have demonstrated that the production of volatile organic compounds 81 (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 82 83 food and beverages, has proven antimicrobial properties (Vimala et al., 2007) (https://www.nlm.nih.gov/). These antimicrobial properties change across species, and are 84 affected by yeast growth stage, culture mode, temperature, medium composition, and other 85 factors (Martins et al., 2010). The aim of the present study was to evaluate the effectiveness of 86

the VOCs from these three yeasts in *vitro* and <u>postharvest</u> controlling of the <u>postharvest</u> decay
of strawberry fruit...

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90 2. Materials and methods

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92 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.

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

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104 2.2. Profiles of yeast volatile organic compounds and higher alcohols, and their production
105 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.

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115 2.3. In vitro antimicrobial activity of yeast volatile organic compounds against decay-causing

116 *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 24-123 h incubation at 25 °C, the lids of the Petri dishes were replaced with plates of potato dextrose 124 agar (PDA), with the plates inoculated with the fungal pathogen alone. These plates with the 125 two colonies were sealed (Parafilm) and incubated at 25 °C for 7 days, after which the fungal 126 127 growth was recorded. Per each fungus, the control treatment was represented by the Petri dishes 128 that did not have the yeast cultures. The percentage of growth inhibition of the fungal mycelia 129 by the yeasts was calculated as compared to the control. Three replicates were used for each yeast/ fungus combination. 130

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132 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 \pm 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.

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143 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 144 145 separately on 30 mL YPD medium for 24 h at 25 °C, and then used to inoculate 1500 mL YPD 146 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 ($70.5 \times 40 \times 16.5$ cm) together with the 147 148 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 149 above the fruit. The plastic boxes were closed with a plastic lid and sealed immediately, then 150 left under shaking at 100 rpm and 25 °C for 48 h. Plastic boxes with the same number of 151 strawberries and with containers including water were used as the controls. After 48 h, the Petri 152 153 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 154 155 daily, and disease severity was recorded according to an empirical scale with six levels degrees: 156 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, \geq 81% fruit surface 157 infected and showing sporulation. The infection index, or McKinney's Index, incorporates both 158 the incidence and severity of the disease, and it is expressed as the weighted means of the 159

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

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

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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.

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170 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. 171 Preliminary trials were carried out to evaluate the optimal concentration of ethyl acetate, which 172 would be effective against postharvest decay and avoid phytotoxic effects on strawberry fruit. 173 Forty-eight strawberry fruit were placed in the plastic boxes used previously in the in vivo VOC 174 efficacy evaluation, testing different concentrations of ethyl acetate, as 0.0718, 0.718 and 7.18 175 176 mg/mL-(the higher concentrations tested in *in vitro* test caused damage to the fruit by changing 177 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 178 were removed and the boxes were kept for 3 d at 25 \pm 1 °C, after which the decay, severity and 179 180 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, aA second trial was carried 181 out according to the optimal concentration of ethyl acetate found in the preliminary test. Boxes 182 containing thirty-four trays with 6 strawberries each were placed with the same arrangement 183 with 0.718 mg/mL ethyl acetate for 48 h. Same bBoxes containing strawberry fruit and water 184

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
<u>disease severity were recorded.</u> strawberry decay, severity and McKinney's Index were
evaluated.

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190 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.

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198 **3. Results**

199 3.1. Solid-phase microextraction gas-chromatography analyses of the volatile organic200 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 and 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 2phenylethanol (10.0 mg/L) and isobutanol (12.2 mg/L) than the other two yeasts (Table 1).

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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 215 activity against B. cinerea, with 87% growth inhibition. Similar behaviours were observed for 216 *M. pulcherrima* and *S. cerevisiae*, which reduced the growth of *B. cinerea* by 56% and 63%, 217 respectively (Table 1). These three yeasts also reduced the development of the fungus M. 218 219 fructicola with 55%, 42% and 57% growth inhibition by W. anomalus, M. pulcherrima, and S. 220 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 221 against A. alternata, with 35% growth inhibition. Little or no antimicrobial activity was 222 observed for W. anomalus, M. pulcherrima, and S. cerevisiae against P. digitatum, 223 Cladosporium spp. and Colletotrichum spp. (Table 2). 224

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226 *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 <u>nullified_none</u> with <u>the leastless_</u>concentrations.

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235 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 236 confirmed the *in vitro* data. The largest effects were seen for the exposure to liquid cultures of 237 W. anomalus, with M. pulcherrima and S. cerevisiae also showing some inhibitory effects. 238 239 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 240 241 incubation with the 6-day-old liquid yeast cultures, the strawberry fruit showed significant 242 reduction of greav 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 243 244 by 89%, 40% and 32% by W. anomalus, M. pulcherrima and S. cerevisiae, respectively, as 245 compared to the control.

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247 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 248 249 preliminary trials, the 0.718 mg/mL ethyl acetate concentration showed significant reductions 250 of the strawberry disease parameters while it did not result in any matrix degeneration of the 251 strawberry fruitphytotoxic effect, which maintained the integrity of their structure. In contrast, 252 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 253 254 were seen for the lowest concentration of (0.0718 mg/mL) of ethyl acetate as compared to the; 255 indeed, in this case, the development of the postharvest fungi on the strawberry fruit paralleled that of the control (data not shown). Whereas, 256

257 Differently, the vapours of 0.718 mg/mL ethyl acetate decreased the strawberry disease, 258 as compared to the control gray mold (Table 3). In particular, strawberry fruit treated with ethyl

acetate had <u>decay_disease</u> incidence, <u>disease</u> severity and McKinney's Index<u>, of</u> 36%, 52%, and
52% lower than the control, respectively.

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263 **4. Discussion**

Volatile organic compounds are carbon-based, gas-phase products, and approximately 250 264 different VOCs have been defined as being produced by fungi. These are produced as mixtures 265 of simple hydrocarbons, heterocyclics, aldehydes, ketones, alcohols, phenols, thioalcohols and 266 thioesters, and/or their derivatives (Korpi et al., 2009; Ortiz-Castro et al., 2009). Due to the 267 268 small sizes of these molecules and their diffusion through the atmosphere and soil, numerous 269 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 270 271 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 272 Saccharomyces isolates can inhibit fungal mycelial growth of B. cinerea, Aspergillus 273 versicolor, Aspergillus caelatus and F. oxysporum, while the VOCs produced by non-274 275 Saccharomyces strains, such as Candida sake and Candida versatilis, inhibited Penicillium 276 commune, Aspergillus terreus and A. carbonarius. Di Francesco and coworkerset al. (2015) 277 identified a group of different alcohol VOCs that are produced by Aureobasidium pullulans that 278 can protect apples from grey-gray mould. Parafati et al. (2015) also-demonstrated inhibitory 279 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 280 modes of action, including the production of VOCs. 281

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

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

286 Our preliminary *in vitro* tests, -carried out without contact between yeast/mould cultures, showed an effectively reduction on the growth of *B. cinerea* and other fungi. -This behaviour 287 excluded killer activity, competition for nutrients or -other modes of action involving cell to 288 cell contact, and we focused our attention on VOCs that are toxic to these fungal pathogens, 289 290 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 291 292 these yeasts showed a reduction of ed McKinney's Index for strawberry grey gray mould, 293 compared to the control fruit. The VOCs naturally produced by W. anomalus were more 294 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 295 296 of VOCs that remain confined in the boxes. The yeast with the highest production of 297 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 298 299 mould was only seen for the 6-day-old yeast cultures since the VOCs produced after 1-day were

300 not sufficient to control the grey mould<u>effective</u>.

301 In the analysis of the VOC profiles of these yeasts, ethyl acetate was the major 302 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 303 304 W. anomalus has been attributed to the production of ethyl acetate in terms of grain storage 305 moulds, 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. 306 anomalus VOCs against the fungus B. cinerea is ethyl acetate even if other volatile molecules 307 308 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 greary mould. 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 graey mould 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.

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321 **Conflict of interest**

322 The authors declare no conflicts of interest.

323 **References**

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

405 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

408 <u>culture method.</u>

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

Table 2. *In vitro* antagonistic activity of yeast VOCs against the fungi, expressed as percentages

- 413 of inhibition of the fungal mycelia, using the dual culture method.

Yeast VOC		Fu	ıngal grov	vth inhibiti	on by yeas	t VOC (%)	
source	Botry	Monili	<u>Alterna</u>	Aspergill	Penicilli	Cladospor	Colletotric
	tis	nia	ria	US	um	ium spp.	hum spp.
	ciner	fructic	alterna	carbona	digitatu		
	ea	ola	t a	rius	m		
Wickerhamo	87.0	55.0	47.0	44.0	1.5 ±0.5	6.0 ±3.5 ^d	0.5 ±0.5 ^d
myces	±3.6 ^a	±11.8 ⁺	±8.2 ^{-be}	±6.9 ^e	đ		
anomalus							
Metschnikowi	56.0	42.1	7.5	6.5 ±0.9	0.0 ± 0.0	4.5 ±1.0 ^{ed}	1.0 ±0.0 ^d
đ	±10.1	±3.1 ⁺	±2.2 ^e	ed	đ		
pulcherrima	a						
Saccharomyc	63.0	57.0	35.0	5.0 ±2.0	1.5 ±0.5	3.0 ±1.7 ^e	2.0 ±2.0 ^e
es cerevisiae	±4.0 *	±4.4 ^a	±5.0 ^{-b}	e	e		

416 Different letters show significant differences of three yeast VOCs related to different fungal

417 growth inhibitions (Fisher LSD tests; p <0.05).

Table 3. Decay, severity and McKinney's Index of postharvest decay, mainly graey mould, of420strawberry fruit exposed for 48 h to 0.8 μ L/mL ethyl acetate, and then kept for 3 d at 25 ± 1 °C.421Different letters indicate significant differences within columns according to the Student's t-422test (p <0.05).</td>

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



	426	Figure	caption
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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).