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Short Communication

Isolation and identification of *Saccharomyces cerevisiae* and evaluation of biodegradation efficiency of acetamiprid during fermentation

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ABSTRACT:

Acetamiprid is a chloronicotinyl neonicotinoid that is widely used in the control of aphids in wheat crop, *Saccharomyces cerevisiae* were isolated from local flour, vegetables and fruits and *Saccharomyces cerevisiae* YF isolates was identified by ITS region of the rRNA. The dissipation of acetamiprid during wheat fermentation (wheat dough) and in the medium by *Saccharomyces cerevisiae* YF was investigated. These results showed the dissipation of acetamiprid in medium were about 97%, while results of dissipating in wheat dough were about 58.5-88.3%.

Keywords:

Pesticides, Acetamiprid, Yeast, Wheat, HPLC, Dough.

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INTRODUCTION

Neonicotinoid insecticides are one of the most important commercial insecticides which is used widespread. They are systemic broad spectrum insecticides that exhibit a novel mode of action because they are agonists of nicotinic acetylcholine receptors (nAChR), leading to paralysis and death of pest organisms, which are more toxic to insects and less toxic to mammals, providing an excellent example of selective toxicity (Bass *et al*., 2015; Tomizawa *et al.*, 2000). Acetamiprid belongs to the class of chloronicotinyl neonicotinoid insecticides. It is being more competitive than conventional insecticide due to its special acting characteristics and has been considered as an important substitute to the organophosphate insecticides which have caused severe environmental pollution and pesticide resistance of insects, it was banned in many countries (Simon-Delso *et al.*, 2015 and Weerathunge *et al.*, 2014). Unsafe food contains hazards that cause disease, including pesticide residues (Kolberg *et al.*, 2011; Unnevehr, 2015). The control of pesticide residues in wheat grain is generally based on MRLs (Maximum Residual Limits). Although the correct use of pesticides does not cause problems of public concern in health and environmental areas, if inappropriate abusive treatments are applied without respecting safety recommendations, undesirable residues can remain on grains and can be transferred to cereal food products. In their presence, there are increasing concerns over the use of organophosphorus insecticides linked to health, as those are the main residues detected in wheat (Balinova *et al.*, 2006; Lozowicka *et al.*, 2014; Yadav *et al*., 2015).

It is very important to employ advanced and fast extraction methods for pesticide residues analysis. The QuEChERS method is a simple, low cost, rapid, selective and sensitive method for the analysis of acetamiprid insecticide residue in various food matrices like fruit (Lazić *et al*., 2014), honey (Codling *et al.*, 2016), crops (Tokieda *et al.*, 1997), etc. Biodegradation is a process that involves the introduction of microorganisms that are capable of degrading target chemicals and represents a useful approach to enhance pesticide degradation and/or detoxification in the environment (Zhang *et al*., 2012; Wang *et al*., 2016a). Some microorganisms that are used for biodegradation of acetamiprid include, *Pigmentiphaga* sp strain AAP-1 degrades acetamiprid (Wang *et al.*, 2016b). *Rhodococcus* sp. BCH2 (Phugare and Jadhav, 2015), *Ensifermeliloti* CGMCC 7333 (Zhou *et al.*, 2014). The current study aimed to investigate the ability of *S. cerevisiae* YF to biodegrade the acetamiprid in medium and during fermentation (wheat dough). The extraction of acetamiprid from wheat dough samples was done using a QuEChERS modified method with insecticide determination and quantification achieved by HPLC-UV.

MATERIALS AND METHODS

Strain isolation

Eleven *S. cerevisiae* YF were isolated from local flour, vegetables and fruits; they were grown in Potato dextrose agar (PDA) and incubated at 30 ºC.

Screening of the potential strain

Each isolates was cultured in 100 ml of medium with at an initial cell density of $1*10^5$ cells/ ml, the medium consisted of 0.5% yeast extract, 0.5% peptone and 0.5% glucose with 10 ppm of acetamiprid, and then incubated at 30ºC for 18 h in an orbital incubator. After

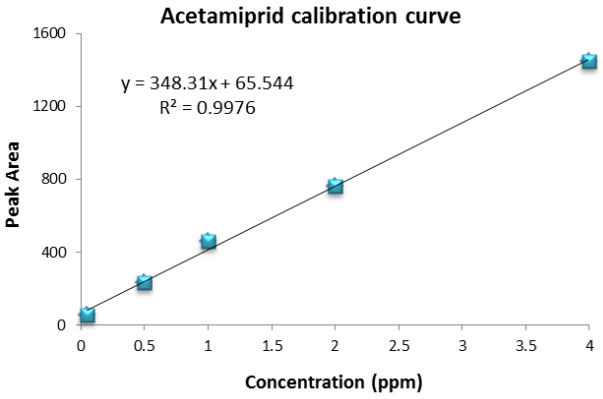

Figure 1. Standard curve of acetamiprid

Table 1. Primers used for ITS region of the rRNA amplification

S. No	Primers	Primer Sequence
1	ITS1	5′TCCGTAGGTGAACCTGCGG 3′
2	ITS4	5′TCCTCCGCTTATTGATATGC3′

that, cells are counted by hemocytometer.

Identification and characterization of the isolated yeast strain

The isolated yeast strain was identified on the basis of morphological and cultural characteristics and genetically identified by the ITS region of the rRNA sequencing (Grangeteau *et al*., 2015).

DNA extraction

DNA was extracted from *S. cerevisiae* YF by the Genomic DNA Purification Kit Wizard (Promega/ USA) according to the manufacturer's instructions.

Polymerase chain reaction amplification

The PCR was used in separate experiments to amplify ITS region of the rRNA from *S.cerevisiae* YF, using the primers described by (Sturaro *et al.*, 2018). The PCR reaction mix contained 2 µl of each primer (Table 1). 5 µl of DNA, master mix and 11 µl of nuclease free water. The PCR amplification cycle of 5 min at 94ºC, 30 cycle of 1 min at 94ºC, 1 min at 55ºC and 1:40 min at 72ºC and additionally 1 cycle of 5 min at 72ºC, finally are cooling at 4ºC.

BLAST comparison

The amplified products were identified using Macrogen/Korean sequencing and the sequences were compared with the NCBI database using BLAST search.

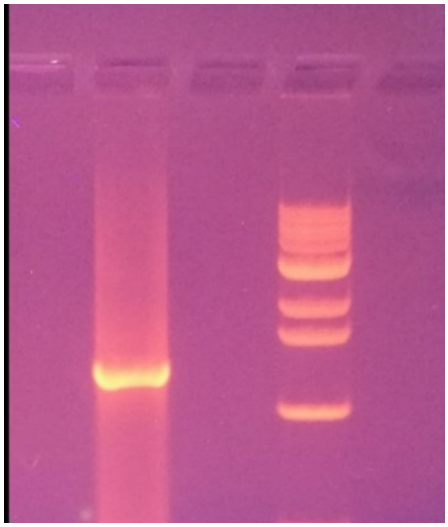

Figure 2. Agarose gel electrophoresis for PCR products with ladder 1 Kp

Degradation of acetamiprid by *Saccharomyces cerevisiae* YF in medium

Isolate was cultured in 100 mL of medium with at an initial cell density of $1*10^5$ cell/ mL, medium consists of (0.5% yeast extract, 0.5% peptone and 0.5% glucose) with 5, 10 and 15 ppm of acetamiprid, control (media with 15 ppm of acetamiprid without *S. cerevisiae* YF), then incubated at 30ºC for 18 h in an orbital shaking incubator. After that, this degradation of acetamiprid was determined by HPLC.

Preparation of wheat dough

Considering the fact that processing studies should simulate commercial or household practices as closely as possible. Initially wheat flour was fortified with acetamiprid 0.14, 0.17, 0.61 and 0.82 ppm, and 1 mL of *S. cerevisiae* YF ($1*10^7$) was added to each 100 g

Table 2. Cell counted of *S. cerevisiae* YF in medium

S. No	Isolates	Number (cell/mL)	Isolates	Number (cell/mL)
1	*S. cerevisiae* Y1	$4.3*10^5$	*S. cerevisiae* Y7	$3.3*10^5$
2	*S. cerevisiae* Y2	$1.9*10^6$	*S. cerevisiae* YG	$2.6*10^6$
3	*S. cerevisiae* Y3	$3.8*10^5$	*S. cerevisiae* YF	$1.5*10^7$
4	*S. cerevisiae* Y4	$3.7*10^5$	*S. cerevisiae* YD	$8.3*10^5$
5	*S. cerevisiae* Y5	$5.2*10^5$	*S. cerevisiae* YS	$1.8*10^6$
6	*S. cerevisiae* Y6	$9.4*10^4$	-	-

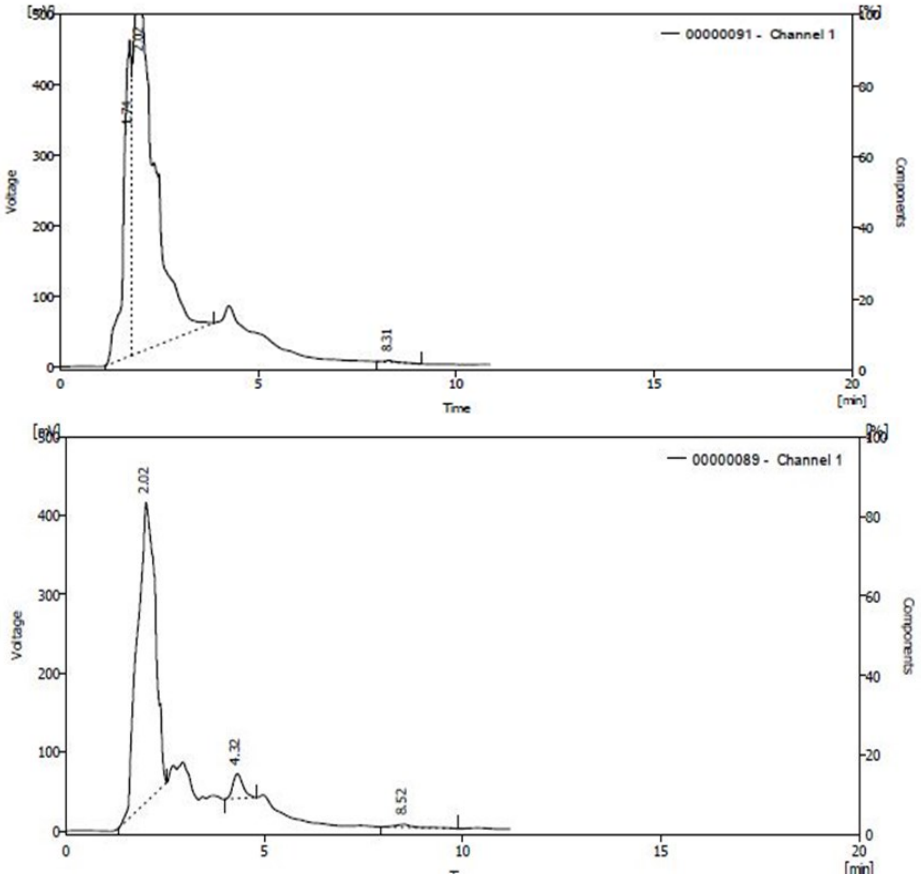

Figure 3. HPLC spectra of acetamiprid biodegradation by *S. cerevisiae* YF (a) 15 ppm, (b) 10 ppm

flour, wheat dough was prepared using conventional procedure according to (AACC, 2000) with some modification. The dough was kept for fermentation at room temperature for 1 h. Finally, it was examined for degradation of pesticide residues during the process by HPLC.

Preparation of standards (Acetamiprid 20 SP)

Acetamiprid technical material was obtained from Dr. Ehrenstorfer GmbH, Germany. The stock solution of 100 ppm was prepared by dissolving 0.01 g of technical material (99% purity) in 100 mL of HPLC grade acetonitrile. From this stock, intermediate stock solutions of 4 ppm and 0.05 ppm were prepared which was shown in Figure 1.

Table 3. Biodegradation of acetamiprid by *S. cerevisiae* YF during wheat fermentation

S. No	Fortification level (ppm)	Residual concentration (ppm)
1	0.82	0.17
2	0.61	0.071
3	0.17	0.044
4	0.14	0.058

Extraction procedure

Extraction of pesticide residue was accomplished according to the QuEChERS method of Anastassiades *et al*. (2003) with some modifications. 10 g of sample, homogenized and humidified, were weighed in a 50 mL centrifuge tube. 10 mL of acetonitrile, containing 1 % (v/v) of acetic acid was added to the sample, and the mixture was vortexed for 1 min. After that, 3 g of $MgSO_4$ was added and vortexed immediately for 20 sec. Later, 1.7 g of sodium acetate and 0.5 g of disodium hydrogen citrate sesquihydrate were added and the tube was hand shaken for 1 min and centrifuged at 4000 x g for 8 min to provide a completely phased separation.

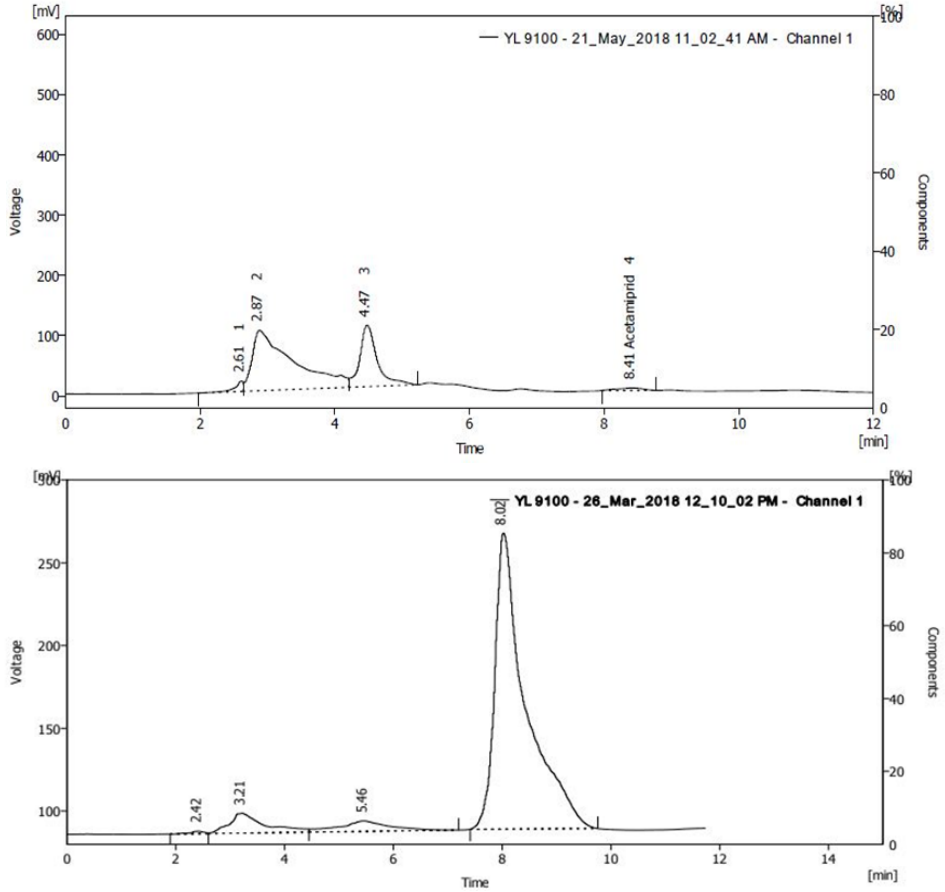

Figure 3. HPLC spectra of acetamiprid biodegradation by *S. cerevisiae* YF (c) 5 ppm, (d) Control

Finally, it was filtered by Buchner funnel to obtain a clear supernatant, then further filtered by using 0.45 µm and transferred to vial before injection into HPLC filtered by using 0.22 µm.

The data were analyzed statistically and the results were expressed in means using Microsoft excel software.

RESULTS AND DISCUSSION

Screening of the potential strain

Cells (yeast) were counted by hemocytometer, the results showed in the Table 2 that one of the isolates $1.5*10^7$ cell/mL is larger in number than other isolates, the isolation was capable of growing on the medium which was selected for further investigation.

Molecular (PCR) identification

It was done through PCR technique using universal fungus-specific ITS1 and ITS4 rRNA with a product of 845 bp, after showing the band in 1% agarose, visualized under UV and after staining with ethidium bromide, as shown in Figure 2. PCR products were sent to Macrogen/ Korean to determine the sequence of the nitrogen bases. The ITS regions were easily amplified with universal primers that are compatible among most fungal species. It has shown sufficient genetic variability for identification at interspecies level and has been adopted as the official standard barcoding region for fungi (Schoch *et al.*, 2012). These sequences were compared with the available information on these genes in the NCBI through the BLAST nucleotide search to identify the isolates. The results of the identification of fungal isolation were concordant with the DNA sequences at 98 % of *S. cerevisiae* sequences available in the NCBI database (Gen Bank).

Degradation of acetamiprid by *S. cerevisiae* YF in medium and wheat dough

Results showed that the dissipation efficiency of acetamiprid by *S. cerevisiae* YF were 97.4%, 97.3% and 97.5% while the control was 1.4% as shown in Figure 3. The cytochrome p 450 enzyme in fungi plays a major role in the biodegradation of acetamiprid *via* N-demethylation of acetamiprid by *Phanerochaete sordida* YK-624 (Wang *et al.,* 2012), cytochromes P450 enzymes in the fungi are responsible for detoxification of environmental pollutants (Črešnar and Petrič, 2011). Studies showed that numerous microorganisms are involved in the degradation of acetamiprid such as *Micrococcus luteus* strain SC 1204 having a maximum consumption of 69.84% of ACE in 24 h (Kanjilal *et al.*, 2015), *Ensifer meliloti* CGMCC 7333 degraded 65.1% of acetamiprid in 96 h Zhou *et al.* (2014), few or rare of study on the use of *S. cerevisiae* in dissipate of acetamiprid while it found several studies previously that dissipate various pesticides by *S. cerevisiae*, which reported that dissipation rate was obtained as 96% of diazinon concentration of 2.5 mg/L with 3.88% of *S. cerevisiae* (Ehrampoush *et al.*, 2017). However, it found in the study 5% concentration of *S. cerevisiae* can significantly biodegrade the DINOCAP and DNOC pesticides. (Zaharia *et al*., 2013).

Results presented in Table 3 indicated that biodegradation of acetamiprid during wheat fermentation by *S. cerevisiae* YF, showed that there was dissipation of 58.5%, 79.2%, 88.3% and 79.2% respectively. In a case contaminated by flour with a concentration of 0.82 ppm of acetamiprid, it cannot be degraded as a whole or within the MRL during fermentation in one hour, while in a concentration 0.61, 0.17 and 0.14 ppm degrade within the MRL, the time of fermentation was according to commercial or household practices as closely as possible. The reduced level will further lower the risk of consuming bread making from acetamiprid contaminated flour (wheat). Reported that degrade of herbicide glyphosphate was approximately 21% within 1 h during the fermentation stage of bread making (Low *et al*., 2005). Đorđević and Đurović-Pejčev (2015) mentioned that there had seen activity of S. *cerevisiae* to dissipate chloropyrifos methyl residues reduction in wheat sample for approximately 14-19%.

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