Effect of baker's yeast separation effluent on barley (*Hordeum vulgare*) germination and growth.

Nadhem Aissani[1]*, Anouar Abidi[2], Souhail Menjli[3], Hichem Sebai[1]

[1]Laboratory of Functional Physiology and Valorization of Bioresources, High Institute of Biotechnology of Beja, University of Jendouba, Beja, Tunisia

[2]Laboratory of Physiology, Faculty of Medicine of Tunis, University of Tunis al Manar, 15, djebel lakhdar la rabta 1007, Tunis, Tunisia

[3]Rayen Food Industries, Ben bachir 8111-Jendouba, Tunisia

Abstract

Industrial waste; whatever the form in which it is released, still present as environmental dangers for the nature and survival of all living beings. Among these toxic products, the focus has been on liquid effluents from the baker's levy industry that cause real environmental problems throughout the Mediterranean region and precisely in Tunisia. In order to minimize these hazards and to take advantage of these wastes for the sake of our environment, the present work consists of valuing the effects of these effluents on the germination and growth of one of the main cereals our country which is barley. This has been put into play, using different doses of effluent solutions with or without dilution.

The results showed that this waste is characterized by its richness in organic matter, and the presence of proteins traces. On the other hand, the microbiological analysis shows high rates of microbial load of coliforms and total germs.

Furthermore, no phytotoxic effect of this effluent has been demonstrated, especially at a volume of 5 ml of diluted and filtered solution, the latter has triggered better germination and growth of the plant almost similar to that of the control.

Keywords: Liquid effluents, Baker's levy industry, Barley, Fertigation practice.

Accepted on December 10, 2018

Introduction

Pollution of the environment is a daily danger to human, animal and plant health, with local, regional and global implications. Pollution has adverse effects on the earth, water and its biotic and abiotic components. Industrial effluents are normally considered as the main industrial pollutants containing organic and inorganic compounds. Among these effluents, liquid waste, which is the least valued in Tunisia, these are directly rejected in nature and endanger its ecological balance.

Like most companies in the agri-food sector, the baker's yeast production industries have problems managing their effluents, especially liquids, and continually add large amounts of wastewater containing high levels of nutrients, heavy metals and hazardous substances to cropland [1,2].

These effluents not only increase the level of nutrients, but also the excessive tolerance limits and in most cases cause toxicity [3,4]. The various metallic and non-metallic elements act as nutrients, but at high concentrations, they have toxic effects on seed germination and seedling growth, ultimately affecting plant growth and yield. On the other hand, various metals/non-metals may not be toxic to the plant, but the combination of these with other products may cause toxic effects [5,6]. In the same context, some agrifood factories specializing in the baker's yeast industry have turned to the valorization of the liquid separation effluent towards the favoring of germination and the growth of plants [7]. On the other hand, they propose to provide farmers with a secondary product of the industry that can improve plant development and preserving the environment.

The aim of this study was to investigate the effects of the liquid effluent of the baker's yeast (*Saccharomyces cerevisia*) on the germination and growth of barley species *Hordeum vulgare*, and subsequently the improvement of field crops, characteristics of North-West Tunisia

Material and Methods

Liquid effluents characterization

The liquid effluents used throughout this work were collected from Rayen Food Industries, a plant specializing in the baker's yeast industry, located in the north-west of Tunisia (Ben Bachir-Bousalem).

Samples were taken at different times of fermentation and were stored under adequate conditions to ensure better stability.

The pH was measured using a pH meter (INOLAB) according to the potentiometric method. Electrical conductivity and salinity were measured using a MEAS / Cond 8 conductivity meter.

Determination of the dry matter was carried out by adding 5 g of liquid effluent to 20 g of dry sand. The whole is dried for 2 h in the oven at 105°C. Total nitrogen was determined by the Kjeldahl method. Total phosphorus was measured calorimetrically. Ca, Mg, Na, K were determined by atomic absorption (Fisher Scientific ice 3000) [8].

Bacterial enumeration of the liquid effluent

Dilution enumeration: This test consists on carrying out successive dilutions of liquid effluent on water at 10%. After dilution, the culture medium specific for the desired germ is added. Following cooling, the dishes are placed in a conditioned incubator (culture medium, temperature, incubation time) for different examined germs (Table 1). After the incubation, bacterial colonies and sometimes molds on the culture media were observed.

Enumeration by filtration: This method is achieved by the use of membrane filtration (Newlystar) devices. 100 ml of the effluent are filtered, and then the membrane is deposited on the specific solid culture medium for each germ (Table 1).

Table 1. Classification of germs according to culture medium, temperature and incubation time

Germs	Culture Medium	Incubation’s Temperature and time
Total germs (AFNOR derived, ISO4833 standard)	GNG (PCA or Agar): Nutritive Agar, Plat Count Agar	30°C for 48 h
Fecal Coliforms (Derived from ISO4832)	VRBA: Violet Red Bile Agar	30°C for 24 to 48 h
Total Coliforms (Derived from ISO4832)	VRBA	44°C for 24 to 48 h
Escherichia coli (Derived from AFNOR SDP : 07/1-07/93)	MBE : Methylene Blue Eosin Agar	37°C for 24 to 48 h
Lactic Bateria (NT 51.25 (2015)	MRS : Man Rogosa and Sharpe	30°C for 48 h
Wild yeasts and molds (NT 51.25 (2015))	Lysine	30°C for 72 h

Phytotoxicity test

Phytotoxicity was measured using Zucconi test by measuring seed germination [9]. 10 barley seeds were placed on a screen in glass Petri dishes with dimensions of 110 mm × 20 mm. These seeds were irrigated with 1.25 ml/g soil of the effluent solutions with or without a dilution to 10% then was capped and kept in a dark incubator at 25°C
temperature for 5 days. A germination index (GI) was calculated by counting the grown seeds and determining the average sum of seeds root elongation in each tested sample. The germination test is carried out on 4 types of effluents (E1, E2, E3 and E4) before and after filtration. The results were finally expressed as a percentage.

Fertigation practice

The main objective of this experiment is to test the effects of liquid effluent on plant growth and to optimize its beneficial concentrations for this species. This essay was conducted in accordance with the natural climatic conditions favourable to the growth of barley. Indeed, all the pots were placed in a greenhouse designed as a growth chamber programmed for a photoperiod of 12 h day 712 night, with a photosynthetic photon flux density of 300 µmol m-2 s^{-1}; temperature, 24 ± 1/18 ± 1°C day / night; and relative humidity, 60/70 ± 3%. The test was carried out in a polystyrene honeycomb plate filled with soil. The seeds are carefully irrigated with 10 ml of water or liquid effluent.

The monitoring of plant growth for 20 days (total plant length and leaves number) allows us to study the possibility of upgrading the liquid effluent.

Result

Microbiological characteristics of liquid effluents

The results showed that all samples are characterized by high microbial load. The microbiological study after filtration confirmed the result of these obtained with dilution.

Physicochemical characteristics

The pollutant effect of the latter resulted in a slightly acidic pH which extends from 4 to 6.99 and a high dry matter content of 5 to 8%. This effluent is characterized by the absence of alcohol which could not therefore be at the origin of a possible toxicity.
The mineral composition of this effluent shows a significant composition of water moisture
(90%) coupled with a large amount of phosphate (6 to 7.44%) and conversely low doses of protein (0.96%) and nitrogen (0.15%).

Phytotoxicity test

The obtained results showed that the germination index for the diluted effluent is more important than the water-treated control. This parameter depends essentially on its concentration, only the low doses have allowed an improvement in germination. This germination index reaches a maximum at the 3rd dilution (10^{-3}) and then gradually decreases.

These results are in agreement with those found by Lan et al. and Perveen et al. [10-11]. We also noted that the germination index is higher after filtration of the effluent (Figure 1).

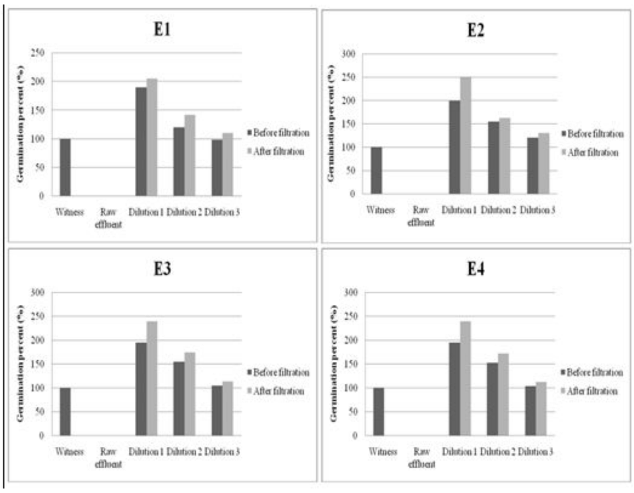

Figure 1. Evolution of the germination index (%) according to different dilutions of effluents (E1, E2, E3 and E4)

These results confirm those obtained by Batako et al. and Rehman et al. [12-13] which demonstrate the inhibitory effects of bacteria and their toxins on the germination of rice and sesame seeds.

Fertigation practice

The obtained results confirm that irrigation with suspended effluent is a good accelerator of plant growth. Indeed, there is an improvement of increasing average lengths of stems compared to the control, this growth in length reaches its maximum for plants irrigated by the dose set at 1.25 ml/g soil, the latter is of the order of 55 cm. Nevertheless, beyond this volume's irrigation, it causes an antagonistic effect resulting in a decrease of this length and confirming, the toxicity of the effluent for large volumes (> 5 ml) (Figures 2 and 3).

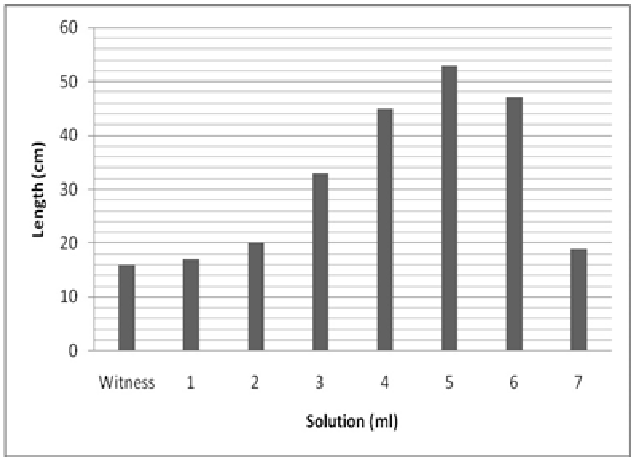

Figure 2. Average stems length of barley plants after 20 days of irrigation by different solutions

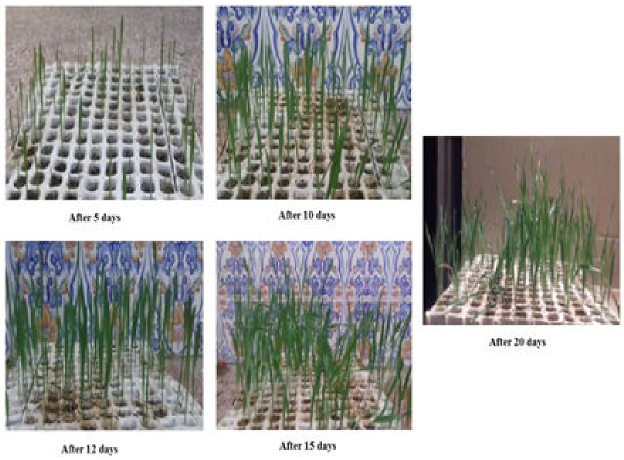

Figure 3. Barley plants Growth during the twenty days of irrigation

Discussion

Microbiological characteristics of liquid effluents

The microbiological characteristics depend on the raw material (molasses) and the manufacturing process (fermentation, separation). The comparison between the different studied samples was grouped in Table 2.

Table 2. Microbiological characteristics of the liquid effluent GT: Total sprouts, CT: Total coliforms, CF: Fecal coliforms, BL: Lactic Bacteria, LS: Wild yeasts, M: Molds

	GT (CFU/mL)	CT (CFU/mL)	CF (CFU/mL)	*E. coli* (CFU/mL)	BL (CFU/mL)	LS and M (CFU/mL)
E1	$6.1\ 10^4$	<10 104	<10 104	<10 104	2.4 104	<10 104
E2	106	10 104	10 104	<10 104	3 104	104
E3	10 104	106	<10 104	<10 104	10 104	<10 104
E4	<10 104	<10 104	<10 104	10 104	10 104	10 104

Physicochemical characteristics

Table 3 summarizes the physicochemical properties of the liquid effluent baker's yeast. The physicochemical characteristics of this effluent before and after filtration are variable and are similar to each other; they depend on the raw material (molasses) and the manufacturing process (fermentation and separation).

Table 3. *Physicochemical characteristics of the liquid effluent before and after filtration*

Before filtration	Before filtration				After filtration			
	E1	E2	E3	E4	E1	E2	E3	E4
pH	5.03	4.73	6.99	6.08	4.73	4.5	7.2	5.9
Conductivity (ms\cm)	21.3	23.7	21.5	23.2	23.9	19.23	19.52	22
Salinity (g\l)	15.1	17.1	15.3	16.5	16.9	13.5	13.7	15.6
Protein (%)	0.96	0.7	0.82	0.84	0.96	0.7	0.8	0.87
Nitrogen (%)	0.15	0.112	0.13	0.134	0.15	0.11	0.12	0.13
Phosphate (%)	7.44	6.99	6.03	7.1	7.22	5.98	6.01	6.97
Alcohol Test (%)	0	0	0	0	0	0	0	0
Water (%)	92.71	93.05	94.13	92.73	91.89	92.57	93.79	91.43
Boux (g\l)	9	9.4	7.3	10.2	-	-	-	-
Dry matter (%)	7.29	6.95	5.87	7.27	-	-	-	-

Phytotoxicity test

The determination of the germination index of the barley grains during 5 days of treatment with the effluent, before and after filtration, approves the phytotoxic properties of the raw effluent since it inhibits the germination of the grains.

This difference can be explained by the richness of the yeast effluent in nutritional elements that stimulate germination on the one hand and the inhibition that can be exerted by bacteria and their metabolites, on the other hand.

Fertigation practice

Plant growth was monitored during the experimental period by measuring the variation in length. From the 7th day of growth monitoring, we observe the appearance of white layers of mold on the surface of the soil and this for solutions of 7 ml and more. This involves a beginning of contamination of the soil and seeds, confirming the growth and migration of the bacteria that appear in the effluent before flaking, in the soil.

From the 12th day, and in accordance with the results noted on the 7th day, we note the death of the 80% of the plants irrigated by 7 and 8 ml per day either by the contamination or by the excess dose of the effluent.

From the fertigation experiment we deduce that the yeast effluent should be used filtered and diluted at the germination stage. These results are consistent with those reported by Al-Dulaimi et al. in their review of the use of urban wastewater for growth and germination stimulation of maize, rice and sorghum [14]. In addition, this product is a moisture conservator; it participates in maintaining a better soil stability and makes us blessed with a new additional source of water that improved agricultural irrigation in our country characterized by its limited water sources [15] staining.

Conclusion

Industrial production of baker's yeasts generates a significant release of liquid waste which ranks among the main environmental hazards in the whole Mediterranean region and not only in Tunisia. These dangers have been aggravated especially with mismanagement and have been causing release of bad smells and thus threatening the contamination of the entire environment.

Our study focused on valorizing these liquid effluents in agriculture, whose main objective was to provide farmers with an efficient biological product. This product has shown no phytotoxic effects and could contribute to an improvement of the diet and indirectly a better preservation of the environment.

In the same context, the use of liquid effluent for fertigation contributes to an increase in yield and a reduction of the costs of the crop.

The physicochemical analysis of this waste shows its richness in organic matter, and the presence of traces of proteins. On the other hand, the microbiological analysis shows high rates of microbial load of coliforms and total germs.

All these results lead us to valorize this effluent in the fertilization of the soil and the stimulation of germination of the plants. Concerning barley grains fertigation, the best volume solution of liquid effluent was fixed at 5 ml and gave rise to a maximum germination rate coupled with optimal growth.

In conclusion, the valorization of liquid waste of baker's yeast in the fertigation of soils can be considered as a promising alternative for organic farming, essentially when using solutions of low doses of effluents. However, this alternative requires more in-depth studies on soil analysis and identification of the active effluent molecules that have been behind these encouraging results.

Acknowledgements

The High Institute of Biotechnology of Beja (Beja,Tunisia) is funded by the Tunisian Ministry of Higher Education (Tunis, Tunisia) and Rayen Food Industries (Jendouba-Tunisia), financed this work. The authors are grateful to all persons who helped to conduct this study.

Conflicts of Interest

All authors declare that there are no conflicts of interest.

References

1. Bielen A, Šimatović A, Kosić-Vukšić J, et al. Negative environmental impacts of antibiotic-contaminated effluents from pharmaceutical industries Water research. Water Res. (2017); 126:79-87.
2. Stanbury PF, Whitaker A, Hall SJ. Principles of Fermentation Technology (Third Edition) (2016).
3. Lyu J, Park J, Kumar Pandey L, et al. Testing the toxicity of metals, phenol, effluents, and receiving waters by root elongation in Lactuca sativa L Ecotoxicology and environmental safety. Ecotoxicol Environ Saf. (2018); 149:225-32.
4. Thomas KV, Barnard N, Collins K, et al. Toxicity characterisation of sediment porewaters collected from UK estuaries using a Tisbe battagliai bioassay. Chemosphere. (2003);53(9):1105-1111.
5. Mahakham W, Theerakulpisut P, Maensiri S, et al. Environmentally benign synthesis of phytochemicals-capped gold nanoparticles as nanopriming agent for promoting maize seed germination. Sci Total Environ. (2016);573:1089-1102.
6. Tolaymat T, Genaidy A, Abdelraheem W, et al. The effects of metallic engineered nanoparticles upon plant systems: An analytic examination of scientific evidence. Sci Total Environ. (2017);579:93-106.
7. Savitha S, Sadhasivam S, Swaminathan K, et al. A prototype of proposed treatment plant for sago factory effluent. Journal of Cleaner Production. (2009); 17:1363-72.
8. Finete Vde L, Gouvea MM, Marques FF, et al. Is it possible to screen for milk or whey protein adulteration with melamine, urea and ammonium sulphate combining Kjeldahl and classical spectrophotometric methods? Food Chem. (2013);141:3649-3655
9. Zucconi F, Pera A, Forte M, et al. Evaluating toxicity of immature compost Biocycle. (1981);22(2):54-7.
10. Lan W, Wang W, Yu Z, et al. Enhanced germination of barley (Hordeum vulgare L.) using chitooligosaccharide as an elicitor in seed priming to improve malt quality. Biotechnol Lett. (2016);38(11):1935-40.
11. Abida Perveen, Iftikhar Imam Naqvi, Rehana Shah, et al. Comparative Germination of Barley Seeds (Hordeum Vulgare) Soaked in Alkaline Media and Effects on Starch and Soluble Proteins. J Appl Sci Environ Manage. (2008); 12(3):5-9.
12. Batoko H, Bouharmont J, H M. Inhibition of rice (Oriza sativa. L) seedling elongation by a Pseudomonas fuscovaginae toxin. Euphytica. (1994); 76:139-43.
13. Rehman, F Deeba, M. I. ul Haque, et al. Inhibition of sesame seedling growth by xanthomonas campestris pv. sesami culture secretions. J Anim Plan. (2013); 23:1207-10
14. Rana ID, Norli BI, Ibrahim MH. The effect of industrial wastewater in seed Growth rate. J Scientific Research. (2012);2(3).
15. Chouchane H, Hoekstra AY, Krol MS, et al. The water footprint of Tunisia from an economic perspective. Ecological Indicators. Sustainability. (2015);52:311-319
16. Ra JS, Jeong TY, Lee SH, et al. Application of toxicity identification evaluation procedure to toxic industrial effluent in South Korea. Chemosphere. (2016);143:71-7.
17. A Review International Journal of Scientific and Research Publications 2:2250-3153.
18. Chapter 11: Effluent treatment Butterworth Heinemann: 687-723.

*Correspondence to:

Dr. Nadhem Aissani

Laboratory of Functional Physiology and Valorization of Bioresources,

High Institute of Biotechnology of Beja, University of Jendouba, Beja, Tunisia

Mobile: +21658617557

E-mail: aissaninadhem@gmail.com