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Baker's Yeast Separation Effluent Effect on Pea (Pisum Sativum) Germination and Growth

Anouar ABIDI[1], Nadhem AISSANI*[2], Souhail MENJLI[3], Hichem SEBAI[2]

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

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

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

Abstract

Like most industrial companies, the baker's yeast production industries find problems in the management of their effluents. These effluents pose serious environmental nuisances that are mainly due to their pollutant load and the release of unpleasant odors.

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 yeast industrial liquid effluent effects on pea (*Pisum sativum*) germination and growth, one of the main agricultural products of Tunisian north-west.

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, Pea, Fertigation Practice

Corresponding author: Dr. Nadhem AISSANI
Laboratory of Functional Physiology and Valorization of Bioresources, High Institute of Biotechnology of Beja, University of Jendouba, Beja, Tunisia. Tel: +21658617557 **Email:** aissaninadhem@gmail.com

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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 (Bielen et al. 2017; Stanbury et al. 2017).

These effluents not only increase the level of nutrients, but also the excessive tolerance limits and in most cases cause toxicity (Lyu et al. 2018; Ra et al. 2016; Thomas et al. 2003). The various metallic and nonmetallic 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 (Mahakham et al. 2016; Tolaymat et al. 2017). 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 (Savitha et al. 2009). 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 pea species Pisum sativum, and subsequently the improvement of this agricultural in Tunisia North-West.

Material and methods

Liquid effluents physiochemical characterization

Liquid effluents used throughout 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 hours 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) (Finete Vde et al. 2013).

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 2).

Germs	Culture Medium	Iincubation'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 48h
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

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

	GT (CFU/mL)	CT (CFU/mL)	CF (CFU/mL)	E .coli (CFU/mL)	BL (CFU/mL)	LS and M (CFU/mL)
E1	(CFU/mL)	(CFU/mL)	(CFU/mL)	(CFU/mL)	(CFU/mL)	(CFU/mL)
E2	106	10 104	10 104	<10104	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

Table 2: Microbiological characteristics of the liquid effluent

In vitro germination test

Germination test was assessed using Zucconi test by measuring seed germination (Zucconi F et al. 1981). 10 pea seeds were placed on a screen in a glass Petri dishes with dimensions of 110 mm × 20 mm. 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 5 types of effluents (S1, S2, S3, S4 and S5) corresponding respectively to 10, 10-1, 10-2, 10-3, 10-4 and 10-5 (g/l) of dilution of the effluent number 1 (E1). The results were finally expressed as a percentage.

The germination index was determined by the following formula:

$$G\ (\%) = \frac{N_E}{N_T} \times \frac{L_E}{L_T} \times 100$$

N_E: number of germinated grains watered by the sample of the diluted effluent
N_T: number of germinated grains in the control irrigated by water
L_E: average length of the radicle of germinated grains for the sample
L_T: average length of the radicle of germinated grains for the control

Fertigation practice

The main objective of this experiment is to test the effects of liquid effluent on plant growth, number of leaves and branches and to optimize its beneficial concentrations for this spice. This essay was conducted in accordance with the natural climatic conditions favorable to the growth of pea. 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.

Plants were irrigated with 1, 2, 3, 4, 5, 6, 7 and 8 ml of effluent/g soil called respectively S1, S2, S3, S4, S5 S6 S7 and S8.

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

Results and 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. 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

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, separation). 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 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%).

	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	-	-	-	-

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

In vitro germination test

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

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 (Figure 1).

These results are in agreement with those found by Lan et al and Perveen et al (Abida Perveen et al. 2008; Lan et al. 2016). This can be explained, on the one hand by the richness of the yeast effluent in nutritional elements that stimulate germination and on the other hand by the inhibition that can be exerted by bacteria and their metabolites

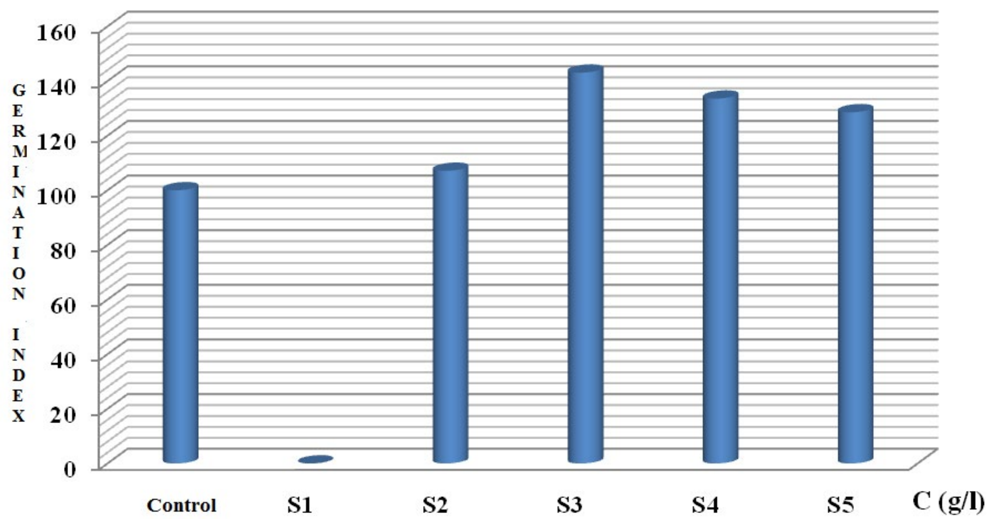

Figure 1: Evolution of the germination index (%) according to different dilutions of effluents (S1 to S5)

Fertigation practice

Plant growth was monitored during the experimental period by measuring the variation in length, number of leaves and branches. 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 5 ml/g soil, the latter is of the order of 46 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 (> 5ml) (Figure 2).

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 7ml 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.

The enumeration of the leaves is carried out after twenty days of irrigation with the different concentrations is illustrated in (Figure 3). Indeed, there is an increasing improvement of the average number of leaves compared to the control, it reaches its maximum for the plants irrigated by the S4 solution of the order of 26 leaves per stem, this value decreases slightly for the strongest low concentrations. The evolution of the number of leaves is generally related to the increase in the number of ramifications of the stems. Indeed, there is an increasing trend in the number of stems which reaches its maximum for plants irrigated by the S4 solution with 8 ramifications (Figure 3).

The use of yeast effluent as a source of irrigation improves soil properties and maintains moisture (water use gains), stimulates germination, and improves plant growth (Figure 4). These results confirm those found by Sharma et al. in 2011 on the use of the paper industry's effluent promote the germination and growth of the pea and also those found by Vishnoi et al. in 2013 on the stimulation of growth of the same plant by leachates.

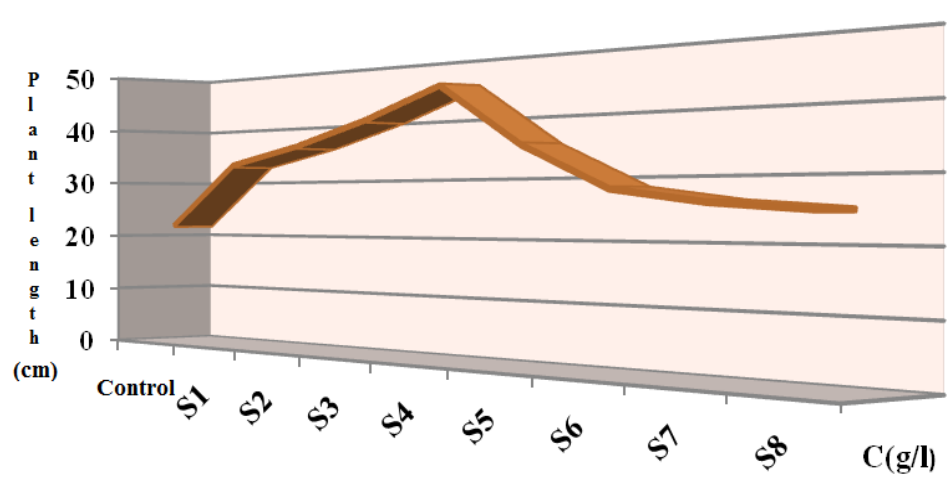

Figure 2: Evolution of plant length according to different dilutions of effluents (S1 to S8)

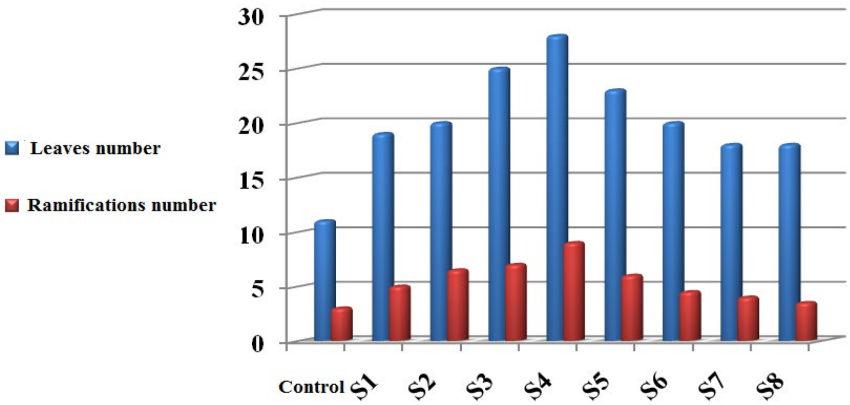

Figure 3: Evolution of leaves and ramifications number according to different dilutions of effluents (S1 to S8)

After 8 days

After 10 days

After 12 days

After 15 days

After 20 days

Figure 4: Pea plants Growth during the twenty days of irrigation

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 pea grains fertigation, the best volume solution of liquid effluent was fixed at 5 ml/g of soil and gave rise to a maximum germination rate coupled with optimal growth.

In conclusion, the valorization of liquid waste of yeast in the irrigation of agricultural plants and the fertilization of soils can be regarded as a promising alternative tool for the biological culture, essentially when using solutions of low doses.

Conflicts of interest

All authors declare that there are no conflicts of interest.

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