EFFECT OF EARTHWORM'S EXTRACTS (*APORRECTODEA MOLLERI*) ON YEAST GROWTH: *IN VITRO* STUDY

S. Houida Researcher | L. Yakkou Researcher | M. Raouane Prof. | A. EL Harti Prof. | S. Amghar* Prof.

Research Team : "Lumbricidae, Improving Soil Productivity and Environment" (LAPSE), Research Centre « Eau, Ressources Naturelles, Environnement et Développement Durable (CERNE2D), Ecole Normale Supérieure (E.N.S.) Mohammed V University, Rabat, Morocco.

*Corresponding author: Dr. Prof. Souad AMGHAR. email: eamghar@gmail.com. GSM: +212661769456.

ABSTRACT

This study was aimed to investigate the effect of earthworms after their decomposition on yeast growth. To achieve this objective, *in vitro* crude soluble extracts were prepared from two earthworms lots: freshly harvested from the field "FHE" and the other previously starved for one week "SE". The dry residues of these two crude extracts were used at different concentrations as a solid media for the growth of two yeast species: one "*Candida tropicalis*" got from another laboratory and the other was isolated by our team from earthworms' casts "*Filobasidium uniguttulatum*", and their growth was evaluated by colonies counting. The results obtained, compared with the Sabouraud Dextrose Agar (SDA), showed that the growth of both yeast isolates was significantly higher on mediums prepared using exclusively earthworms' crude extracts and agar. Optimal growth was obtained at a concentration corresponding to 0.375g/100 mL of earthworms' soluble matter of the two types of earthworms' lots. These results affirmed the richness and the diversification of those extracts, in nutrients and growth factors, that comes mainly from the intrinsic composition of earthworm's body biomass. The efficiency of the FHE and SE extracts for cultivation of yeast shows their possible use as a culture media that may be applied to other soil microorganisms and even *in vivo* as soil amendments or biofertilizers.

Keywords: crude extracts – earthworms' decomposition – yeast growth – culture media.

تأثير مستخلصات دودة الأرض (*APORRECTODEA MOLLERI*) على نمو الخميرة: دراسة في المختبر

صوفيا حويدة الباحثة | لمياء ايكو باحثة | محمد روان استاذ | عبد اللطيف الحارتي استاذ | سعاد امغار* استاذ

فريق البحث: " الخرطونيات، تحسين إنتاجية التربة والبيئة"

المدرسة العليا للأساتذة، جامعة محمد الخامس، الرباط، المغرب.

المستخلص

يهدف هذا البحث إلى دراسة تأثير مستخلصات ديدان الأرض بعد تحللها على نمو الخميرة. ولتحقيق هذا الهدف، تم إعداد مستخلصات خامة قابلة للذوبان *في المختبر* من دود الأرض. تم تقسيمهم الى مجموعتين: واحدة تم حصادها حديثا من حقل "FHE" والأخرى تم تجويعها من قبل لمدة أسبوع واحد بهدف افراغ محتوى انبوبهم الهضمي "SE". استخدمت المستخلصات المحصل عليها من كلا المجموعتين في تركيزات مختلفة كوسائط مغديه لنمو نوعين من الخميرة: أحدهما حصلت عليها من مختبر آخر" *Candida tropicalis*" والآخرى تم عزلها من قبل فريقنا من محيط ديدان الأرض *Filobasidium uniguttulatum*". تم تقييم النمو عبر حساب المستعمرات الخميرية. أظهرت النتائج التي تم الحصول عليها، مقارنة مع Sabouraud Dextrose Agar (SDA)، أن نمو كل من الخميرتين كان أعلى بكثير على الوسائط المعدة باستخدام المستخلصات الخامة لكلى المجموعتين من ديدان الارض. تم الحصول على النمو الأمثل بتركيز يعادل 375.1000g/ مل من مستخلصات ديدان الأرض وذلك في كلى المجموعتين. أكدت هذه النتائج ثراء وتنوع هاته المستخلصات، بالمغذيات وعوامل النمو، التي تأتي أساسا من الكتلة الحيوية لدود الأرض. كفاءة المستخلصات FHE وSE في نمو الخميرة يظهر امكانية استخدامها كوسائط النمو لفطر الخميرة، كما يمكن استعمالها في نمو كائنات حية دقيقة أخرى متواجدة بالتربة، زيادة على امكانية استعمالهم كأسمدة الحيوية للتربة.

الكلمات المفتاحية: مستخلصات خامة، تحلل ديدان الأرض، نمو الخميرة، وسائط النمو.

Received:18/12/2020, Accepted:22/2/2021

INTRODUCTION

Earthworms constitute a very important mass of soil fauna, their activity plays a key role in the decomposition of organic matter, the renewal of nutrients and the soil structuring (5, 10). In addition, they are the main boosters of the formation of soil microbial communities, as yeasts, bacteria, and fungi. However, compared to bacteria and mycorrhizal fungi, the potential of using yeasts as plant growth-promoting agents (PGP) and as soil amendments has been misapplied for a long time. Recently, researches had proved their important roles in the transformation of nutrients, the maintenance of soil structure (4), as plant growth-promoting agents (PGP) (2, 8, 16, 17), biocontrol agents (11) and biodegradation agents of toxic benzamine in the environment (20). Besides studies on the movement of N from dead earthworm tissues toward microbial biomass and plants (7, 22), few studies have been interested in the postmortem effect of earthworms on the soil microflora, particularly on yeasts. The aim of our study is to demonstrate experimentally the effect of crude earthworms extracts on yeasts growth. To achieve this objective, we have prepared crude extracts from earthworms by maceration in water from two different lots of worms: freshly harvested earthworms (FHE) and Starved for one-week earthworms (SE). These extracts were tested on the growth of two yeast species (*Candida tropicalis* and *Filobasidium uniguttulatum*) that belong to the Plant Growth Promoters groups. The growth of yeasts were evaluated in solid media. Otherwise, the effect of the two extracts of FHE and SE will be compared between them, in order to verify the possible effect of the gut contents of earthworms on *in vitro* yeasts growth. Finally, evaluate the effectiveness of the worm extracts, by the relative concentration and the relative growth compared to the control media.

MATERIALS AND METHODS

Biological materials

Earthworms: Earthworm samples were collected near the Moulay Abdeallah water dam in the region of Akrach – Rabat (latitude: 33.97947700, longitude -6.82635500), Morocco. They have been identified by Dr. Jorge Dominguez at "Grupo de Ecologia Animal (GEA)" in Vigo University – Spain. The molecular identification of the earthworms based on cytochrome c oxidase subunit I (COX1) gene revealed that the earthworm's specimens belong to *Aporrectodea molleri* specie. The sequence was submitted to the NCBİ Genbank and it was given the accession number MT878074.1. The earthworms were separated into two lots: Freshly Harvested Earthworms (FHE) and Starved Earthworms (SE) which devoid of soil and food for a week.

Yeasts

Candida tropicalis: comes from the collection of clinical strains from the Higher Institute of Nursing Professions and Health Techniques (ISPITS) in Rabat-Morocco.

Filobasidium uniguttulatum: was isolated, from the soil where earthworms were harvested, in our laboratory: *Lumbricidae*, Improvement of Soil Productivity and Environment (LAPSE) at ENS—Rabat (Morocco). Then, identification was done by Internal Transcribed Spacer Sequencing, at the National Center for Scientific and Technical Research (CNRST) in Rabat. The sequence was therefore submitted to the NCBİ Genbank and had the following accession number MW369490.

Methods

Preparation of crude extracts from earthworms : The lots of FHE and SE (100 g of wet biomass) were immersed in concentrated methanol and placed directly in the oven at 70°C for 3 days. After drying, the worms were ground using a mortar until a powder is obtained, weighed to determine the dry biomass of the earthworms (FHE_{db} and SE_{db}). Extracts preparation was carried out in an oven (70°C), by maceration in successive baths of hot distilled water. The macerates obtained were filtered progressively on glass cotton, then through Wattman paper. The resulted filtrate was evaporated in an oven (70°C) until the obtention of a powder, weighed to determine the crude extract (FHE_{ce} or SE_{ce}). It is from this extract, consisting mainly of water-soluble matter, that solid culture media will be prepared at different concentrations by successive dilutions. FHE_{ce} and SE_{ce} are stored at -6°C until use in the experiments.

Preparation of culture media

Culture medium: Sabouraud Dextrose Agar (HIMEDIA) contains 4 g/100mL of Dextrose, 1 g/100mL of Peptone, and 1.5 g / 100 mL of agar (Biokar) that was used as control. The extract of each earworms lots (6 g, FHE or SE) is suspended in 200 mL of distilled water (stock solution) and the 6 dilutions were prepared. Worm extracts (FHE and SE) were used as unique source of nutrients at different concentrations (7 in total) by successive ½ dilutions. The pH was adjusted to 5.6 ± 0.2 units then 1.5 g / 100 mL of agar was added, and the solid mediums were autoclaved at 121°C for 15 min.

Yeast culture and growth evaluation

Inoculum preparation: The inoculum of each yeast's types (*Candida tropicalis, Filobasidium uniguttulatum*) was prepared in order to obtain a suspension giving on the solid medium a countable colonies number between 30 and 300.

Evaluation of yeast growth: On solid medium prepared exclusively by FHE_{ce} or SE_{ce} and the control media, 0.1mL of each yeast inoculum was put into media using the spread plate method (triplicate), and incubated for 72 h at 30°C and 26°C for *Candida tropicalis* and *Filobasidium uniguttulatum*, respectively. The growth of yeasts was evaluated by counting the colonies number developed on medium surface.

Methods for evaluating the efficiency of earthworms' extracts

The effectiveness of FHE and SE extracts on the growth of both yeast types tested by two indexes:

Relative concentration (RC): the ratio percentage of dry matter content for FHE_{ce} or SE_{ce} concentration and nutrients content (dextrose, peptone) in SDA control media.

$RG = C_{FHE}$ or $C_{SE}/ C_{SDA} \times 100$

Relative Growth (RG): the percentage of the growth of yeasts (colonies number per Petri dish) in FHE_{ce} or FE_{ce} (G_{FHE} or G_{FE}) compared to the growth in the conventional media SDA (G_{SDA}).

$RG = G_{FHE}$ or $G_{SE}/ G_{SDA} \times 100$

The Relative Concentration as well as the Relative Growth on the control medium SDA were considered as 100%. Thereby, FHE_{ce} or FE_{ce} are considered more effective than the conventional medium, when simultaneously the value of RC is less than or equal to 100% and the value of RG is greater than or equal to 100%.

Statistical analysis and data treatment

The results were the subject of the variance analysis (ANOVA). The comparisons between the values obtained for each type of media were made by a unidirectional analysis of variance, followed by the Tukey multiple test (IBM statistics SPSS 22). The differences are considered significant for $p < 0.05$.

RESULTS AND DISCUSSION

Soluble matter yield from the two varieties of earthworms: FHE and SE: The dry weights of the different lots (FHE_{dw} and SE_{dw}), as well as those of the soluble matter contained in the FHE_{ce} and SE_{ce}, are showed in Table 1. The FHE gut contents are evaluated by the difference of the dry weight between the lots FHE_{dw} and SE_{dw}. This quantity, equal to 6g, represented 24% of the FHE lot dry weight. The dry weight of the crude extracts $FHE_{dw\text{-}ce}$ and $SE_{dw\text{-}ce}$ is respectively equal to 11g (57.89% of FHE_{db}-6g) and 13g (68.42% of SE_{db}). This slightly higher percentage of soluble matter for FE lot is likely due to the condition of earthworm after the fasting period. Our observations showed that the worms, which have undergone the fasting period, become more flaccid and less turgid, which probably indicates a loss of intracorporeal water.

Table 1. Dry biomass and crude extract amounts obtained from two lots of earthworms starting by 100g of wet biomass

Earthworms	Biomass		Crude extract
	Wet	Dry	
FHE	100	25	11
SE	100	19	13

Yeast growth evaluation

The culture results of the two yeast species *Candida tropicalis* and *Filobasidium uniguttulatum*, on media prepared only by earthworms' extracts (FHE and FE), showed that yeasts were able to grow and assimilate these extracts as unique source of nutrients.

Crude extract of freshly harvested earthworms (FHE): The results of the effect of FHE_{ce} at different concentrations (C_n) on the growth of *Candida tropicalis* and *Filobasidium uniguttulatum* are shown in

Table 2. For the yeast *Candida* tropicalis**,** all the concentrations of FHE crude extracts allowed its growth with an average number of colonies formed per Petri dish, significantly similar at the concentrations C_1, C_2, C_6 and C_7, and much higher at C_3, C_4 and C_5 compared with the number obtained in the control SDA media allowed the development of 228.33 ± 1.45 colonies ($p < 0.05$). The optimal efficiency was obtained at the concentration C_4 (0.375g/100 mL of crude extracts), with 283.00 ± 2.08 colonies per Petri dish, a relative concentration compared to that of SDA equal to only 7.5% and a relative growth of 124.12%. On the other hand, *Filobasidium uniguttulatum* couldn't grow at the first concentration C_1 of FHE extract and the growth on lowest concentrations C_6 and C_7 was significantly inferior. The optimal growth was obtained as well as *Candida tropicalis* at C_4, with a value of 230.33 ± 1.86 colonies per Petri dish, and which was the only concentration giving a number of colonies significantly higher from that of SDA (201.33 ± 1.76). The optimal efficiency was recorded at C_4 (0.37g/100 mL of FHE extract) corresponding to a RG of 114.4% and a RC of only 7.5%.

Table 2. Colony forming units (CFU) of *Candida tropicalis* and *Filobasidium uniguttulatum* in mediums based on different concentrations of earthworms' dry matter (FHE_{CE}) compared to the control media SDA. CFU values are expressed as means ± standard errors. Different letters (a, b, c, d) represent significant differences in treatments at P<0.05. RC = Relative Concentration compared to that of SDA (which represent 100%). RG = Relative Growth compared to that on the SDA medium

Yeast strain	Medium (FHE_{CE})	C_1	C_2	C_3	C_4	C_5	C_6	C_7	SDA
Candida tropi	Dry matter (g/100mL)	3	1.5	0.75	0.375	0.19	0.09	0.05	5
	RC %	60	30	15	7.5	3.74	1.875	0.937	100
	CFU	231.00 ± 1.73 [a]	233.00 ± 1.73 [a]	241.67 ± 1.45 [b]	283.00 ± 2.08 [c]	244.33 ± 2.03 [b]	233.33 ± 0.88 [a]	227.00 ± 1.73 [a]	228.33 ± 1.45 [a]
	RG %	101.17	102.05	105.84	124.12	107.00	102.19	99.42	100
Filobasidium uniguttul	CFU	0 ± 0 [a]	202.00 ± 2.31 [c]	201.00 ± 2.08 [c]	230.33 ± 1.86 [d]	211.67 ± 3.28 [c]	182.33 ± 2.40 [b]	188.00 ± 3.06 [b]	201.33 ± 1.76 [c]
	RG %	0	100.33	99.84	114.40	105.14	90.56	93.38	100

Different letters (a, b, c, d) represent significant differences in treatments at P< 0.05.

Crude extract of starved earthworms (SE)

The results of SE_{ce} effect at different concentrations on the growth of *Candida tropicalis* and *Filobasidium uniguttulatum* are presented in Table 3. The ability of the yeast *Candida tropicalis* to grow on media based on starved earthworms' extracts was slightly different as the growth was significantly low on the highest and the lowest concentrations C_1, C_2 and C_7. All the other concentrations gave an average number of colonies formed per Petri dish, significantly much higher (C_3, C_4, C_5 and C_6) than the number obtained in SDA media, which is 228.33 ± 1.45 colonies per dish ($p < 0.05$). In fact, the efficiency zone varied between C_3 = 0.75g/100mL and C_6 = 0.093g/100mL with an optimal efficiency obtained once more at C_4 (0.375g/100 mL of crude extracts), with 274.33 ± 2.96 colonies formed per dish, and a RG of 120, 15%. The isolated yeast *Filobasidium uniguttulatum* only SE extracts at concentrations C_3 and C_4 allowed a growth significantly higher than that obtained on SDA media ($p < 0.05$). Crude extracts' concentrations C_2, C_5 and C_6 showed results comparable to SDA, while for C_1 and C_7, corresponding to the extreme concentrations (3g/100 mL and 0.05g/100 mL), the growth was significantly lower. Thus, the optimal efficiency of FE extract is observed anew at the concentration C_4 = 0.375g/100 mL, the RG was 112.75% compared to the SDA.

Table 3. Colony forming units (CFU) of *Candida tropicalis* and *Filobasidium uniguttulatum* in mediums prepared using different concentrations of Starved Earthworms' dry matter compared to the control media SDA. CFU values are expressed as means ± standard errors. Different letters (a, b, c, d) represent significant differences in treatments at P<0.05. RC = Relative Concentration compared to that of SDA (which represent 100%). RG = Relative Growth compared to control

Yeast strain	Medium (SE_{CE})	C_1	C_2	C_3	C_4	C_5	C_6	C_7	SDA
Filobasidium uniguttulatum	Dry matter (g/100mL)	3	1.5	0.75	0.375	0.19	0.09	0.05	5
	RC %	60	30	15	7.5	3.74	1.875	0.937	100
	CFU	220.67 ± 2.03 ^{b}	228.00 ± 2.08 ^{b}	241.67 ± 3.18 ^{c}	274.33 ± 2.96 ^{d}	270.33 ± 2.96 ^{d}	271.00 ± 1.53 ^{d}	203.00 ± 3.61 ^{a}	228.33 ± 1.45 ^{b}
	RG %	96.64	99.85	105.84	120.15	118.39	118.68	88.91	100
Candida tropicalis	CFU	182.33 ± 1.76 ^{a}	204.33 ± 2.40 ^{b}	226.67 ± 2.08 ^{c}	227.00 ± 1.76 ^{c}	206.00 ± 2.31 ^{b}	204.67 ± 2.40 ^{b}	187.33 ± 2.60 ^{a}	201.33 ± 1.76 ^{b}
	RG %	90.56	101.49	112.59	112.75	102.32	101.66	93.05	100

Different letters (a, b, c, d) represent significant differences in treatments at P< 0.05

Our results showed that the low concentrations of FE and EHF media gave significantly lower growth than the reference medium (C_7 of the SE medium for *Candida tropicalis*; C_1 and C_7 of the SE medium and C_6 and C_7 of the medium FHE for *Filobasidium uniguttulatum*). These decreases would be due to dilutions which deplete the media. In contrast, for *Filobasidium uniguttulatum*, the high concentrations also affected growth, either by inhibiting growth (C_1 of the FHE media) or by giving significantly less growth than in SDA (C_1 of the SE media). That would probably be due to the excess in vitamins and growth factors, which are known for their toxic effects at high concentration. By comparing the efficiencies of earthworms' crude extracts (FHE) and (SE) (Fig. 1), we noticed that for both yeasts' species, the optimal concentration for yeast growth was C_4=0.375g/100 mL that corresponds to a relative concentration (RC) equal to 7.5%. The optimum relative growths (RG), whatever the nature of the media (FHE or SE), were similar for both yeasts, but its value for *Candida tropicalis* was always higher than that of *Filobasidium uniguttulatum*. The obtained results show that the tolerance of *Candida tropicalis* for culture media based on earthworms is slightly higher than that of *Filobasidium uniguttulatum.* Also, the richness of the extracts, in nutritive matters and growth substances comes, mainly, from the intrinsic composition of the body mass of the worms, and not that of their gut contents.

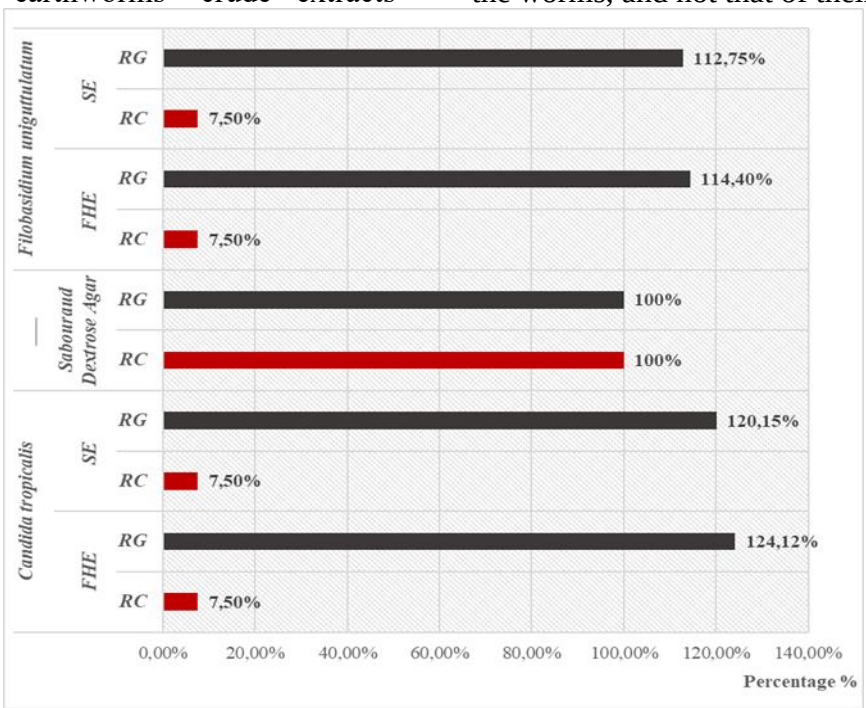

Figure 1. Efficiency of crude extracts (FHE) and (SE), at the obtained optimal concentrations (C_4=0.375g/100mL), on the growth of *Candida tropicalis* and *Filobasidium uniguttulatum*, in solid media

Many studies on the nutritional composition of dry earthworm powder has shown that it contains proteins at a rate of 60–70%, 8 to 20% of carbohydrates, 7–10% of lipids, 2–3% of minerals, various vitamins and rhizogenic indole compounds (3, 9, 12, 14, 21). This rich and diverse composition of earthworms could explain the optimal growth of the two yeasts tested, obtained at concentrations of FHE and FE crude extracts of only 0.375g/100 mL compared to the SDA media, which contains 5g/100 mL of nutrient matter (peptone and dextrose). Projecting our results to the natural environment would help understand the postmortem effect of earthworms in the rhizosphere. As a matter of fact, these invertebrate animals which constitute approximately 80% of the animal biomass of the ground, have a potentially very long life cycle, up to 10 to 12 years, although on the ground, many species live only 1 or 2 seasons, due to their sensitivity to a wide range of predators (10). After their death, their decomposed tissues enrich the soil with nutrients an d probably growth factors with high added value, particularly in N (7, 10, 22). This enrichment would improve soil fertility by promoting the activity and growth of microorganisms, especially yeasts. Indeed, a diversified range of PGP yeasts, have characteristics favoring the growth of plants, and participate to this effect in several ways such as the biocontrol abilities (1, 8, 11, 13, 17, 18, 19, 20). In this work, we tested two yeasts species were tested, which are part of soil yeasts. *Candida tropicalis* is frequently found in decomposing plant matter and has been reported to be a PGP yeast (2). *Filobasidium uniguttulatum*, determined by Kwon-Chung (15) in 1977 and formerly named *Cryptococcus uniguttulatum*, is a species which has not been reported in subsequent studies, but the genus *Cryptococcus* is part of the PGP yeasts, and its implication in the improvement of plant productivity has been reported (8, 11, 16).

ACKNOWLEDGMENT

Our thanks to Prof. Dr. Jorge Dominguez for performing the earthworm molecular identification sampled from Rabat – Morocco. This work was supported by the National Center for Scientific and Technical Research (CNRST – Morocco) under the Research Excellence Scholarship Program.

REFERENCES

1. Alonso, M. L.; D. Kleiner and E. Ortega . 2008. Spores of the mycorrhizal fungus glomus mosseae host yeasts that solubilize phosphate and accumulate polyphosphates. Mycorrhiza 18(4): 197–204

2. Amprayn, K.; M.T. Rose, M. Kecskés,L.; Pereg, H.T. Nguyen and I.R.Kennedy. 2012. Plant Growth Promoting Characteristics of Soil Yeast (*Candida Tropicalis HY*) and Its Effectiveness for Promoting Rice Growth. Applied Soil Ecology 61: 295–99. http://dx.doi.org/10.1016/j.apsoil.2011.11.009

3. Anitha, J. and A. J. Indira. 2012. Nutritional and Antioxidant Evaluation of Earthworm Powder (Eudrillus Euginae). International Research Journal of Pharmacy 3(2): 177–80

4. Botha, A. 2011. The importance and ecology of yeasts in soil. Soil Biology and Biochemistry 43(1): 1–8. http://dx.doi.org/10.1016/j.soilbio.2010.10.001

5. Brown, G. G. and B.M. Doube. 2004. Functional Interactions between Earthworms, Microorganisms, Organic Matter, and Plants. In Earthworm Ecology 2nd ed., (ed.) Edwards, C. A. CRC Press LLC: Boca Raton, FL, pp: 213–239

6. Buzzini, P.; M.A. Lachance and A. Yurkov . 2017. Yeasts in Natural Ecosystems: Diversity. Springer International Publishing. http://link.springer.com/10.1007/978-3-319-62683-3

7. Christensen, Ole. 1988. The Direct Effects of Earthworms on Nitrogen Turnover in Cultivated Soils. Ecological Bulletins. pp: 41–44

8. Cloete, K. J.; A J.Valentin , M.A. Stander, M. A.; Blomerus, and A. Botha. 2009. Evidence of symbiosis between the soil yeast *Cryptococcus laurentii* and a Sclerophyllous medicinal shrub, *Agathosma Betulina* (Berg.) pillans. Microbial Ecology 57(4): 624–32

9. Edwards, C. A. 1985. Production of feed protein from animal waste by earthworms. Philosophical Transactions of the Royal Society of London. B, Biological Sciences 310(1144): 299–307

10. Edwards, C. A. and P.J. Bohlen. 1996. The role of Earthworms in Organic Matter and Nutrient Cycles." In Biology and Ecology of

Earthworms., Chapman and Hall, New York., pp: 155–180

11. El-Tarabily, K. A. and K.Sivasithamparam. 2006. Potential of yeasts as biocontrol agents of soil-borne fungal plant pathogens and as plant growth promoters. Mycoscience 47(1): 25–35

12. ElHarti, A and M.Raouane, . 2009. Détermination de la région d'excrétion des substances rhizogènes chez *Lumbricus Terrestris L*. Biotechnology, Agronomy and Society and Environment 13(1): 85–92

13. Falih, A. M. and M. Wainwright. 1995. Nitrification, S-oxidation and P-solubilization by the soil yeast *Williopsis californica* and by S*accharomyces cerevisiae.* Mycological Research 99(2): 200–204. http://dx.doi.org/10.1016/S0953-7562(09)80886-1

14. Köse, B. and E. Öztürk, .2017. Evaluation of worms as a source of protein in poultry. Selcuk Journal of Agricultural and Food Sciences 31(2): 107–11. http://sjafs.selcuk.edu.tr/sjafs/article/view/886

15. Kwon-Chung, K. J. 1977. Perfect state of *Cryptococcus Uniguttuzatus*. International Journal of Systematic and Evolutionary Microbiology 27(3): 293–99

16. Medina, A.; P. Caravaca, A.; Roldán and R. Azcón, R. 2004. Improvement of soil characteristics and growth of *Dorycnium pentaphyllum* by amendment with agrowastes and inoculation with AM fungi and/or the yeast *Yarowia lipolytica.* Chemosphere 56(5): 449–56

17. Nassar, A. H.; K.A. El-Tarabily . and K. Sivasithamparam, 2005. Promotion of plant growth by an auxin-producing isolate of the yeast *Williopsis saturnus* endophytic in maize (*Zea mays L*.) roots." Biology and Fertility of Soils 42(2): 97–108

18. Prabina, B. J.; K. Kumutha.; R. Anandham, and P. Durga. 2019. Isolation and characterization of multifunctional yeast as plant probiotics for better crop nutrition in pulses. International Journal of Current Microbiology and Applied Sciences 8(01): 2711–18

19. Sansone, G.;I. Rezza, V. Calvente, D.; Benuzzi and M.L.Sanz de Tosetti. 2005. Control of *Botrytis cinerea* strains resistant to iprodione in apple with rhodotorulic acid and yeasts. Postharvest Biology and Technology 35(3): 245–51

20. Silambarasan, S. and A.S. Vangnai. 2017. Plant-growth promoting *Candida Sp. AVGB4* with capability of 4-nitroaniline biodegradation under drought stress. Ecotoxicology and Environmental Safety 139:472–80. http://dx.doi.org/10.1016/j.ecoenv.2017.02.018

21. Sogbesan, A. O. and a.A. Ugwumba, . 2008. Nutritional values of some non-conventional animal protein feedstuffs used as fishmeal supplement in aquaculture practices in Nigeria. Turkish Journal of Fisheries and Aquatic Sciences 8(1): 159–64

22. Whalen, J. K.; R.W. Parmelee D.A. McCartney . and J.L. Vanarsdale. 1999. Movement of N from decomposing earthworm tissue to soil, microbial and plant N pools. Soil Biology and Biochemistry 31(4): 487–92.