EFFICACY OF SOME ORGANIC COMPOUNDS AGAINST ROOT ROT WILT DISEASES OF OLIVE TRANSPLANTS AND THEIR GROWTH RESPONSE IN NEW VALLEY GOVERNORATE, EGYPT

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Abstract

Root rot and wilt disease complex were detected during disease survey carried out through two successive seasons (2011 & 2012) in several olive transplants nurseries and new orchards at El-Kharga, Baris, Balat, El-Dakhla and El-Farafrah districts of New Valley governorate. Percentage of disease incidence and severity on olive transplants in surveyed districts varied. The highest means either in disease incidence or severity (60.8 and 48.7%, respectively) were recorded at El-Dakhla district followed by El-Kharga (52.1 and 43.6%). *Fusarium oxysporum* was the most frequently isolated fungus at the five locations tested, followed by *F. solani* and *Rhizoctonia solani*, while *Macrophomina phaseolina* was the least one. Efficiency of seven organic compounds and one fungicide, as soil drench treatment, varied in reducing disease incidence and severity under greenhouse conditions. All organic compounds tested reduced the diseases severity than the control. Humic acid, Inicium, Fulvital and Bio-Health compounds gave the highest protection compared to untreated transplants (control). Humic acid, however, was significantly superior than the other, whil EMI was the least. All treatments significantly increased plant height, number of leaf plant $^{-1}$, leaf area and fresh & dry weights.

Keywords: Olive transplants, root rot and wilt diseases, fungicides, organic compounds *F. oxysporum* , *F. solani*, *R. solani*, and *M. phaseolina*.

INTRODUCTION

Olive (*Olea europaea* L.) is considered one of the most important economic fruit crops in the world as well as in Egypt. It is grown extensively in the Mediterranean Basin, the subtropical regions of Australia, Southern Africa, and North and South America (Barreto *et al*., 2003). In Egypt, the total area planted with olive trees being 150,000 feddan and in the New Valley governorate is about 4806 feddans (Anonymouss, 2011).

Olive transplants are subject to attack by several soil-borne pathogens, causing severe deterioration in nurseries and new orchards. Root rot and wilt diseases of olive transplants are primarily caused by several pathogens, *Fusarium oxysporum*, *F. solani*, *Rhizoctonia solani*, *Macrophomina phaseolina* and other fungi (Radwan *et. al.,* 1995, Sergeeva, *et. al.,* 2005, Mousa, *et. al.,* 2006, El-Morsi, *et. al.,* 2009 and

Sanei and Razavi 2011). These pathogens are capable of surviving in the soil in the absence of their host plants, and might become destructive under favorable conditions.

In Egypt, under New Valley governorate conditions of high temperature and low relative humidity, root rot and wilt of transplants and young olive trees has been observed at the early stages of plant development in nurseries or after being transplanted to new orchards. In nurseries and new orchards survey carried out in 2011-2012, most of the affected plantations had water-logged and/ or salinity soil.

To overcome this problem, organic compounds and chemical control of disease were tried to reduce losses. Successful control of such disease has been obtained by using a wide array of fungicides (El-Morsi, *et. al.,* 2009). The extensive application of chemical fungicides is harmful to human, living organisms and environment. A promising strategy for the replacement of chemical pesticides has been the implementation of biological control. Organic supplements however, are one of the chief biological means for the control of soil borne diseases. Bioagents produce biologically active compounds (antibiotics & toxic substances) that have antifungal activity, besides bioactive compounds including plant growth regulators like gibberellin, auxin, cytokinin, ethylene, abscisic acid, jasminc acid, protein, vitamins and minerals (Noble and Coventry, 2005). Alga Grow 4, Baker's yeast, Bio-Health, EM1, Fulvital, Humic acid and Inicium are organic compounds which can be applied successfully in many areas of plant production as a plant growth stimulant or soil conditioner for enhancing natural resistance against plant diseases, stimulation of plant growth through increased cell division, as well as optimizing uptake of nutrients and water. Moreover, such treatments stimulated growth of the useful soil microorganisms as mentioned by Chen and Aviad (2004) , Scheuerell and Mahaffee (2004), El-Morsi, *et. al.* (2009) and Kamble, *et. al*. (2012).

The present work was planned to assess root rot & wilt occurrence and to evaluate the effect of certain organic compounds as single treatments on controlling the disease as well as their effects on growth parameters of olive transplants in New Valley governorate.

MATERIALS AND METHODS

1. Diseases survey

Disease survey was carried out in nurseries and new orchards at El-Kharga, Baris, Balat, El-Dakhla and El-Farafrah districts in New Valley governorate during two successive years (2011 and 2012). Percentages of diseased transplants, showing

symptoms of root rot and/or wilt diseases were recorded .On the other hand, diseased root samples were collected for isolation trials on PDA plates. Disease severity was assessed on transplants exhibiting symptoms. Foliar symptoms, including dull, internally rolled or necrotic leaves, defoliated and death twigs, were evaluated on a scale of 0-4 based on the percentage of the affected foliage, where 0= transplants healthy, 1= from 0 to 25% (miled symptoms), 2= from 26 to 50 % (intermediate symptoms), 3= from 51 to 75% (severe symptoms), 4= more than 76% diseased foliage (transplants nearly dead to dead).The disease severity (D.S.) was calculated according to El-Morsi *et. al.* (2009) as: D.S. (%) = (∑ (n x v)/ N x V) x 100 Where, (n) = the number of diseased transplants per category, (v) = category number, N = total number of the transplants, (V) = maximum of category.

2. Isolation and identification of the causal fungi

Diseased roots of olive transplants showing rotting or discoloration were collected for isolation. The root samples were thoroughly washed under running tap water, cut into small pieces (1 cm), and surface sterilized with dipping in 0.3% sodium hypochlorite for 2 minutes, then washed in several changes of sterile distilled water. The surface sterilized pieces were blotted dry on sterilized filter paper, and transferred individually to Petri dishes, containing potato dextrose agar (PDA) medium, then incubated at 25°C for 5 days. The developed fungal colonies were purified using hyphal tip or single spore techniques. The purified fungi were identified according to the fungal morphological and microscopical characteristics as described by Booth (1977), Barnett and Hunter (1986) and Sneh *et al.* (1991) and confirmed by Botany Department, Faculty of Science, Assiut University. The obtained culture isolates were maintained on PDA slants and kept in refrigerator at 5°C for further study. The frequency of the isolated fungi was calculated separately for each of the collected samples.

3. Pathogenicity tests

The pathogenic capability of the isolated fungi (6 isolates) was carried out under greenhouse conditions in El-Kharga Agricultural Research Station. Clay pots (30 cm in diameter) sterilized by dipping in 5% formalin solution for 15 min. Soil was sterilized with formalin solution (5%) through covering with a polyethylene sheet for 7 days and left to dry for 2 weeks. The sterilized pots were filled with sterilized soil (5 K/pot). The tested fungi were grown on autoclaved barley grain medium at 27 ±1 °C for 15 days. The sterilized potted soil was individually infested with the tested fungi at the rate of 5% of soil weight. The pots were irrigated regularly three times a week before planting to ensure even distribution of the inoculated fungus in the soil. One olive transplants (six-month old) of Toffahi cultivar were planted in each pot and six transplants (pots) were used as a replicates for each treatment. Other group of pots

contained uninfested soil was kept as control. Percentages of incidence and severity of the disease were recorded after three months from planting in pots. Re-isolation was carried out from infected transplants showing disease symptoms and the isolated fungi were compared with the original fungal cultures used.

4. Disease control

Seven organic compounds and one fungicide (Table, 1) were evaluated to control root rots and wilt diseases on olive transplants. This experiment was carried out on healthy - looking olive transplants (cv. Toffahi) under greenhouse conditions during 2012. Six Pots as replicates containing sterilized soil previously infested with inoculum of each fungus were drenched with each tested organic compounds (250 ml per pot) 7 days later soil infestation. Plants were irrigated regularly for three times a week. Three months later, percentages of disease severity (DS) and efficaey values were calculated according to the following formula:

$$\text{Protection of disease severity (\%)} = \frac{\text{DS of control transplants} - \text{DS of treated transplants}}{\text{DS of control transplants}} \times 100$$

Vegetative growth parameters *i.e.* plant height (cm), number of leaf plant, leaf area (cm^2) according to Ahmed and Morsy (1999) as well as fresh weight (gm plant) and dry weight (gm plant) were recorded.

Statistical analysis

Statistical analysis was carried out using analysis of variance and means were compared using L.S.D. test (Steel and Torrie, 1980).

Table 1. Trade name, composition, active ingredient (%) and recommended doses of some organic compounds and fungicide.

Trade name	composition	% Active ingredient	Recommended concentration in field
Alga Grow 4	Algae extract, nitrogen, phosphorus pent oxide and potassium oxide.	16% FS*	1 ml / L
Baker's yeast	*Saccharomyces cerevisiae*	100% WSG**	2.5 gm / L
Bio-Health	*Trichoderma harzianum*, *Bacillus subtilis*, *Ascophyllum nodosum*, Amino acids, Trace elements, Humic acid, Fulvic acid, Vitamins, Auxin and Cytokinin	100% WSG	2.5 gm / L
EM1	Photosynthetic bacteria, Lactic acid bacteria and Yeasts.	100% FS	5 ml / L
Fulvital	Fulvic acid, Free Amino acids and Trace elements.	11% FS	5 ml / L
Humic acid	Humus	85% WSG	2.5 gm / L
Inicium	Amino acids and phosphorus pentoxide .	10% FS	5 ml / L
Rizolex-T	Tolclofosm methyl + Thiram	50% WP	3 gm / L

* FS = Flowable concentrate for seed treatment.

** WSG = Water soluble granules.

RESULTS

1. Survey of disease

Disease survey was carried out during two successive years (2011 and 2012) based on typical symptoms of olive transplants with wilt and/ or root rots in five examined districts at new valley governorate. Data in Table (2) show the disease incidence and severity of wilt and root rot disease complex affecting olive transplants in different inspected locations in New Valley governorate. Disease incidence ranging from 22.4% to 59.5% during 2011 and from 24.8% to 62.2% in 2012 were recorded. While, the disease severities ranged from 17.0% to 46.8% during 2011, and 19.4% to 47.6% during 2012. Generally, the disease incidence and severity were slightly high in 2012 (45.2 and 37.0%, respectively) than 2011 (42.1 and 35.0%). Also, the highest percentage means of disease incidence and severity were found at El- Dakhla (60.0% & 82.0%) and El- Kharga (52.1% & 43.7%) during both seasons of survey, while the least at El-Farafrah (23.6% & 18.2%).

Table 2. Occurrence of root rot/wilt disease complex of olive transplants in the nurseries of New Valley governorate during 2011 and 2012 successive seasons.

Locations	Disease incidence		Mean	Disease severity		Mean
	2011	2012		2011	2012	
El-Kharga	50.7	53.5	52.1	42.7	44.6	43.7
Baris	35.6	39.5	37.6	30.6	34.0	32.3
Balat	42.5	45.8	44.2	37.7	39.5	38.6
El-Dakhla	59.5	62.2	60.9	46.8	47.6	48.2
El-Farafrah	22.4	24.8	23.6	17.0	19.4	18.2
Mean	42.1	45.2	43.6	35.0	37.0	36.2

2. Isolation, identification and frequency of the causal fungi

Fusarium oxysporum, *F. solani*, *Rhizoctonia solani*, *F. moniliforme*, *F. equiseti* and *Macrophomina phaseolina* were isolated from olive transplants, collected from different nurseries and new orchards of five locations in New Valley governorate (table 3). Frequency of the isolated fungi varied between locations. Generally, *Fusarium oxysporum* was the most frequent (29.41%) followed by *F. solani* (22.8%) and *R. solani* (18.38%). Also, *F. moniliforme* and *F. equiseti* were recorded at moderate frequencies (9.56 and 12.5%, respectively), while *M. phaseolina* was recorded at low frequencies (7.35 %).

Table 3. Frequency of fungi isolated from naturally infected samples of olive transplants collected from two five locations in New Valley Governorate.

Isolated fungi	Kharga		Baris		Balat		Dakhla		Farafrah		Total	
	No. of isolates	% Frequency	No. of isolates	% Frequency	No. of isolates	% Frequency	No. of isolates	% Frequency	No. of isolates	% Frequency	No. of isolates	% Frequency
Fusarium equiseti	3	11.54	2	8.33	5	15.15	4	12.5	3	14.29	17	12.50
F. moniliforme	2	7.69	3	12.5	4	12.12	2	6.25	2	9.52	13	9.56
F. oxysporum	8	30.77	7	29.17	9	27.27	10	31.25	6	28.57	40	29.41
F. solani	6	23.08	5	20.83	7	21.21	8	25	5	23.81	31	22.80
Macrophomina phaseolina	2	7.69	3	12.5	2	6.06	2	6.25	1	4.76	10	7.35
Rhizoctonia solani	5	19.23	4	16.67	6	18.19	6	18.75	4	19.05	25	18.38
Total	26	100	24	100	33	100	32	100	21	100	136	100

4. Pathogenicity tests

Data presented in Table (4) show that all the tested fungi were pathogenic to olive transplants. The pathogenic fungi isolates were found to have different degrees of pathogenic capabilities. However, the transplants inoculated with the tested fungi exhibited rots on crown and roots characterized by light to dark color and foliar wilting symptoms. *Fusarium oxysporum*, *F. solani* and *Rhizoctonia solani* caused the highest disease incidence (100 %) and severity (83.4, 75.4 and 89.6%, respectively) on tested olive transplants. Disease incidence and severity caused by *F. moniliforme*, *Fusarium equiseti* and *M. phaseolina* were lower. Reisolation from infected tissues yielded mainly the same fungi originally inoculated.

Table 4. Pathogenicity tests of fungi isolated from diseased samples of olive transplants (cv. Toffahi), under greenhouse conditions.

Fungi	Disease incidence (%)	Disease severity (%)
F. equiseti	33.3	18.9
F. moniliforme	33.3	22.8
F. oxysporum	100	83.4
F. solani	100	75.4
M. phaseolina	33.3	17.3
R. solani	100	89.6
Control	0.0	0.0
L.S.D. at 0.05	1.17	1.07

5. Effects of some organic compounds on disease severity and vegetative growth parameters of olive transplants

Results in Tables (5, 6 and 7) show that all the organic compounds tested and Rizolex-T significantly reduced incidence of root rot and wilt diseases on olive transplants caused by *F. oxysporum, F. solani* and *R. solani*. Efficiency for controlling the diseases and improving vegetative growth parameters varied. Generally, Humic acid, Inicium, Fulvital, Bio-Health, Alga Grow 4 and Baker yeast gave the least disease severity and the highest disease protection, while Rizolex-T and EM1 gave the least reduction in disease severity and disease protection. Humic acid, however, was significantly superior than the others except in case of Fulvita with *R. solani*, in minimizing disease severity and maximizing protection (%). It gave 89.57%, 87.81% and 85.84% reduction in disease severity caused by *F. oxysporium, F. solani and R. solani, respectively.*

Table 5. Effects of the tested organic compounds as soil drench on disease severity caused by F. oxysporum and growth parameters of olive transplants.

Tested organic compounds	Disease severity %	Protection %	Plant height (cm)	Number of leaf Plant^{-1}	Leaf area (cm^2)	Fresh weight plant^{-1} (gm)	Dry weight plant^{-1} (gm)
Alga Grow 4	15.69	81.18	21.67	26.33	3.89	23.16	11.65
Baker's yeast	18.96	77.25	16.33	21.33	3.82	21.09	10.25
Bio-Health	14.96	82.05	24.33	28.00	3.99	25.36	12.45
EM1	22.36	73.18	10.67	14.00	3.73	15.63	7.06
Fulvital	14.56	82.53	25.67	31.33	4.00	28.56	13.25
Humic acid	8.69	89.57	29.33	34.67	4.22	33.69	14.96
Inicium	12.36	85.17	26.67	31.67	4.09	30.14	13.55
Rizolex-T	16.69	79.98	18.33	25.00	3.86	22.15	10.48
Control	83.36	-	5.00	8.00	3.36	8.36	4.56
LSD at 0.05	3.09	-	4.31	4.01	0.23	4.82	2.87

Table 6. Effect of the tested organic compounds as soil drench on disease severity caused with Fusarium solani as well as growth parameters on olive transplants.

Tested organic compounds	Disease severity %	Protection %	Plant height (cm)	Number of leaf Plant	Leaf area (cm^2)	Fresh weight plant^{-1} (gm)	Dry weight plant^{-1} (gm)
Alga Grow 4	16.59	78.01	22.33	30.33	4.12	32.15	15.42
Baker's yeast	16.59	78.01	22.00	28.33	4.09	29.65	14.56
Bio-Health	18.79	75.10	20.67	26.67	3.98	28.59	14.00
EM1	25.47	66.24	11.33	17.00	3.69	19.63	8.96
Fulvital	12.40	83.56	24.00	30.33	4.19	35.49	17.05
Humic acid	7.69	89.81	28.33	37.33	4.29	44.26	21.96
Inicium	11.96	84.15	26.00	36.67	4.21	36.56	17.85
Rizolex-T	20.49	72.84	18.33	25.00	3.96	25.36	12.96
Control	75.45	-	8.33	12.67	3.29	14.00	7.00
LSD at 0.05	3.81	-	3.33	5.63	0.24	4.09	2.40

Table 7. Effect of the tested organic compounds as soil drench on disease severity caused by R. solani and growth parameters of olive transplants.

Tested organic compounds	% Disease severity	% Protection	Plant height (cm)	Number of leaf Plant	Leaf area (cm^2)	Fresh weight (gm)	Dry weight (gm)
Alga Grow 4	22.91	74.44	21.67	30.67	4.00	23.69	11.09
Baker's yeast	24.47	72.70	19.67	28.00	3.82	21.36	10.23
Bio-Health	20.45	77.19	21.67	30.67	4.05	25.96	12.05
EM1	30.56	65.91	17.67	26.67	3.57	19.76	9.25
Fulvital	15.59	82.61	28.00	36.00	4.09	33.00	16.05
Humic acid	12.69	85.84	34.00	47.67	4.19	41.08	19.09
Inicium	16.59	81.50	25.00	34.67	4.01	26.09	12.15
Rizolex-T	28.75	67.93	18.33	25.00	3.69	20.05	9.50
Control	89.65	-	11.67	17.33	3.55	13.09	6.21
LSD at 0.05	3.50		3.04	5.89	0.34	4.05	2.76

All treatments significantly improved plant height and also, increased number of leaf plant $^{-1}$, leaf area fresh and dry weights compared with those of the control treatments.

DISCUSSION

Olive transplants are subject to attack by several soil-borne pathogens, causing severe losses in nurseries and new orchards in New Valley governorate, Egypt. Survey of root rot and wilt disease complex in different locations of New Valley governorate was conducted during two successive seasons (2011 and 2012), indicated that root rot and wilt disease complex is the most important diseases in New Valley governorate, since it caused a major problem on transplants and young olive trees. The disease incidence and severity differed at five inspected locations. The high values of disease occurrence and severity my attributed to warm and dry conditions in these districts as well as long-term olive transplants cultivation in the same soils without using the correct, strict sanitation methods and preventive therapeutic control measures. The highest means of disease incidence and severity were recorded on the transplants grown at El-Dakhla followed by El-Kharga while the least on those grown at El-Farafrah. Also, these results are in agreement with those reported by Mousa *et. al.,* (2006) and El-Morsi, *et. al.,* (2009) .

The results of the present study revealed that several fungi were isolated from rotted and/or wilted samples of transplants and young olive trees these were *F.*

oxysporum, *F. solani*, *F. moniliforme*, *F. equiseti*, *R. solani* and *M. phaseolina*. *Fusarium oxysporum* was the most frequently isolated fungi, followed by *F. solani* and *R. solani* on all surveyed locations. While, *M. phaseolina* was found in low frequency. Similar results were obtained by several other investigators (Radwan *et. al.* 1995: Barreto, *et. al.,* 2003, Sergeeva, *et. al.,* 2005, Mousa, *et. al.,* 2006, El-Morsi, *et. al.,* 2009 and Sansei and Resave 2011). Pathogenicity tests proved that all the isolated fungi were pathogenic to olive transplants (Tiffanies var.), however F. okays., F. solani and R. solani were the most destructive. Symptoms of root rot and wilt diseases on olive transplants as previously reported by Barrera, *et. al*., (2003), Barreto, *et. al*., (2003) and Sanei and Razavi 2011) were observed.

Efficiency of the tested substances in controling root rot and wilt diseases and improving vegetative growth parameters varied. All the substances tested significantly reduced disease occurrence and severity. In this respect, Humic acid, Inicium, Fulvital, Bio-Health, Alga Grow 4 and Baker's yeast were the most effective. Humic acid, however, was significantly superior than the others, except in case of Fulvital with *R. solani*, while, EMI and the fungicide Rizolex T were less effective, though significant. On the other hand, all treatments significantly improved plant height, increased number of leaf plant^{-1}, leaf area, fresh weight and dry weight compared with the control. The organic compounds tested have been applied successfully in many ways in plant production as a plant growth stimulant, soil conditioner. They enhanced natural resistance against plant diseases and pests, stimulated plant growth through increased cell division, as well as optimized uptake of nutrients and water as well stimulating as soil microorganisms playing role in reducing root rot and with diseases (Chen and Aviad 2004, Scheuerell and Mahaffee, 2004, Noble and Coventry 2005: El-Morsi, *et. al.,* 2009 and Kamble, *et. al*., 2012).This positive action of organic compounds tested may be primarily attributed to their stimulating bioagants such as *T. harzianum* and *B. subtlis*, besides their role as effective fertilizers and plant growth promoters. The fungicide Rhizolex-T reduced greatly incidence of the disease and this positive action may be due to its mode of action which affected of lipids and membrane synthesis (FRAC, 2012). The present result is in agreement with that reported by El-Morsi, *et. al.* (2009).

In conclusion, Results of the present study could suggest that soil drench with organic compounds can be used as a safe control measure of the disease on olive transplants and as a stimulant of vegetative growth parameters.

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فعالية بعض المركبات العضوية فى مكافحة أمراض أعفان الجذور والذبول لشتلات الزيتون وتأثيرها على النمو فى محافظة الوادى الجديد

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تم تسجيل وجود مرض أعفان الجذور والذبول المركب على شتلات الزيتون المنزرعة فى المشاتل والبساتين المنشأة حديثا بكل من مراكز الخارجة ، وباريس ، وبلاط، والداخلة والفرافرة بمحافظة الوادى الجديدخلال الحصر الذي اجري في عامي 2011و2012 م. ولقد وجد المرض فى تلك المشاتل والبساتين بنسب وشدة متفاوتة فى المناطق التى شملها الحصر، حيث بلغ اعلي متوسط لها في مركز الداخلة (60.8%، 48.7% على التوالي) بينما جاء مركز الخارجة في المرتبة الثانية في هذا المجال (52.1%، 43.6% على التوالي). و تم عزل العديد من الأنواع لأجناس فطرية مختلفة من العينات المصابة، من هذه الفطريات هى فطر فيوزاريوم اوكسيسبورم، فيوزاريوم سولانى، فيوزاريوم مونليفورم، فيوزاريوم أكوايسيتى، ريزوكتونيا سولانى ، ماكروفومينا فاصولينا. واختلف التوزيع التكرارى لتلك الفطريات تبعا لاختلاف أجناسها والمواقع المعزولة منها. وكانت أكثر الفطريات توجدا : فيوزاريوم اوكسيسبورم، وفيوزاريوم سولانى، وريزوكتونيا سولانى . وأثبتت اختبارات القدرة المرضية للفطريات المعزولة تحت ظروف الصوبة والعدوى الصناعية أن الفطريات فيوزاريوم اوكسيسبورم، وفيوزاريوم سولانى، وريزوكتونيا سولانى هى اكثرها احداثا لأعفان الجذور والذبول على شتلات الزيتون.

تم دراسة تأثير سبعة مركبات عضوية (حمض الهيومك، أنيسيوم، فولفيتال، بيوهلث، الجاجرو4–خميرة الخبيز–إى إم 1) ومطهر فطرى للمقارنة (ريزولكس– تى) على شدة المرض، وقياسات النمو الخضرى لشتلات الزيتون (الصنف التفاحى) تحت ظروف الصوبة. و أظهرت المركبات العضوية المختبرة كفاءة عالية فى مكافحة أمرض أعفان الجذور والذبول كما تفوق حمض الهيومك معنويا علي المعاملات الاخري في هذا المجال بينما كان اقلها فعالية مركب EMI ،كما حسنت قياسات النمو، وكان أفضلها حمض الهيومك، أنيسيوم، فولفيتال، بيوهلث، الجاجرو 4– خميرة الخبيز.