Introduction

During the last three decades a great attention was paid towards aquaculture as an attempt to fulfill the gap between the increased population and their demand from animal protein. Aquaculture is still the fastest growing food producing sector, compared to other food commodities with an annual increase of approximately 12% (**FAO, 2004 and FAO, 2009**). Nile tilapia, *Oreochromis niloticus* (L.) is an important species for freshwater aquaculture and the most widely cultured in tropical and subtropical countries. Consumers like tilapia's firm flesh and mild flavor, so markets have expanded rapidly (**FAO, 2005**).

Aquatic animals in large-scale production facilities are exposed to stress conditions, diseases and deterioration of the environmental conditions, leading to serious economic losses (**FAO 2004, and Subasinghe 2005**).

The use of antibiotics in aquaculture as disease prevention and growth promotion may introduce potential hazard to public health and to the environment by the emergence of drug-resistant microorganisms and antibiotic residues. Furthermore, the normal microbial flora in the digestive tract, which is beneficial to fish, is also killed or inhibited by oral chemotherapy (**Sugita et al., 1991 and FAO/WHO/OIE 2006**). With increasing demand for environment friendly aquaculture, the use of probiotics in aquaculture is now widely used instead of chemotherapy and antibiotics to increase safety protein production for human.

The positive effects of probiotic uses include the production of inhibitory compounds against pathogens, competition for nutrients and adhesion sites and the stimulation of both local and systemic immune responses (**McCraken and Gaskins 1999**). Probiotics include bacteria and yeasts, the beneficial role of yeasts being of particular interest because they represent an important source for nonspecific immuostimulants as β-glucans (**Sahoo and Mukherjee 2002**), chitin (**Vecchiarelli 2000**), nucleic acids as well as mannan oligosaccharides (**Li et al., 2004**) and acts as well as growth promoters (**Lara-Flores et al., 2003; Li and Gatlin, 2003, 2004, and 2005**) of various fish species.

Yeasts are a rich source of protein and B-complex vitamins. They have been used successfully as a complementary protein source in fish diet. Also, they have been used as a supplement in animals feed to compensate for the amino acid and vitamin deficiencies of cereals, and are recommended as substitute soyabean oil in diets for fowl (**Gohi, 1991**). In addition they are considered a cheaper dietary supplement as they are easily produced on an industrial level from a number of carbon-rich substrate by product (**Lee and Kim, 2001**).

Yeasts are ubiquitous microorganisms, which disseminate with animals, air and water currents, and which can grow in various environments where organic substrates are available. Their presence has been noted in fish guts for some time in wild, as well as farmed animals, but this natural occurrence has been generally considered as incidental. Industrial yeast is commonly used in aquaculture, either alive to feed live food organisms, or after processing, as a feed ingredient (**Stones and Mills, 2004**).

The aim of the present study is directed to isolate yeasts from cultured fresh water fishes, identified the isolated yeasts, make Invitro test the antibacterial effect of isolated yeasts, make minimal inhibition concentration determination (MIC) of isolated yeasts, make pathogencity test of isolated yeasts, evaluate the effect of isolated yeasts as probiotics in cultured *Oreochromis niloticus*, and Test fish resistance to some bacterial disease.

REVIEW OF LITERATURE

Tilapia fish:

In the Arabian region, tilapias are the most favorable fish species for consumers. Moreover, tilapias, specially the *Oreochromis spp*.are ideally for semi-intensive and intensive culture in tropical and subtropical environments. Their tolerance to high stocking densities in relatively poor water quality has generated considerable interest in areas where water is limited **(Rana and Suliman, 1993).**

The culture of tilapia in ponds has a long history which dated back to ancient Egyptian, yet it is only a few decades ago since its real potential for commercial aquaculture was fully recognized. Africa is the natural origin of tilapias but during the last 40 years many tilapia species were cultured in several water systems**.** Rapid growth rates, high tolerance to low water quality, efficient feed conversion, ease of spawning, resistance to disease and good consumer acceptance make tilapia a suitable fish for culture, especially in the tropics and subtropics, including most of the areas suffering from a lack of animal protein. **(Mires, 1995)**

The tilapia species have become important in fish culture, especially in warmer climates. According to FAO data, the annual world aquaculture production of tilapias and other cichlids in 2003 was about 1677751 MT. The most important species in terms of percentage production by weight is undoubtedly *Oreochromis niloticus* 1349954 MT (80.46%) (**FAO**, **2003**).

Nile tilapia, *Oreochromis niloticus* (L.) is an important species for freshwater aquaculture. Improving fish performance and disease resistance of cultured organisms are major challenges facing fish culturists. Moreover,

bacterial diseases are one of the limiting factors for fish culture including Nile tilapia (**Rahman et al., 1997and Li et al., 2006**).

Egypt fish production is 889035 tons in year 2005. The total production of bolti is 333479.66 tons and the production of bolti from aquaculture is 217018.66 tons in year 2005 **(GDFA, 2006)**.

Bacterial diseases:

Lewin et al., 1992 decided that bacterial resistance to antimicrobial drugs has become widespread in aquaculture. Cultured fish have been reported to be infected with resistant strains of *Aeromonas hydrophila, Aeromonas salmonicida, Edwardsiella ictaluri, Pasteurella piscicida*, *Vibrio anguillarum, Yersinia ruckeri,* and *streptococci* resistant to many drugs used in aquaculture, including nalidixic acid, oxolinic acid, tetracycline, sulfa drugs, and chloramphenicol .

Yambot and Inglis 1994 reported that the acute mortality among Nile tilapia in which the most apparent clinical signs included opaqueness in one or both eyes, accompanied by exophthalmia and eventual bursting of the orbit. Motile aeromonads were isolated from the eyes, liver and kidneys of inffected fish.

Greenlees et al., 1998 reported that the bacterial diseases may cause heavy losses from mortality, reduced growth and unmarketable appearance in various fish species. Some reported occurrences of the human disease were associated with puncture wounds or abrasions and handling of infected fish or contaminated water.

Robert 2000 recorded that *Aeromonas hydrophila , A. sobria,* and *A. caviae* are the causative agents of Motile Aeromonad Septicemia. Synonyms: hemorrhagic septicemia, infectious dropsy, infectious abdominal dropsy, red

sore. *Vibrio anguillarum, Listonella anguillarum* and *Vibrio ordalii* are the causative agents of vibriosis. Synonyms: salt water furunculosis, boil disease, ulcer disease, red pest of eels. *Pseudomonas anguilliseptica* is the causative agent of red spot disease of eels, hemorrhagic septicemia of fish. *Pseudomonas fluorescens* is the causative agent of Pseudomoniasis. Synonyms: bacterial tail rot, fin rot, hemorrhagic septicemia.

Lotz et al., 2005 recorded that the most bacteria that can potentially infect fish are gram-negative bacteria, including *Aeromonas hydrophila*, *Aeromonas salmonicida*, *Flavobacterium columnare* (which causes columnaris), *Vibrio*, and *Pseudomonas* species.

Ya and Shuang 2010 reported *A. sobria* was the pathogenic agent of tail-rot disease in *Oreochromis niloticus*. *A. sobria* was multi-resistant to the most frequently used antimicrobial drugs in China.

Yeast studies:

Yeasts may be more numerous than cultivable bacteria, like in the deep-sea eel *Synaphobranchus kaupi* (**Ohwada et al., 1980**), or in mature masu salmon in freshwater (**Yoshimizu et al., 1976**). These examples seem exceptional, but yeast may be of physiological importance even when accounting for less than 1% of the total microbial isolates.

Barnett et al., 1990 reported that most of the yeasts, except *Saccharomyces* spp., can grow on cellulosic materials, however; only few genera are able to degrade starch. Among disaccharides, lactose is one of the most refractory carbon substrate to most of the yeasts. Among hundreds of genera, only few are lactose positive, many *Cryptococcus* spp., *Trichosporon* spp., some *Debaryomyces* spp., *Kluyveromyces* spp.,

Myxozyma spp., and occasionally *Bullera* spp., *Candida* spp., *Rhodotorula* spp. and *Tremella* spp.

Dealler 1991 reported that the yeast identification procedures start with germ tube test in clinical laboratories, which is a rapid method to differentiate *Candida albicans* from other *Candida* species. Although this is a rapid test, it may lead to both false positive and false negative results.

Few types of yeast have been reported as responsible for disease in fish such as Candida sp. caused internal lesions in *Oncorhynchus tshawytscha* (**Mueller and Whisler 1994**) and gas Swim bladder swollen with dense material in *Sparus aurata* (**Galuppi et al., 2001**), *Cryptococcus* sp. caused surface and internal lesions (gas swim bladder, kidney) in *Oncorhynchus tshawytscha* (**Mueller and Whisler 1994**), *Sporobolomyces salmonicolo* caused ascites, visceral mycosis in *Oncorhynchus tshawytscha* (**Muench et al., 1996**), and *Trichosporon* sp. caused internal lesions (gas swim bladder, kidney) in *Oncorhynchus tshawytscha* (**Mueller and Whisler 1994**) .

Ismail et al., 1994 reported that a total of 16 mould genera representing 29 species and one species variety were isolated from forty five salted fish samples. The dominant genera were *Aspergillus* (53.3%, and 75.6%) and *Penicillium* (44.4%, 68.8%) on malt extracts agar and halophilic malt agar respectively. Other frequently isolated moulds were *Cladosporium*, *Acremonium*, *Alternaria*, *Eurotium*, *Rhodotorula* and *Rhizopus* in a varying percentage.

Sun and Sun, 1998 found that the yeast *Torulopsis mogii* is the pathogen to some shrimp in China.

Warren and Hazen, 1999 said that the yeast can named with further tests such as culturing on cornmeal agar, carbohydrate assimilation and fermentation tests and automated identification procedures are done.

Alliot et al., 2000 and **Groll and Walsh. 2001** reported that *Rhodotorula spp.* are rarely isolated as causative agents of opportunistic mycoses. In vulnerable hosts, Causes of meningitis (**Gyaurgieva et al., 1996**) endocarditis ventriculitis, peritonitis, endophthalmitis, central venous catheter-infections, fungemia, and sepsis.

Galuppi et al., 2001 emphasized that the yeast and other fungi may be potentially harmful in an immunocompromised host, or in adverse environmental conditions. Sporidiobolaceae like *Cryptococcus* spp., *Sporobolomyces salmonicolor* and *Trichosporon* spp. also seem to be opportunistic, as they occasionally cause disease in fish. Most yeasts are likely harmless to healthy fish reared in good conditions, but new candidate probiotics should be carefully tested, while watching for any sign of casual mycosis.

Zlatanov et al., 2001 evaluated that *Rhodotorula minuta* contained unsaturated fatty acids, mainly oleic and linoleic, predominated in triacylglycerols, also contains Sterols and tocopherols (mainly y- and 6-tocopherol).

Emilina et al. 2003 established that the stationary phase of the growth cycle of *Rh. Rubra* reached at the 6th day.

Ginka et al. 2004 reported that the stationary phase of the growth cycle of *Rh. Rubra* reached at the 5th day.

Moore and Strom, 2003 said that few types of yeast have been reported as responsible for disease in fish. *Metschnikowia bicuspidata* vA. *bicuspidata* caused mortality in chinook salmon fry fed infected *Artemia*

franciscana. They succeeded to reproduce the disease by intraperitoneal injection of the pathogen.

Reuter et al., 2003 reported that Black yeast-like *Exophiala* spp. are parasites of fish, since they are clearly distinct from the yeasts hosted by healthy fish.

Magliani et al., 2004 found that killer toxin produced by some yeast strains is a low molecular mass protein or glycoprotein toxin which kills sensitive cells of the same or related yeast genera without direct cell–cell contact. Up to now, toxin-producing killer yeasts have been identified in genera *Candida, Cryptococcus, Debaryomyces*, *Hanseniaspora, Hansenula*, *Kluyveromyces, Metschnikowia, Pichia, Saccharomyces, Ustilago*, *Torulopsis, Williopsis and Zygosaccharomyces* indicating that the killer phenomenon is indeed widespread among yeasts.

Nahvi and Moeini, 2004 found that yeast can easily grow at room temperature and can utilize (assimilate and/or ferment) a variety of carbohydrates such as pentoses, hexoses, disaccharides and, rarely polysaccharides.

Patterson and McGinnis, 2006 reported that yeasts occurring in fish microbiota may be classified in two distinct phyla of the fungal kingdom: *Ascomycota*, among which *Saccharomycetaceae* are probably the most important family, but also *Basidiomycota*, which include the genus *Rhodotorula* – these red yeasts are commonly observed in fish microbiota.

Gatesoupe, 2007 reported that yeasts have been commonly isolated in the gastrointestinal tract, and high population densities were sometimes noted in healthy fish, but the data were quite variable in terms of colony counts and taxonomical diversity. *Rhodotorula* spp. seemed relatively frequent in both marine and freshwater fish, and *Debaryomyces hansenii* has

been found to be dominant in rainbow trout. Some other dominant strains have been described, such as *Metschnikowia zobelii, Trichosporon cutaneum, and Candida tropicalis* in marine fish, and Candida spp., *Saccharomyces cerevisiae*, and *Leucosporidium* spp. in rainbow trout. The natural proliferation of yeasts in fish mucus may be generally considered as commensalisms, in spite of a few cases of pathological infections mainly due to opportunistic strains. Several strains were documented to settle and grow in fish intestine after experimental introduction, particularly *S. cerevisiae* and *D. hansenii* in rainbow trout. There have been a few instances of competition among yeasts in fish intestine, while the effect of yeast on associated bacteria is still unclear.

Gaëtan et al., 2011 isolated species in the genus Candida from an unidentified deep-sea coral collected near Rainbow hydrothermal vent, from water samples near Menez Gwen hydrothermal field and from the stomach of a marine fish.

Yeasts probiotic activities:

Rusmesy et al., 1990 and 1992 reported that natural immune stimulants are biocompatible, biodegradable and safe for the environment and human health. Moreover, they possess an added nutritional value. In this way, yeasts have been tested in fish diets as a possible alternative to commonly used animal protein.

Vazquez-Juarez et al., 1993 isolated yeast from the intestines of wild rainbow trout and introduced it with feed into the digestive tracts of domestic rainbow trout. They recorded that a significant increasing in the growth of the cultured trout.

Noh et al., 1994 said that when *S. cerevisiae* was tested alone, growth and feed efficiency were improved in Israeli carp and Nile tilapia. In tilapia fed a control diet, survival and digestibility were reduced by increasing the population density, while this stress did not affect the groups treated with the yeast.

Peter et al., 1994 reported that *Aureobasidium pullulans, Citeromyces matritensis, Cryptococcus laurentii, Rhodotorula glutinis,* and *Sporobolomyces roseus* produced antibacterial compounds inhibitory to both *Pseudomonas fluorescens* and *Staphylococcus aureus* in an overlay bioassay. In contrast, isolates of *Candida albicans, Filobasidium uniguttulatum, Saccharomyces cerevisiae, Torulaspora delbruckii, Tremella foliacea, Trichosporon beigelii,* and *Trichosporon dulcitum* did not produce inhibitory compounds. Two distinct antibiotics were produced by an isolate of *Aureobasidium pullulans* in liquid culture during both the logarithmic and the stationary phases of growth.

Siwicki et al., 1994 tested several immunostimulants on rainbow trout, lyophilised *Candida utilis*, lyophilised *S. cerevisiae*, ß-glucans (MacroGard®, ß-1,3/1,6-linked polymers of D-glucose, extracted and purified from baker's yeast, *S. cerevisiae*), deacylated chitin (Chitosan®, ß-1,4-linked polymer of D-glucosamine), a premix of selenium and vitamins C and E, and a premix of butane and amino acids. The additives increased cellular immune response and immunoglobulin serum titres, and the most significant stimulations were generally observed with the two yeasts. After challenge with *Aeromonas hydrophila*, the best protection was obtained in rainbow trout fed either *S. cerevisiae*, ß-glucans, or deacylated chitin, while *C. utilis* seemed less efficient.

Sung et al., 1994 reported an enhanced resistance to vibriosis in P. monodon postlarvae administered with β-glucan. Increased post-challenge survival was observed in glucan-fed *Penaeus monodon* to white spot syndrome virus.

Andlid et al., 1995 observed that reduced numbers of bacteria when yeast colonization peaked in rainbow trout intestine, though the effect of yeasts on bacteria associated in fish intestine is not clear.

Sakai et al., 1995 reported that the non-specific immune system can be stimulated by probiotics. It has been demonstrated that oral administration of *Clostridium butyricum* bacteria to rainbow trout enhanced the resistance of fish to vibriosis, by increasing the phagocytic activity of leucocytes.

Carver and Walker, 1995 found that yeast is generally considered a good source of proteins, nucleic acids, vitamins and polysaccharides. A part from cell wall glucans, the nucleotide content of the yeast might also contribute to immunostimulation.

Duncan and Klesius (1996) compared the effects of ß-D-glucan from barley and dried baker's yeast, *S. cerevisiae*. Cellular immunity was increased by both feed additives, but to a lesser extent with the yeast. In particular, glucan from barley, but not the yeast, stimulated the chemo tactic response to *Edwarsiella ictaluri* exoantigen by macrophages and neutophils, and the chemiluminescent response to *E. ictaluri* by peritoneal exudates' phagocytes. However, the fish were not protected against edwardsiellosis in a challenge experiment, even when they were fed glucan.

Gatesoupe et al., 1997 and Calvente et al., 1999 reported that the yeasts produced extracellular proteases and siderophores, and they bound lactoferrin. Iron availability is a key issue for fish microbiota and such

features may play a role for antagonism to some pathogens, the virulence of which is iron-dependent .

Song et al., 1997 reported that theβ-1,3-glucans of certain fungi and yeasts have been successfully used as immunostimulants to enhance the defence potential of fish and shellfish against bacterial and viral infection .

Gedek, 1999 and Castagliuolo et al., 1999 who reported that the yeast may be antagonistic to entero-pathogenic bacteria, due to adhesion of bacterial cells or by secreting proteases which inhibit bacterial toxins. Also yeasts produced extracellular proteases and siderophores, and they bound lactoferrin.

Salminen et al., 1999 defined a probiotic as "a live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance". This definition is still widely referred to, despite continual contention with regard to the correct definition of the term. Current probiotic applications and scientific data on mechanisms of action indicate that non-viable microbial components act in a beneficial manner and this benefit is not limited just to the intestinal region.

Scholz et al., 1999 compared the efficacy of five different yeast-supplemented diets in shrimp and reported that *Phaffia rhodozyma* incorporated into the feed gave better performance in terms of bacterial clearance and increased phenoloxidase activity in haemolymph.

Thompson et al., 1999; and Verschuere et al., 2000 pointed out that one of the main modes of action and beneficial effects of probiotics in aquacultured organisms is enhancement of nutrition of host species through the production of supplemental digestive enzymes and higher growth and feed efficiency, prevention of intestinal disorders and pre-digestion of anti nutritional factors present in the ingredients. In addition to several modes of

action: competitive exclusion of pathogenic bacteria through the production of inhibitory compounds; improvement of water quality; enhancement of immune response of host species; and enhancement of nutrition of host species through the production of supplemental digestive enzymes.

Burrells et al. (2001) reported that dietary nucleotides, extracted from brewers yeast, could enhance resistance to various pathogenic infections in Atlantic salmon. However, the extent to which RNA in brewers yeast contributes to the beneficial influences of dietary brewers yeast on immune responses and resistance to *S. iniae* infection of hybrid striped bass is not clear.

Sakai et al., 2001 reported that Nucleotides from yeast RNA were evaluated for their ability to enhance the non-specific immune responses in carp, *Cyprinus carpio*. Oral administration of nucleotides to fish daily for 3 days resulted in enhanced responses of phagocytic and nitroblue tetrazolium (NBT) activities in kidney phagocytic cells. This activation of kidney cells was observed for at least 10 days post-treatment. The serum complement and lysozyme activities also increased in fish treated with nucleotides. Furthermore, the number of *Aeromonas hydrophila* in nucleotide-treated fish significantly decreased in the blood, kidney and liver after intraperitoneal injection. Thus yeast nucleotides appear to enhance non-specific immune responses in fish.

Esteban et al., 2001 decided that the advantages of using yeast cell their fast growth, low cost, high stability and the fact that they are not common constituent fish feed. This makes it easier to work with them at known concentrations, compared with using soluble substances such as vitamins, which exist as micronutrients in feed.

Tovar et al., 2002 found that the addition of live yeast improved diet and protein digestibility, which may explain the better growth and feed efficiency seen with yeast supplements.

Ortuño et al., 2002 suggested that lyophilized whole cells of a commercial strain of S. cerevisiae BMA64-1A could produce a more general immune response, due to other cell wall compounds besides ß-glucans, like mannoproteins, and chitin as a minor component. RNA extracts and partially autolysed brewers yeast may be also efficient.

Peng et al., 2003 found that enhanced weight gain and feed efficiency were generally observed in fish fed the diets supplemented with yeast compared to the basal diet in both trials. In the second trial, body composition of whole fish, hemocrit and serum lysozyme levels were observed to be within normal ranges and not influenced by the various dietary treatments. After 9 weeks of feeding in the second trial, exposure to *S. iniae* resulted in no mortality and reduced signs of disease in fish fed diets supplemented with 2% and 4% brewer's yeast, while 20% mortality was observed in fish fed the control diet. They concluded brewers yeast positively influenced growth performance and feed efficiency of hybrid striped bass as well as resistance to *S. iniae* infection. In addition, results of immune response assays demonstrate that brewers yeast can be administered for relatively long periods without causing immunosuppression.

Lara-Flores et al., 2003 evaluated the effect of the supplementation of 0.1% of bacterial mixture containing *Streptococcus faecium* and *Lactobacillus acidopilus*, a second group was supplemented at 0.1% with yeast, *Saccharomyces cerevisiea*, and a third, a control diet without supplements to a diet of Nile tilapia (*O.niloticus*). They found that fish fry fed on diets with a probiotics supplement exhibited greater growth than

those feed with the control diet, and fish feed on yeast containing diet showed better growth than those fed on the microbial mixture containing diet. They also found that the diets supplemented with probiotics produced an individual weight gain and specific growth rate significantly higher than the control diet.

Pedro et al., 2004 decided that from 60 strains only Saccharomyces sp. M26 presented an inhibitory halo in *Lactobacillus fermentum* culture and significant reduction in the culture turbidity (71%) and specific growth rate (56%) when compared to the control. Freezing did not affect the antibacterial activity of the *Saccharomyces* spp. M26 extract and heating at 90 °C for 20 min completely destroyed this activity. It is expected the decrease of lactic acid bacteria growth in the *S. cerevisiae* alcoholic fermentation should allow for better control of these bacteria in the process.

Burgents et al., 2004 reported enhanced disease resistance in the pacific white shrimp *Litopenaeus vannamei* against experimental *Vibrio* infection when fed with *S. cerevisiae* supplemented feed.

Li and Gatlin 2004 evaluated the effect of prebiotics, on the growth of hybrid striped bass (*Morone chrysops* x *Morone saxatilis*). Two levels (1% and 2% of diet) of GroBiotic TM AE and brewers yeast were added to the basal diet with menhaden fish meal and menhaden oil adjusted to provide isonitrogenous (40%) and isolipidic diets (10%). Each diet was fed to juvenile hybrid striped bass in 110 liter aquaria twice daily for 7 weeks. The found that enhanced growth performance was generally observed in fish fed the diets supplemented with GroBiotic TM AE or brewers yeast compared to the basal diet.

Tovar-Ramirez et al., 2004 tested the absence and two of live yeast in sea bass (*Dicentrorch labrax*) larvae diets. Fish were fed from time of

month opening to 37 days after hatching a diet of 0, 1.1, or 5.7% wet weight of live yeast (*Debaryomyces hansenii* CBS/8339). They found that final mean weight in groups fed 1.1% yeast was twice that of other groups.

Li and Gatlin 2005 evaluated the commercial prebiotic GroBiotic®-A (amixture of partially autolyzed brewers yeast) at a rate of 1 or 2% brewers yeast GroBiotic®- A for 21 weeks in a recirculating system. They found that growth performance was generally higher in fish fed diets supplemented with GroBiotic®- A or brewers yeast compared to fish fed the basil diet. They also found that fish fed brewers yeast had significantly higher weight gain than the other diets.

Hansen and Olaf-sen 1999, Ringø and Olsen 1999; Tanaka et al. 2003; Al-Harbi and Uddin 2004, and Ringø et al. 2006a and 2006b reported that the community of intestinal microbiota in cultured fish is affected by many factors such as dietary nutrition, aquaculture conditions, and feeding systems.

Barnes et al., 2006 evaluated The addition of a yeast culture food supplement during feeding of McConaughy strain rainbow trout *Oncorhynchus mykiss*. The yeast culture was added to commercial trout starter at levels of 0.125 and 0.25 g / kg. They found that the number of rainbow trout that died during the first 4 weeks of rearing was significantly reduced in the tanks receiving either of the yeast culture-supplemented diets compared with the tanks receiving a control starter diet. They also found that weight and weight gain were significantly and consistently greater in the tanks fed the 0.25 g / kg yeast culture diet also mortality rates were significantly less only in the tanks receiving 0.25 g kg)1 yeast culture supplementation. Individual fish lengths and weights at the end of the second trial were significantly greater in the tanks receiving either level of

yeast culture compared with the control diet only in 1 year. Total tank weights and feed conversion were not significantly different between any of the treatments.

EL-Haroun et al., 2006 conducted an experiment to examine probiotic treatment in the fingerling diet of Nile tilapia *Oreochromis niloticus* (L.). A total of 240 of Nile tilapia fingerlings (weight ranged from 22.96 to 26.40 g) were divided into five experimental groups. The experiment was conducted for 120 days. Experimental diets were identical in all, except for the variation in probiotic levels. A probiotic (Biogens) was used at 0% (diet 1), 0.5% (diet 2), 1.5% (diet 3), 2.0% (diet 4) and 2.5% (diet 5) inclusion rates in the experimental diets. They found that The growth performance and nutrient utilization of Nile tilapia including weight gain, and specific growth rate were significantly ($P \leq 0.01$) higher in the treatment receiving probiotic (Biogens) than the control diet.

Kumari and Sahoo, 2006; Irianto and Austin, 2002 and Villamil et al., 2002 reported that the capacity of yeast or structural polysaccharides to improve disease resistance in fish showed their capacity to reduce mortalities associated with infection by pathogens such as *Aeromonas* and *Vibrio anguillarum.*

Sajeevan et al., 2006 demonstrated that marine yeast *Candida sake* at 10% in diet (w/w) may be used as an effective source of immunostimulants in *Fenneropenaeus indicus*.

Taoka et al., 2006 who investigated the effect of live and dead probiotic cells on the non-specific immune system of Nile tilapia. They found that probiotic including *S. cerevisiae*, *Bacillus subtilis*, *Lactobacillus acidophilus*, and *Clostridium butyricum* treatment enhanced non-specific immune parameters such as lysozyme activity, migration of neutrophils and

plasma bacteriocidal activity, resulting in improved resistance to *Edwardsiella tarda* infection.

Waché et al., 2006 tested of two strains of *Saccharomyces cerevisiae* as probiotics on rainbow trout, *Onchorhynchus mykiss,* fry during the first month of feeding. Each strain was introduced into separate diets, at the rate of 10^6 CFU/g and their effects were compared with those of the control diet. they found that the addition of *Saccharomyces cerevisiae* improved diet and protein digestibility, which may explain the better growth and feed efficiency.

Magnadóttir, 2006 reported that the innate immune system was the only defence weapon of invertebrates and a fundamental defence mechanism of fish, and the main parameters of the innate system were commonly divided into physical parameters, cellular and humoral factors.

Gatesoupe, 2007 said that yeasts can stimulate the immune response in fish. ß-glucans is likely the most important compound in this regard, but some other cell-wall components or soluble factors may also play a role. Both cellular and humoral responses have been educed by dietary yeast, depending on the experimental conditions. Other benefits may be expected for the host, especially the intestinal colonization of early feeding fry with yeast, which may have some effect on development, e.g. by accelerating the maturation of the digestive system. In older fish, dietary yeast may stimulate metabolism and growth.

Panigrahi et al., 2004., Misra et al., 2006; Bonaldo et al., 2007 and Yuan et al., 2008 reported that Lysozyme activity in fish increased after fed supplemented probiotic, or injection of β-glucan and polysaccharides.

Martha et al., 2008 found that the live yeast *Debaryomyces hansenii* strain CBS 8339 appears to have a strong positive impact on the immune

system in juvenile leopard. Diet supplementation with this yeast resulted in lower mortality rates by enhancing humoral and antioxidant immune responses represented by the activity of the superoxide dismutase enzyme.

Marzouk et al., 2008a indicated that *Bacillus subtillis* and *saccharomyces cerevisiae* frequently used probiotics that are able to adhere and colonize the *O.niloticus* gut preventing the adhesion and colonization of specific fish pathogens. Also the diets supplemented with living *Bacillus subtillis*, dead or living and *saccharomyces cerevisiae* improve the non specific immune response which reflected on the stimulation of macrophage cells and increasing their phagocytic activity. histologically, the spleen and liver showed great activation of melano-macrophage centers and kupffer cells. The probiotics fed fish groups showed high resistance to the challenged pathogenic microorganisms.

Marzouk et al., 2008b pointed out that the diets supplemented with living *Bacillus subtillis*, dead or living and *saccharomyces cerevisiae* frequently used probiotics that are able to improve the growth parameter (body weight gain, feed conversion ratio and protein efficiency ratio). on the other hand, the examination of the intestinal microbial flora pre and post addition of probiotics showed that, the intestinal of the group fed on diet supplemented with live *Bacillus subtillis* at the end of experimental period showed failure in reisolation of some pathogenic bacteria. Dead *saccharomyces cerevisiae* supplemented diet has no effect. Histological, many oval individual cells of yeast colonizing the intact intestinal epithelium and free in the lumen.

Mohsen et al., (2008) reported that *Saccharomyces cerevisiae* yeast positively enhanced growth performance and feed utilization of Nile tilapia

as well as its resistance to *A. hydrophila* infection and the optimum level of dietary live bakers' yeast is about 1.0 g per kg diet.

Edskes et al., 2009 mentioned that *S. castelli* has URE2 gene in which it was toxic to *Escherichia coli*.

Barnes and Durben 2010 evaluated the addition of a proprietary, fully-fermented yeast Saccahromyces *cerevisiae* culture supplement during long-term feeding of McConaughy strain rainbow trout *Oncorhynchus mykiss*. Beginning at initial feeding and continuing for 408 days of hatchery rearing, the trout received either a commercially-manufactured feed or the same feed containing 0.125 g kg−1 *Saccahromyces cerevisiae*. This study was conducted at a production level as part of normal (real-world) hatchery operations, with the fish periodically inventoried and moved into different rearing units. Although no rearing-tank replication occurred during the first 54 days of feeding, multiple tanks and raceways were used thereafter. Fish in rearing units receiving *Saccahromyces cerevisiae* supplementation exhibited less mortality, particularly during the earlier rearing stages. During the final 177 days of rearing in six raceways, *Saccahromyces cerevisiae* fed McConaughy strain trout were significantly larger and had a significantly improved feed conversion ratio. The overall feed conversion ratio for the entire duration of the study was 1.17 in the fish receiving DVAqua supplementation compared to 1.21 in the control group.

El-Boshy et al., 2010 mentioned that, serum bactericidal activity in the fish injected with different dosages of β -glucan was always significantly higher than in control.

Osman et al., 2010 evaluated that the effect of dietary commercial brewer's yeast, (Beaker's yeast), *Saccharomyces cerevisiae* on growth performance, survival and immunostimulation of Nile tilapia, *Oreochromis*

niloticus. Brewer's yeast supplemented at 0, 1, 2, 3 and 6 gm/kg diet. Each diet was fed to triplicate group of *O. niloticus* with initial body weight at 77.39 ± 5.33 g at 8 weeks feeding period. Control group fed non supplemented diet at total period of experiment. They found that the optimum growth performance were obtained with 3.0 g yeast/kg diet. Also they found that Physiological and biochemical parameters (RBCs count, Hb concentration, HCT value, glucose and lipids of fish), cellular immune parameters (total leucocytic count, phagocytic activity) and hormonal immune parameters (Total protein, albumin, globulin and lysozyme concentration) were significantly elevated than the control group and improved in *O. niloticus* fed brewer's yeast up to 3.0 g/kg diet. After experimental period (8 weeks) fish from each group were challenged by pathogenic *Aeromonas hydrophila* IP, kept under observation for 7 days, and found that the mortality percent decreased with the increase of yeast level in fish diets.

Tukmechi et al., 2011 reported that diets supplemented with whole cell yeast (*Saccharomyces cerevisiae*) in all treatment groups significantly promoted the growth performance compared to control group. A significantly increase was also observed in immune responses in juvenile fish fed beta-mercapto-ethanol (2ME) treated yeast diet. More ever, the lowest fish mortality was obtained in this treatment group. The present results show that a diet supplemented with 2ME-treated yeast stimulates the immune system and growth of juvenile rainbow trout thus enhancing their resistance against *Yersinia ruckeri*.

Welker et al., 2011 found that diets supplemented with yeast or yeast subcomponents as commercial preparations of β-glucan and mannan oligosaccharide or whole-cell *Saccharomyces cerevisiae* at the

manufacturers' recommended levels appears to have a strong positive impact on the immune system in channel catfish. Antibody titres in the second week feeding group were significantly higher *E. ictaluri* challenge in relation to catfish fed with the control diet and increased the lysozyme activity.

Zargham et al., 2011 evaluated that the effect of an allochthonous strain, Saccharomyces cerevisiae PTCC5052 was investigated purely as a probiotic in the case of increasing percentage of survival and enhancing the growth factors of Rainbow trout (Oncorhynchus mykiss) larvae during winter 2010 in shahid Motahhary aquaculture research center that is a new experiment on rainbow trout larvae in Iran. Feeding trial was conducted for 7 weeks since start feeding of larvae. They found that the weight gain, length enhancement, and specific growth rate (SGR) in all of the treatments are significantly higher than the control group ($P<0.05$). and also they reported that that using this type of probiotic would be effective in rainbow trout larviculture.

Material and methods

1. Materials:

1.1. Fish:

1.1.1. Fish for yeasts isolation:

Forty five fish (ten of Nile tilapia (*Oreochromis niloticus*), ten of common carp (*Cyprinus carpio*), ten of silver carp (*Hypophthalmichthys molitrix*) and ten of Cat fish (*Clarias Garipeneaus*) and five from Snail carp (*Mylopharyngodon piceus*)) with different body weight were randomly collected from the production ponds of Central Lab for Aquaculture Research in Abbassa, Abu-Hammad, Sharkia Governorate, Egypt. Fishes were transported to Fish Diseases dry Lab for yeasts isolation.

1.1.2 Fish for safety of isolated yeast:

One hundred and twenty fish (*O. nilotecus*) apparently healthy were collected randomly with body weight 30-33g from Abbassa Fish Farm for study the safety of the isolated yeasts to fish.

1.1.3. Fish for feeding experiments:

Two hundreds and twenty five apparently healthy *O. niloticus* (20 g body weight) were collected randomly from Abbassa Fish Farm for feeding experimental study.

1.2. Aquaria:

Twelve full prepared glass aquaria (77×37×48cm) were holding to study the safety of the isolated yeasts. Another fifteen glass aquaria (77×37×48cm) were holding for feeding experiment. The aquaria were supplied with chlorine free water and aerators.

1.3. Media :

1.3.1. Media used for isolation and identification of yeast:

Media used for isolation and identification of yeast according to (**Barnett et al., 2000**).

A. Sabouraud' Dextrose agar medium (Britania).

B. Corn-meal agar medium (CMA):

Crushed Yellow corn-meal	12.5 g
Agar	3.8 g
Distilled water	300 ml
pH 5.2	

C. Gorodkowa agar medium:

D-glucose	1.0 g
Peptone	10.0 g
NaCl	5.0 g
Agar	20.0 g
Distilled water	1000 ml
pH 5.2	

D. Sugar fermentation medium:

Two percent of sugar (glucose, galactose, sucarose, maltose, salicine, xylose, arabinose, lactose, trehalose and manitol) were prepared in 1% peptone water, bromothyamol blue was added as an indicator.

E. sugar assimilation medium:

Ammonium sulphate	5.0 g
Potassium dihydrogen phosphate	1.0 g
Magnesium sulphate	0.5 g
Agar	20.0 g
Distilled water	1000 ml

Cooked and sterilized at 121^{o}C by autoclaving for 15 min.

F. nitrate assimilation medium:

Glucose	20 g
Potassium dihydrogen phosphate	1.0 g
Magnesium sulphate	0.5 g
Agar	20.0 g
Distilled water	1000 ml

Cooked and sterilized at 121^{o}C by autoclaving for 15 min.

G. Yeast Glucose Peptone Broth medium:

Glucose	20.0 g
peptone	10.0 g
yeast extract	5.0 g
Distilled water	1000 ml

Autoclaved at 121 ^{o}C for 15 min.

H. Peptone water

Peptone	10.0 g
Sodium chloride	5.0 g
Distilled water	1000 ml

Autoclaved at 121 ^{o}C for 15 minutes.

1.3.2 Tryptone-glucose yeast agar medium for bacterial counting (APHA, 1995)

Tryptone	5.0 g
Yeast extract	2.5 g
Sodium chloride	5.0 g
Dextrose	1.0 g
Agar	15.0 g
Distilled water	1000 ml

PH 7.0

Autoclaved at 121 ^{o}C for 15 minutes.

1.3.3Tryptic Soya Broth medium for bacterial growth (Difico)

1.3.4Tryptic Soya Agar (Difico)

1.4.Reagent, Chemicals, antibiotcs and Stains:

-Nitro Blue Tetrazolium Chloride (PARK)

-Logul´s iodine

-Bromothymol blue

-*Micrococcus lysodeikticus* (ATCC No. 1698 Sigma)

- N-N-dimethyl formamide (Adwic).

- Heparin 5000I.U./ml (Nile Co.)

-Hematocrit capillaries.

-Buffer physiological saline.

- Ciprofloxacin antibiotic.

-Oxytetracycline (sigma).

-Fungistatin. (Delta Pharma).

2. Methods

This study was carried out in The Central Laboratory for Aquaculture Research at Abbassa, Agriculture Research Center, Ministry of Agriculture.

2.1. Yeast isolation:

Under complete aseptic condition, 1 g of (muscles, liver, kidney and intestine) was homogenized in mortar containing 9 ml of sterile 0.1% peptone water. One ml of homogenate was inoculated in Sabaurud' Dextrose agar containing antibiotic and incubated at 25^{o}C for three days according to **(ICMSF 1978).** The yeast culture was re-inoculated again in Sabaurud' Dextrose agar until obtain separated colonies of yeast.

2.2. The identification of yeast: (Barnett et al., 2000).

2.2.1. Colony character:

Shape, color, texture, edge, raising and diameter of separated colonies were recorded according to **(Barnett et al., 2000).**

2.2.2. Microscopical examination:

The purpose of examining vegetative yeast cells microscopically was the determination of cell shape, filamentous formation and budding. Yeast culture at one day old was inoculated into 30 ml of sterile yeast

glucose peptone broth in a 100 ml conical flask. The microscopical examination was had occurred after incubation at 25◦C for 2-3 days.

2.2.3. Ascospores examination:

fresh culture, actively growing over night, or 2 days, at 25^{o}C is inoculated into Gorodkowa agar and incubated at 25^{o}C for 3 days and examined microscopically for ascospores formation. The yeast isolates which, ascospores were not seen are incubated further at 25^{o}C and examined every week for at least 6 weeks.

2.2.4. Sugar fermentation:

Sugar media contained 2% of (glucose, galactose, sucarose, maltose, salicine, xylose, arabinose, lactose, trehalose and manitol) were inoculated with suspected isolates and incubated for 3 days at 25^{o}C. Bromothymol blue was used as indicator. Positive sugar fermentation was indicated by production of acid and gas or acid only.

2.2.5. Sugar assimilation:

Sterilized filter paper discs were saturated with solution contained 2% of sugar (glucose, galactose, sucrose, maltose, salicine, xylose, arabinose, lactose, trehalose and manitol), used and gently pressed on the agar surfaces (specific media for sugar assimilation) inoculated with the examined yeast using sterile forceps, incubated at 25^{o}C for 2-4 days. The presence of growth around the disk gave indication of sugars utilization.

2.2.6. Nitrate assimilation:

Sterilized filter paper discs were saturated with solution contained 2% potassium nitrate were used for detecting nitrate assimilation. A specific medium for nitrate assimilation was inoculated with the examined yeast and the nitrate discs was used and fixed on the agar surface. The presence of nitrate was examined after incubation at 25^{o}C for 2-4 days.

2.2.7. Production of extra cellular starch like compound:

This test helps to identify certain species, which characteristically form extra cellular starch-like polysaccharide, forming a blue complex with iodine. The examined yeast was inoculated in sugar medium contained glucose and incubated at 25^{o}C for 2-3 days. One drop of logul´s iodine solution was added to a positive growth medium and checked the medium color changes. A blue, purple or green color indicates that the examined yeast produced starch.

2.2.8. Growth at high osmotic pressures:

Certain yeasts are able to grow in media with high concentrations of sugar or salt. Slopes are prepared of yeast extract agar, some contained 50 and 60% (w/v) D-glucose and 5, 10 and 16% (w/v) NaCl. The slopes are inoculated lightly by streaking of examined yeasts, incubate at 25^{o}C and examined for growth for up to 4 weeks.

2.3. Antibacterial activity of yeasts in vitro:

The antibacterial activity of isolated yeasts was carried out using disk diffusion methods to study the antibacterial activity of yeasts against harmful bacteria in fish, according to (**Gonsales *et al.*, 2006**). The bacterial strains which were used for testing antibacterial activities of isolated yeasts and artificial antibiotic discs were obtained from Fish Diseases Department, Central Lab for Aquaculture Research in Abbassa. These bacterial strains were isolated previously from diseased Nile tilapia (*Oreochromis niloticus*). The pathogenicity of these bacterial strains was tested and the results confirmed that most bacterial strains were pathogenic for *O. nilotecus*. These bacterial isolates were *Aeromonas hydrophila, Aeromonas veronii, Aeromonas sobria, A. jandaei, Pseudomonase anguilliseptica, Pseudomonas fluorescence, Citrobacter frundii, Enterobacter spp., Aerobacter spp., Moraxalla kingil* and *Actinobacter anitratus.* Disks of sterile filter paper were immersed in yeast culture and then inoculated on TSA (Treptic Soya Agar). The yeast culture was incubated at 25^{o}C for 48 hours, after that 1 ml of TSB containing the bacterial strains at 24 h live was added. The inhibition zones were determined after incubation at 30^{o}C for 24 h.

2.4. Sensitivity test using Antibiograms discs:-

Sensitivity of the previous bacterial strains to Antibiograms (Pencilin G, Ciprofloxacin, Kanamycin, Streptomycine, Erythomycin, Vancomycin, Ampicillin, Negram, Tetracycline, Amoxil and Septrin) was carried out using disc diffusion method. The culture of the tested organism was flooded on the surface of tryptic soy agar and the excess were removed. As well as each of the above antibiotic disks were used and gently pressed on the agar

surfaces using sterile forceps. The plates were incubated at 30˚C for 24 hrs. The results were interpreted according to the criteria given by (**Finegold and Martin, 1982**).

2.5. Minimal inhibition concentration determination (MIC):

The minimum inhibitory concentration, of the yeasts to the pathogenic bacteria was determined by broth micro dilution method. 0.5 g of the yeast was suspended in 1 ml distilled water. Serial dilutions from (1:10) using tryptic soya broth (TSB) was prepared for each isolate, and inoculated with one loopful of *A. sobria* or *Pseudomonase flurescence* broth (24 h live). The inoculum's containing 10^2 bacterial cells. Test tubes were incubated at 30^oC for 18–24 hrs and checked for bacterial growth. A loopful, from each tube, was streaked on TSA to check the bacterial growth. The plates were incubated at 30^oC for 24 hrs according to (**Ruangpan et al., 1997**).

2.6. Safety of the isolated yeasts to *Oreochromis niloticus*:

One hundred and twenty apparently healthy *O. nilotecus* were divided into four groups after acclamization for two weeks to study the safety of isolated yeasts which, had antibacterial activity against the previous bacteria. Each group divided into three replicas, each contained 10 fish. Fish of Groups (G1, G2 and G3) was injected Intrapretonial (IP) with 0.2 ml $\times 10^5$ cells/ml of 24 hours lives of the three yeast isolates. Group 4 was injected IP with sterile saline as a control group. The fish was recorded for mortality and clinical signs for 14 day. All groups received Ciprofloxacin in water medication 50ppm. This experiment was carried out in winter (under stress).

Re-isolation of injected yeasts was occurred from freshly dead and moribund fish. (**Shaheen, 1991)**

2.7. Yeasts growths curve:

One suspected colony at 24 hrs live was inoculated in 10 ml corn-meal medium and incubated at 25^{o}C for 48hrs. The ten ml culture yeast was inoculated in 100 ml corn-mail medium and incubated at 25^{o}C. Medium turbidity was increased by increasing cultured yeast growth. The optical density of growth culture was measured every 24 hrs by using a spectroFigmeter at wave length 620 nm according to **(Polonelli et al., 1997).**

2.8. Yeast biomass Production:

A yeast culture at 48 h grow in 400 ml corn-meal medium was inoculated in 4 liters corn-meal medium and incubated at 25^{o}C. Non pathogenic yeast cells were harvested at 4th day according to the growth curve by centrifugation at 3000 rpm for 15 minuets according to **Peter et al. (1994).** The pathogenic yeast was harvested after ad 0.2% formaline with 24 hrs incubation to kill yeast cells. The sterility of yeast was tested by replicates inoculation of formalized yeast in three Petri dishes contained sabaurud's agar medium and incubated at 25^{o}C for 2-3 days. The dead yeast cells were harvested by centrifugation at 3000 rpm for 15 minuets and washed two times by saline (0.85% NaCl).

2.9. Yeast dry weight:

10 ml of saline soln containing yeast cells (of each) were put on filter paper with known its weight and dried in oven at 60ºC. The filter paper with resulting powder was weight until gave five fixed weight respectively. The difference between the filter paper weight alone and the filter paper weight with yeast powder was gave the amount of dry yeast in 10 ml saline contained the yeast cells. The data were given as mg/100ml yeast according to (**Leganes et al. 1987**).

2.10. Yeasts probiotic activities:

2.10.1. Diet preparation:

Commercial basal diet (crude protein 30%) was crushed, and then divided into five parts. The first part was basal diet mixed with sterile saline as a control group. The second and third parts were basal diet mixed with (5 and 10 g of yeast / kg diet) of living harmless yeast. The fourth and fifth parts were basal diet mixed with (5 and 10 g of yeast / kg diet) of whole died cells of harmful yeast. The diets were reformed into pellets; spread to air dry and stored at 4˚C for the feeding experiment. Anther part of diet which contained live yeast was stored at room temperature, for study yeast viability according to (**Ortuño et al., 2002**).

2.10.2. Viability of living yeast:

The diet containing living yeast will stored in room temperature and other part of diet was stored at 4^{o}C. The viability of the yeast in the diets was assessed every week for 4 weeks after that every month following storage.

For this 1 g of diet was homogenized in 9.0 ml of sterile saline and 6-fold serial dilutions of this suspension prepared in saline and 0.1 ml of each dilution was spread onto triplicate plates of sabaurud's dextrose agar and the colony count was determined after 4 days incubation at 25°C according to (**ICMSF 1978**).

2.10.3. Feeding experimental:

Two hundreds and twenty five *Oreochromis niloticus* (20±2 g/fish) were randomly collected from earth ponds of Abbassa Fish Farm. The fish were acclimatized for two weeks, and then divided into five equal groups. Fifteen glasses aquaria (60x50x70 cm), the aquaria were supplied with chlorine free water and continuous aeration using air pumping compressors. Each group, in three replicates, each contained 15 fish. The first group (T_1) fish was fed with the control diet (basal diet free from yeast cells). The second and third groups were fish fed with basal diet contained (5 and 10 g yeast/kg diet) whole living harmless yeast cells (T_2 and T_3). The fourth and fifth groups were fish fed with basal diet containing (5 and 10 g yeast/kg diet) whole died harmful yeast cells (T_4 and T_5). The fish were hand-fed for 28 days at a daily rate 3% of body weight. The water of the aquaria was changed daily. The fish were weighed in 0, 7, 14, 21 and 28 day from the beginning of the feeding experiment.

2.10.3.1. Growth performance

The average weight-gain (AWG), specific growth rate (SGR), feed conversion ratio (FCR) and feed efficiency ratio (FER) were calculated according to the following equations:

AWG (g/fish) = Average final weight (g) _ Average initial weight (g)/experimental period (day)

SGR (%/day) = 100(In final body weight (g) _ ln initial body weight (g))/experimental period (day)

FCR = Feed intake (g) /weight gain (g)

FER = Body weight gain (g)/Feed intake (g)

Organ-somatic index:

The fish were killed by rapid cervical chopping, and the fish were weighed. The liver and spleen were removed and weighed. Moreover, the hepatosomatic and splenosomatic indices were calculated according to **(Fox, et al., 1997).** Organ-somatic index = (organ-weight (g) / bodyweight (g)) x 100.

2.10.3.2. Blood and serum sampling:

At the second week and the fourth week of the feeding experiment, the fish were anaesthetized by immersing the fish in water containing 0.1 ppm tricaine methane sulphonate (MS-222) and 0.5 ppm sodium bicarborate for neutralize anesthesia toxicity. Blood-samples were collected from the caudal vein of fish, by using needles previously rinsed in heparin (15unit/ml) for the evaluation of hematocrit value and respiratory burst activity. For plasma separation the heparinzed blood was centrifuged at 1000 rpm for 5 minutes. The plasma was stored at -20˚C in screw cap glass vials until used for lysozyme and Serum bactericidal activities. For separation serum, 0.5 ml blood samples were withdrawn from caudal vein from fish that challenged

by pathogenic bacteria *A. sobria* and *Ps. fluerscence* into blood eppendorf tubes. The eppendorf tubes contain blood sample were centrifuged at 3000 rpm for 15 min and the supernatant serum was collected. The serum was stored at -20˚C in screw cap glass vials until used to determine antibody titer.

2.10.3.2.1. Hematocrit level:

A haematocrit is a method which used to determine the volume of packed cells in the blood. The haematocrit value will vary, depending on the health and physiological condition of the individual fish. Hematocrite capillary tubes are filled 2/3 with whole blood, tube were centrifuged in hematocrite centrifuge for 5 minute, after centrifugation. The percentage of erythrocyte volume is measured by hematocrite tube reader **(Smith, 1967)**.

2.10.3.2.2. Respiratory burst activity by measuring nitroblue titrazolium activity (NBT):

The NBT (yellow) is reduced to formazan (blue) in the reaction with oxygen radicals from neutrophils and monocytes, the analysis of the production of oxygen radicals by the use of NBT can done by spectroFigmeter.

0.1ml blood was placed into microtiter plate then equal amount of 0.2% NBT solution was added and incubated for 30 min at room temperature, 0.1 ml of NBT blood cell suspension was taken and added to a glass tube contain 1 ml N, N- dimethyl formamide and centrifuged for 5 min at 3000 rpm, the supernatant fluids was read in spectroFigmeter at 620 nm in 1 ml cuvettes **(Siwicki et al. 1985).**

2.10.3.2.3. Lysozyme activity:

The lysozyme activity was measured using Figelectric colorimeter with attachment for turbidity measurement. A series of dilution was prepared by diluting the standard lysozyme from hen egg-white (Fluka, Switzerland) and mixed with *Micrococcus lysodeikticus* (ATCC No. 1698 Sigma) suspension for establishing the calibration curve. Ten ml of standard solution or serum were added to 200 ml of *Micrococcus* suspension (35 mg of *Micrococcus* dry powder/95 ml of 1/15 M phosphate buffer 5.0 ml of NaCl solution). The changes in the extinction were measured at 546 nm by measuring the extinction immediately after adding the solution which contained the lysozyme (start of the reaction) and after a 20 min incubation of the preparation under investigation at 40˚C (end of the reaction). The lysozyme content is determined on the basis of the calibration curve and the extinction measured **(Schaperclaus et al. 1992)**.

2.10.3.2.4. Serum bactericidal activity (SBT):

The SBT integrates both pharmacokinetic and pharmacodynamic properties in a single set of determinations that examines the ability of the fish's serum. Bacterial cultures of *A. sabria* and *Ps. fluorescence* were centrifuged and the pellet was washed and suspended in phosphate buffer saline. The optical density of the suspension was adjusted to 0.5 at 546 nm. This suspension was serially diluted (1:10) with PBS five times. Serum bactericidal activity was determined by incubating 2 µl of this diluted bacterial suspension with 20 µl of serum in a micro-vial for 1 h at 37˚C. In the bacterial control group, phosphate buffer saline replaced the serum. After incubation, the number of viable bacteria was determined by counting the colonies after culturing on TSA plates for 24 hrs at 37˚C.

2.10.3.5. Total bacterial count of fish intestine:

Three fish samples from each replicates were collected randomly and under complete aseptic condition the fish samples were dissected and weighted one gram of intestine and grinding with 9.0 ml sterile saline. Six-fold serial dilutions of this suspension prepared in saline and 0.1 ml of each dilution was spread on to Tryptone-glucose yeast agar medium as recommended by **APHA (1995).** The colonies were counted after incubation at 30oC for 48 hours.

2.10.4. Challenge test:

At the end of the feeding experiment, the fish of each group were divided into three subgroups (distributed in 3 aquaria). The fish were challenged by intraperitoneally injection with 0.5 ml 10^7 cells of 24 h cultures of live *Aer. sobria, and Ps. fluorescence*. The challenged fish were kept under observation for 14 days. The moribund fish was used for bacterial re-isolation. The mortalities were recorded and the relative level of protection (RLP) among the challenged fish was determined according the following question:

RLP =1 -[percentage of treated mortality/ percentage of control mortality] x100.

2.10.5. Indirect hemagglutination activity (Antibody titre):

The Indirect hemagglutination is based on the principle that surface of the erythrocytes can be changed or sensitized with an antigen. If these erythrocytes react with antigen-specific antibodies, there occurs a distinctly perceptible agglutination **(**hemagglutination**).** A dilution sequence from the antigen extract under consideration is prepared in buffer physiological saline and an equal quantity of erythrocytes suspension is added to each dilution

stage. The preparation is shaken and kept for 1-2 hours in the water bath at 37^{o}C. Dilution are then prepared from the antiserum and control. An equal amount of erythrocyte suspension and antigen were pipeted into each tube. The preparations were incubated in water bath at 37^{o}C for 30-120 minutes. A hemagglutination occurs with the antiserum and sedimentation occurs with the control serum. The maximum antigen dilution showing hemagglutination has a high titer. The antigen concentration serves to sensitize the erythrocytes in the main investigation **(Schaperclaus et al. 1992).**

2.11. Statistical analysis:

Statistical analysis was performed using the one way analysis of variance (ANOVA). It was performed with SPSS statistical software (version 10.0, SPSS). The data were subjected for test of homogeneity of variances and Duncan post-hoc test. Data were considered significantly different when $P < 0.05$.

Results

Yeast isolation

The collected freshwater fish (*Oreochromis niloticus*, *Mylopharyngodon piceus*, *Hypophthalmichthys molitrix* and *Clarias Garipeneaus*) for yeasts isolations had no clinical signs or postmortem findings. Data represented in table (1) showed the prevalence of isolated yeasts from freshwater fishes. The results indicated that the number of infected *Oreochromis niloticus*, *Mylopharyngodon piceus*, *Hypophthalmichthys molitrix* and *Clarias Garipeneaus* were 30, 40, 50 and 70% respectively. *Rhodotorula minuta* was isolated from *Oreochromis niloticus* intestine; *Saccharomyces castelli* from *Mylopharyngodon piceus* intestine; *Zygosaccharomyces* sp from *Hypophthalmichthys molitrix* intestine and two different species of *Candida* were isolated from *Clarias Garipeneaus* muscles. It could be noticed that the *Cyprinus carpio* samples were free from yeast.

Table (1) Prevalence of isolated yeasts from freshwater fishes

Fish species	**No., of examined fish**	**Infected fish**		**Organs**	**Yeast species**
		No	%		
Oreochromis niloticus	10	3	30	Intestine	*Rhodotorula minuta*
Cyprinus carpio	10	0	0		non
Mylopharyngodon piceus	5	2	40	Intestine	*Saccharomyces castelli*
Hypophthalmichthys molitrix	10	5	50	Intestine	*Zygosaccharomyces* sp
Clarias Garipeneaus	10	7	70	Muscles	*Candida* spp

Morphological and biochemical characterization of isolated yeasts:

The results revealed that the yeast isolates were suspected as *Saccharomyces castelli*, *Zygosaccharomyces* species, *Rhodotorula minuta*, and *Candida* species**.**

1- The suspected *Saccharomyces castelli* was white to cream color, butyrous, raised, glisten, and circular, not have center, entire edges, ethanol flavor and 3-4 mm in diameter. The vegetative reproduction occurred by budding, no filaments formation as shown in Fig (1) , persistent asci containing 1 or 2 rough, round ascospores as shown in Fig (2). The isolated yeast fermented glucose and failed to ferment galactose, sucrose, maltose, salicine, xylose, arabinose, lactose, trehalose and manitol. It can be grown in the presence of glucose, galactose, sucrose, maltose, salicine, xylose, arabinose, lactose, trehalose and manitol and starch. It was able to assimilate nitrate. It could not form starch. The yeast isolate was able to grow in the presence of 50, 60 % glucose and 5% NaCl. It grew fairly on 10% NaCl and could not grow on 16% NaCl. It grew at 25 and 30^{o}C, while it grew weakly at 35 and 37^{o}C. It did not grow at 40, 42 and 45^{o}C. On the basis of the recommended keys for the identification, following the scheme of identification and characterization of yeast and in view of the comparative study of isolate yeast as shown in table (2) the strain could be identified as *Saccharomyces castelli*.

2- The suspected *Zygosaccharomyces* species was white to cream color, butyrous, raised, glisten, circular, entire edges, ethanol flavor and 2-3 mm in diameter. The vegetative reproduction occurred by budding as shown in Fig (3). The persistent asci formed by conjugation cells, each with 1 or 2 rough and round ascospores as shown in Fig (4). It fermented glucose and failed to ferment galactose, sucrose, maltose, salicine, xylose, arabinose, lactose,

trehalose and manitol. It grew in the presence of glucose, galactose, sucrose, maltose, salicine, xylose, arabinose, lactose, trehalose and manitol and starch. It was able to assimilate nitrate. It could not form starch. The isolate was able to grow in the presence of 50, 60% glucose and 5 and 10% NaCl. It grew weakly on 16 % NaCl. The isolate grew at 25 and 30^{o}C while; it grew weakly at 35and 37^{o}C and can not grew at 40, 42 and 45^{o}C. On the basis of the recommended keys for the identification, following the scheme of identification and characterization of yeast and in view of the comparative study of isolate yeast as shown in table (2) the strain could be identified as *Zygosaccharomyces* species.

3- The suspected *Rhodotorula minuta* was pink color, mucoid to butyrous, raised, glisten, circular, entire edges and 3 - 4 mm in diameter. The vegetative reproduction occurred by budding, no filaments growth and no sexual production as shown in Fig (5 and 6). The isolate failed to ferment glucose, galactose, sucrose, maltose, salicine, xylose, arabinose, lactose, trehalose and manitol. It grew in the presence of glucose, galactose, sucrose, maltose, salicine, xylose, arabinose, lactose, trehalose and manitol and starch. It failed to assimilate the nitrate. It could not secret starch. The isolate was able to grow in the presence of 50, 60% glucose and 5% NaCl while, weakly on 10% NaCl and did not grow on 16% NaCl. The isolate can grow at 25, 30 and 35^{o}C while, it grew weakly at 37^{o}C and did not grow at 40, 42 and 45^{o}C. On the basis of the recommended keys for the identification, following the scheme of identification and characterization of yeast and in view of the comparative study of isolate yeast as shown in table (2) and the strain could be identified as *Rhodotorula minuta*.

4- The suspected *Candida* species (I) was white to cream color, raised, glisten, normal in texture, circular, have dark center, entire edges and pin-

headed in diameter. The vegetative reproduction was by budding, have filaments, elaborate pseudo hyphae as shown in Fig (7 and 8). The isolate fermented glucose, galactose, sucrose, maltose, trehalose and failed to ferment salicine, xylose, arabinose, lactose and manitol. It grew in the presence of glucose, galactose, sucrose, maltose, salicine, xylose, arabinose, lactose, trehalose, manitol and starch. It assimilated the nitrate and did not excreted starch. The isolate grew in the presence of 50, 60% glucose and 5 % NaCl and weakly on 10% NaCl but did not grow on 16% NaCl. The isolate grew at 25, 35, 30 and 37^{o}C while; it grew weakly at 40 and 42^{o}C but did not grow at 45^{o}C. On the basis of the recommended keys for the identification, following the scheme of identification and characterization of yeast and in view of the comparative study of isolate yeast as shown in table (2) and the strain could be identified as *Candida species.*

5- The suspected *Candida* species (II) was white to cream color, raised, glisten, normal in texture, circular, had center, entire edges and 0.2 mm in diameter. The vegetative reproduction occurred by budding, had filaments, elaborated pseudo hyphae as shown in Fig (9 and 10). The isolate fermented glucose, galactose, salicine, sucrose, maltose, trehalose and failed to ferment xylose, arabinose, lactose and manitol. It grew in the presence of glucose, galactose, sucrose, maltose, salicine, xylose, arabinose, lactose, trehalose and manitol and starch. It assimilated the nitrate but it did not form starch. The isolate grew at 50, 60 % glucose and 5 % NaCl. It did not grow on 10 % and 16 % NaCl. The isolate grew at 25, 35, 30 and 37^{o}C and did not grow at 40^{o}C. On the basis of the recommended keys for the identification, following the scheme of identification and characterization of yeast and in view of the comparative study of isolate yeast as shown in table (2) and fig (9 and10) the strain could be identified as *Candida species.*

Table (2): Physical and biochemical characters of the isolated yeast

Items	*Saccharomyces castelli*	*Zygosaccharomyces* species	*Rhodotorula minuta*	*Candida* (I)	*Candida* (II)
Sugar fermentation					
Glucose	+	+	-	+	+
Galactose	-	-	-	+	+
Sucarose	-	-	-	+	+
Maltose	-	-	-	+	+
Salicine	-	-	-	-	+
Xylose	-	-	-	-	-
Arabinose	-	-	-	-	-
Lactose	-	-	-	-	-
Trehalose	-	-	-	+	+
Manitol	-	-	-		
Glucose	+	+	+	+	+
Galactose	+	+	+	+	+
Sucarose	+	+	+	+	+
Maltose	+	+	+	+	+
Salicine	+	+	+	+	+
Xylose	+	+	+	+	+
Arabinose	+	+	+	+	+
Lactose	+	+	+	+	+
Trehalose	+	+	+	+	+
Manitol	+	+	+	+	+
starch	+	+	+		
Nitrate assimilation	+	+	-	+	+
Starch formation	-	-	-	-	-
5%NaCl	+	+	+	+	+
10%NaCl	w	+	w	w	-
16%NaCl	-	w	-	-	-
50%D-Glucose	+	+	+	+	+
60%D-Glucose	+	+	+	+	+
25^{o}C	+	+	+	+	+
30^{o}C	+	+	+	+	+
35^{o}C	w	w	+	+	+
37^{o}C	w	w	w	+	+
40^{o}C	-	-	-	w	-
42^{o}C	-	-	-	w	-
45^{o}C	-	-	-	-	-

(+) positive (-) negative (w) weak

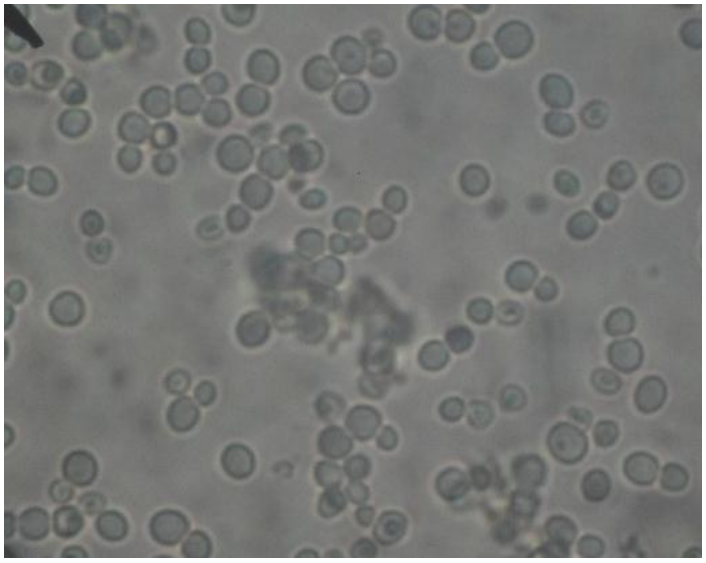

Fig (1): Shows *Saccharomyces castelli* budding, no filaments formation after 72 hours at 25^{o}C on Sabouraud' Dextrose agar medium.

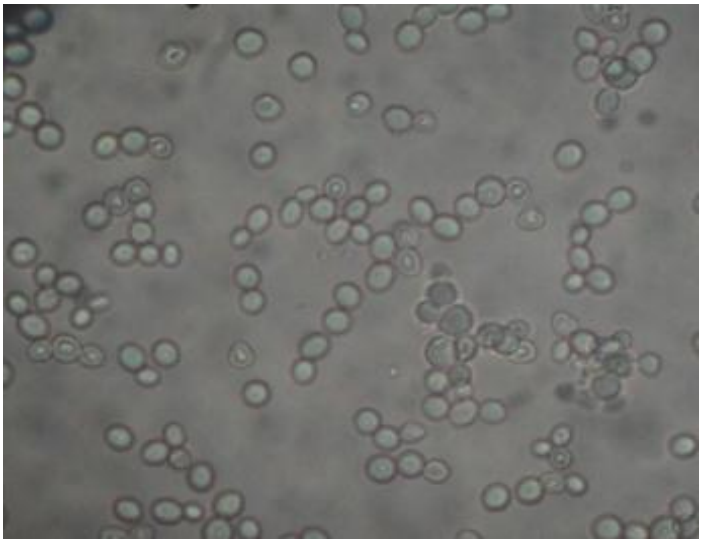

Fig (2): shows *Saccharomyces castelli* asci containing 1 or 2 rough, round ascospores after 48 hours at 25^{o}C on Gorodkowa agar.

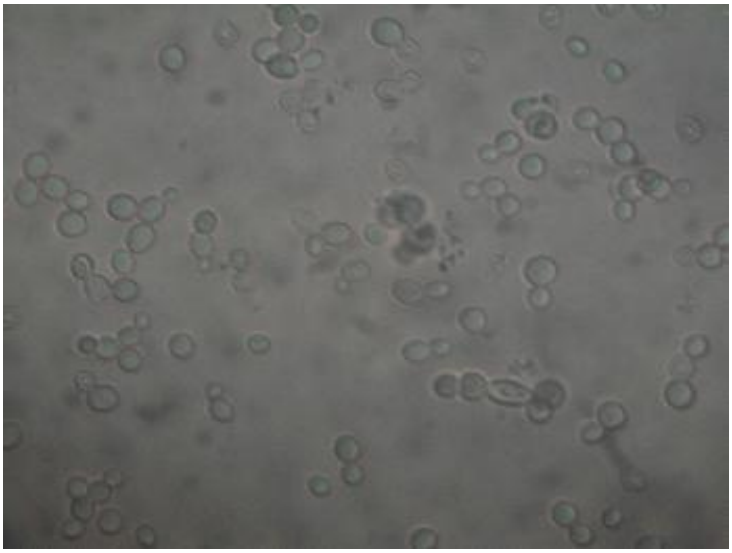

Fig (3): shows *Zygosaccharomyces* budding after 72 hours at 25^{o}C on Sabouraud' Dextrose agar medium.

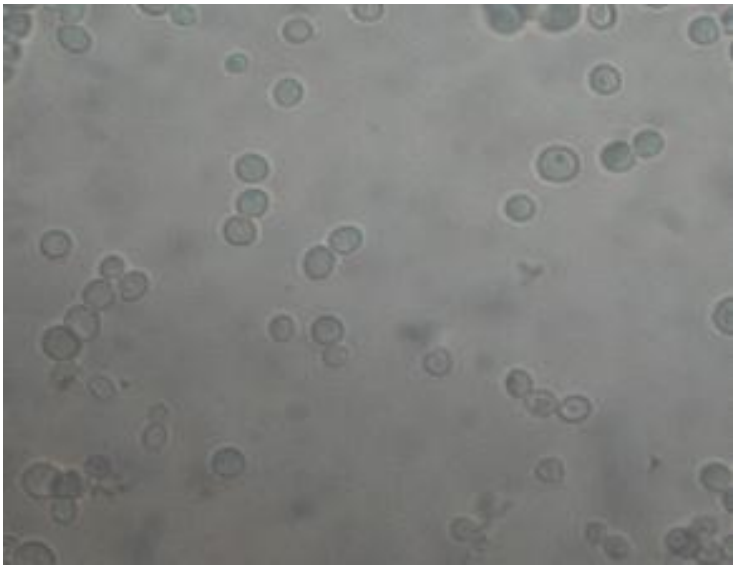

Fig (4): shows *Zygosaccharomyces* asci formed by conjugation cells, each with 1 or 2 rough and round ascospores after 48 hours at 25^{o}C on Gorodkowa agar.

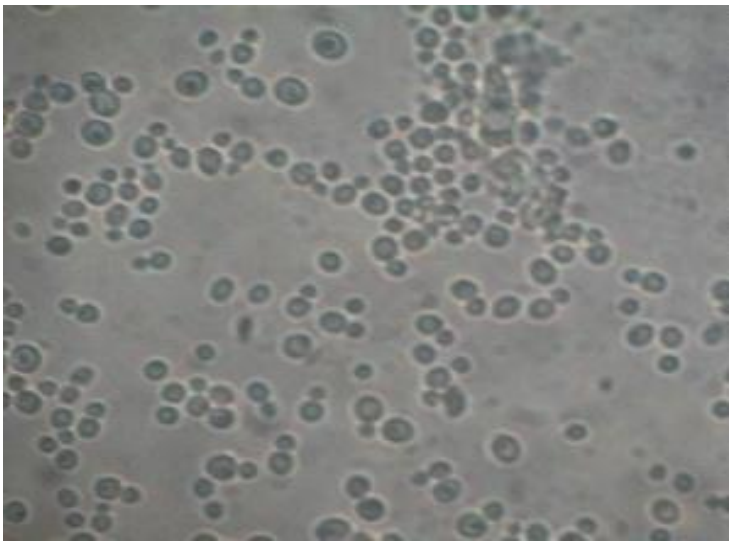

Fig (5): shows *Rhodotorula minuta* budding, no filaments growth after 72 hours at 25^{o}C on Sabouraud' Dextrose agar medium.

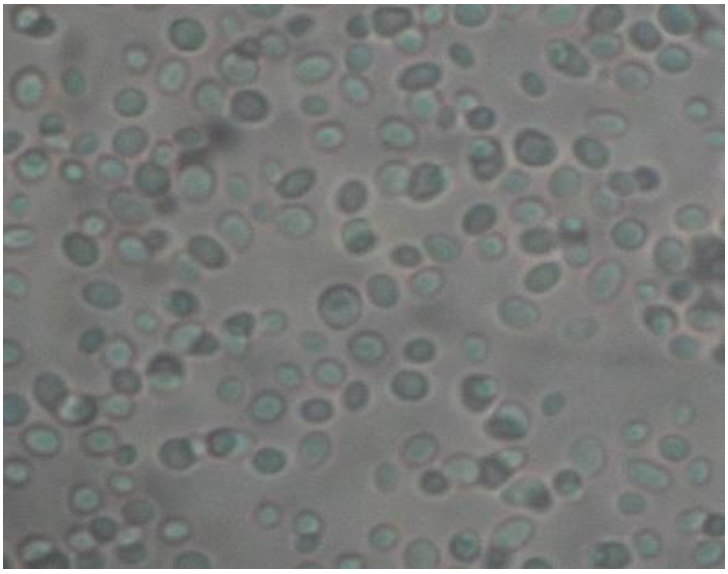

Fig (6): shows *Rhodotorula minuta* no sexual production after 48 hours at 25^{o}C on Gorodkowa agar

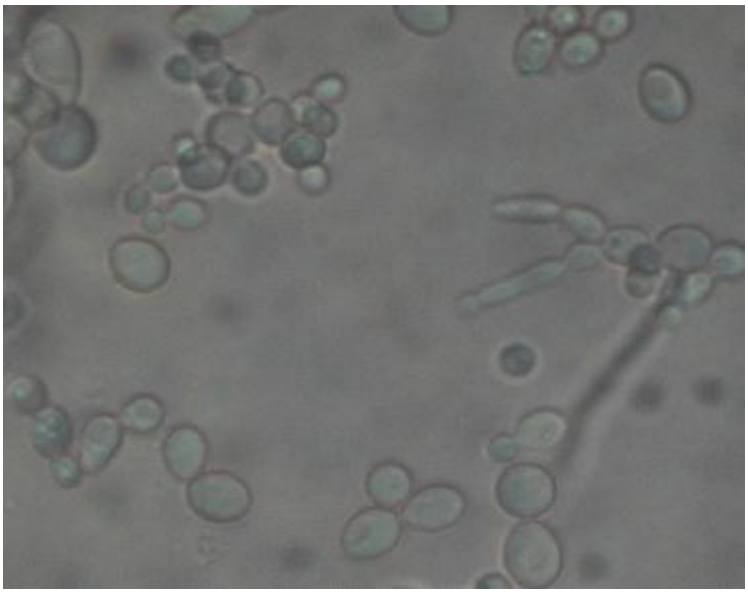

Fig (7): shows *candida* (I) budding, have filaments, elaborate pseudo hyphae after 72 hours at 25^{o}C on Sabouraud' Dextrose agar medium.

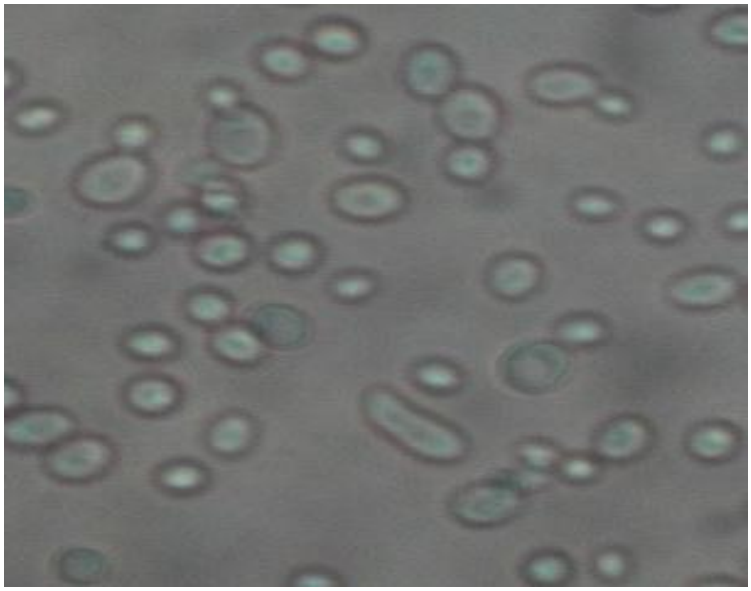

Fig (8): Shows *candida* (I) no sexual production after 48 hours at 25^{o}C on Gorodkowa agar.

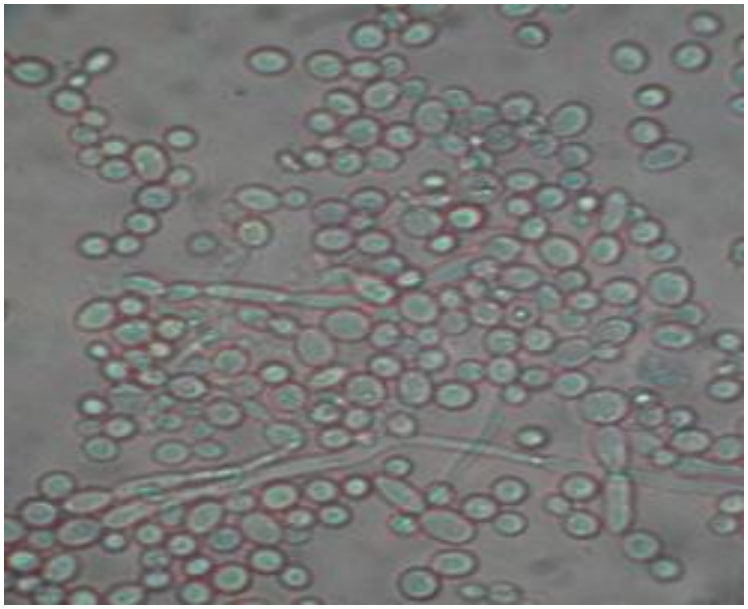

Fig (9): Shows *candida* (II) budding, had filaments, elaborated pseudo hyphae after 72 hours at 25^{o}C on Sabouraud' Dextrose agar medium.

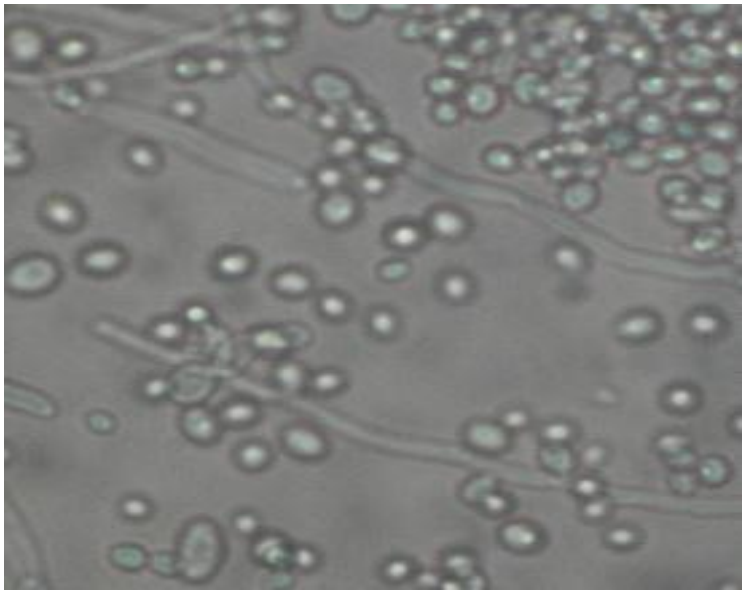

Fig (10): Shows *candida* (II) no sexual production after 48 hours at 25^{o}C on Gorodkowa agar.

Antibacterial activity of yeasts in vitro

Data in table (3), fig (11) and Fig (12) illustrated that *Rhodotorula minuta, Zygosaccharomyces* species and *Saccharomyces castelli* had antibacterial activity against *Aeromonas hydrophila, Aeromonas veronii, Aeromonas sobria, Aeromonas jandaei, Pseudomonase anguilliseptica and Pseudomonas fluorescence*. Where; they had not any effect against *Citrobacter frundii, Enterobacter sp., Aerobacter sp., Moraxalla kingil,* and *Actinobacter anitratus.* The inhibition zones of *Saccharomyces castelli* against *Pseudomonase anguilliseptica, Pseudomonas fluorescence, Aeromonas veronii* and *Aeromonas sobria* were 38, 40, 13 and 42 mm in diameters respectively. On the other hand the inhibition zones of *Zygosaccharomyces* species against *Pseudomonase anguilliseptica, Pseudomonas fluorescence* and *Aeromonas sobria* were 39, 39 and 41.6 mm in diameter respectively. The inhibition zones of *Rhodotorula minuta* against *Pseudomonase anguilliseptica, Pseudomonas fluorescence, Aeromonas veronii, Aeromonas sobria* and *Aeromonas jandaei* were 37, 37, 28, 34 and 30 mm in diameters respectively. The two Candida species had not any activities against the tested bacterial isolates.

Table (3): Inhibition zone diameter of *Saccharomyces castelli, Zygosaccharomyces* and *Rhodotorula minuta* against some pathogenic bacteria.

Bacterial isolates	***Saccharomyces castelli***	***Zygosaccharomyces* species**	***Rhodotorula minuta***
Pseudomonase anguilliseptica	38 ± 0.66a	39 ± 0.33 a	37 ± 3.17a
Pseudomonase fluorecence	40 ± 0.33 a	39 ± 0.66 a	37 ± 2.8a
Aeromonas veronii	13 ± 3.33 b	0 ± 0.00c	28 ± 0.00 a
Aeromonas Sorbia	42.5 ± 1.45 a	41.6 ± 1.66 a	34 ± 2.40 b
Aeromonas jandaei	0	0	30 ± 0.00 a
Aerobacter sp.	0	0	0
Moraxalla kingil	0	0	0
Actinobacter anitratus	0	0	0
Enterobacter sp	0	0	0

Mean ± SE having the same letter in the same row are not significantly different at $P<0.05$.

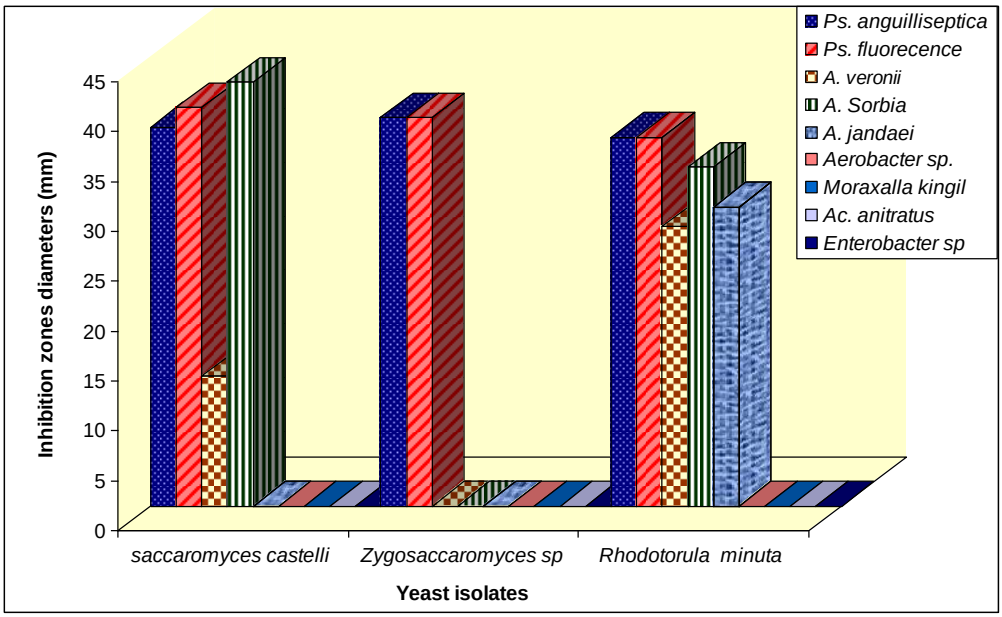

Fig (11): Showed the antibacterial assay of *Saccharomyces castelli*, *Zygosaccharomyces* and *Rhodotorula minuta* against some pathogenic bacteria.

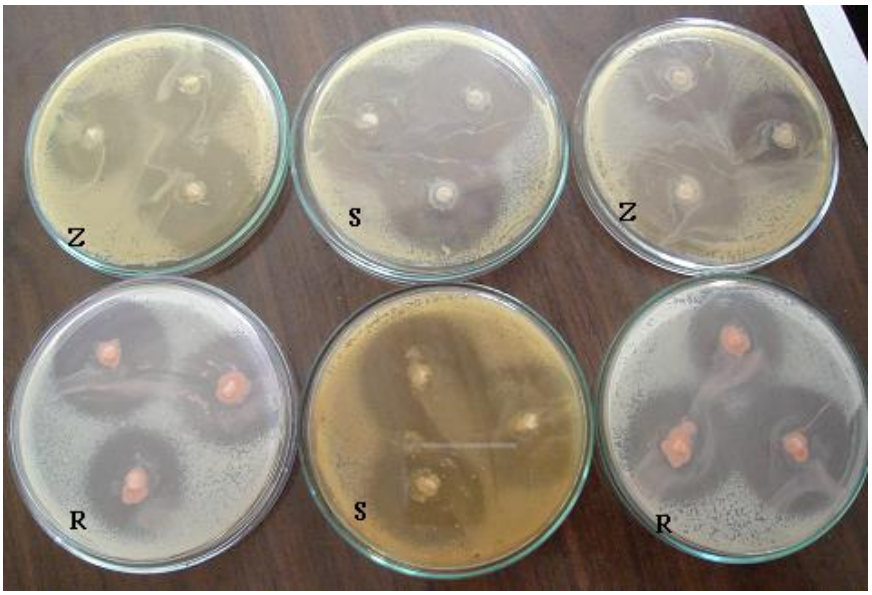

Fig (12): Showed the inhibition zones diameter (mm) of *Saccaromyce castelli* (S), *Zygosaccharomyces* species (Z) and *Rhodotorula minuta* (R) against some pathogenic bacteria on triptic soya agar.

Sensitivity test using Antibiograms disc:

Data in table (4) illustrated that *Aeromonas veronii* resistance to Ampicllin, Erthromycine, Tetracycline, Vancomycin and Pencilin G and sensitive to Ciprofloxacin, Kanamycin and Streptomycin. *Aeromonas Sorbria* resistance to Ampicllin, Erthromycine, Kanamycin, Tetracycline, Vancomycin and Pencilin G and sensitive toward Ciprofloxacin and Streptomycin. *A. jandaei* resistance to Ampicllin, Erthromycine, Streptomycin, Tetracycline, Vancomycin and Pencilin G and sensitive to Ciprofloxacin and Kanamycin. *Pseudomonase anguilliseptica* resistance to Ampicllin, Ciprofloxacin, Kanamycin, Tetracycline, Vancomycin and Pencilin G and sensitive to Erthromycine and Streptomycin. *Pseudomonase fluorescence* resistance to Ampicllin, Tetracycline, Vancomycin and Pencilin G and sensitive to Ciprofloxacin, Erthromycine, Kanamycin and Streptomycin.

Table (4): Sensitivity reaction of the same bacterial isolates which used with yeasts to the antibiogram with the inhibition zone diameters (mm) measurments.

Antibiotic againt	symbol	Concentration	Susceptible zoon	Inhibition zoon (mm) and sensitivity reaction of *bacterial isolates* to antibiograme				
				A. veronii	*A. Serbia*	*A. jandaei*	*Ps. anguillise ptica*	*Ps. fluorecen ce*
Ampicllin	AM	10	≥29	18.3 (R)	14.00(R)	0.00(R)	24.0 (R)	0.0 (R)
Ciprofloxacin	CIP	5	≥21	27.0 (S)	24.33 (S)	25.0 (S)	0.0 (R)	36.0 (S)
Erthromycine	E	15	≥18	0.0 (R)	0.0 (R)	0.0 (R)	23.67 (S)	18.67 (S)
Kanamycin	K	30	≥18	23.0 (S)	11.0 (R)	18.67(S)	10.33(R)	18.33 (S)
Streptomycin	S	10	≥15	28.3 (S)	15.0 (S)	14.0 (R)	25.67 (S)	17.0 (S)
Tetracycline	TE	30	≥19	8.0 (R)	13.0 (R)	14.3 (R)	15.0 (R)	17.33(R)
Vancomycin	VA	30	≥12	0.0 (R)	0.0 (R)	0.0 (R)	0.0 (R)	0.0 (R)
Pencilin G	P	10	≥29	0.0 (R)	0.0 (R)	0.0 (R)	0.0 (R)	0.0 (R)

S= sensitive, R= resistance

Minimal inhibitory concentration determination (MIC):

The MIC of *Saccharomyces castelli* were 0.6, 0.008, 0.008 and 0.8 µg against *A. veronii*, *A. Sobria*, *Ps. anguilliseptica*, *Ps. fluorecence* respectively as showed in table (5).

The MIC of *Rhodotorula minuta* were 0.8, 1.0, 1.0, 0.4 and 1.0 µgm/ml against *A. veronii*, *A. Sobria*, *A. jandaei*, *Ps. anguilliseptica*, *Ps.fluorecence* respectively as showed in table (5).

Table (5): The MIC of *Saccharomyces castelli* and *Rhodotorula minuta* (µg) against five test organisms (at 10^2 bacterial cells).

Yeasts	Minimum inhibitory concentration (µg/10^2 bacterial cells)				
	A. veronii	*A. Sobria*	*A. jandaei*	*Ps. anguilliseptica*	*Ps. fluorecence*
Saccharomyces castelli	0.6	0.008	0.0	0.008	0.8
Rhodotorula minuta	0.8	1.0	1.0	0.4	1.0

Growth curve of yeasts:

The results in (table 6) and (fig 13) showed that the value of optical density due to the growth of *Saccharomyces castelli*, *Zygosaccharomyces* and *Rhodotorula minuta* per day. The growth of yeast was stopped at the 5th and 6th day of growth with *Saccharomyces castelli* and *Rhodotorula minuta* while *Zygosaccharomyces* was at 7th and 8th day. The yeast harvesting was occurred at 4th day of growth.

Table (6): The growth curve of yeasts / day using spectrephtometer (optical density).

Time (day)	*Saccharomyces castelli*	*Zygosaccharomyces* species	*Rhodotorula minuta*
0	0.225	0.0633	0.0967
1	0.418	0.1493	0.1987
2	0.4403	0.3813	0.342
3	0.5613	0.452	0.4583
4	0.6307	0.699	0.591
5	1.0033	0.805	0.7803
6	1.0417	0.8213	0.81
7	1.009	0.853	0.796
8	1.1127	0.9187	0.817
9	1.0637	1.0473	0.852
10	1.0403	1.0233	0.9613

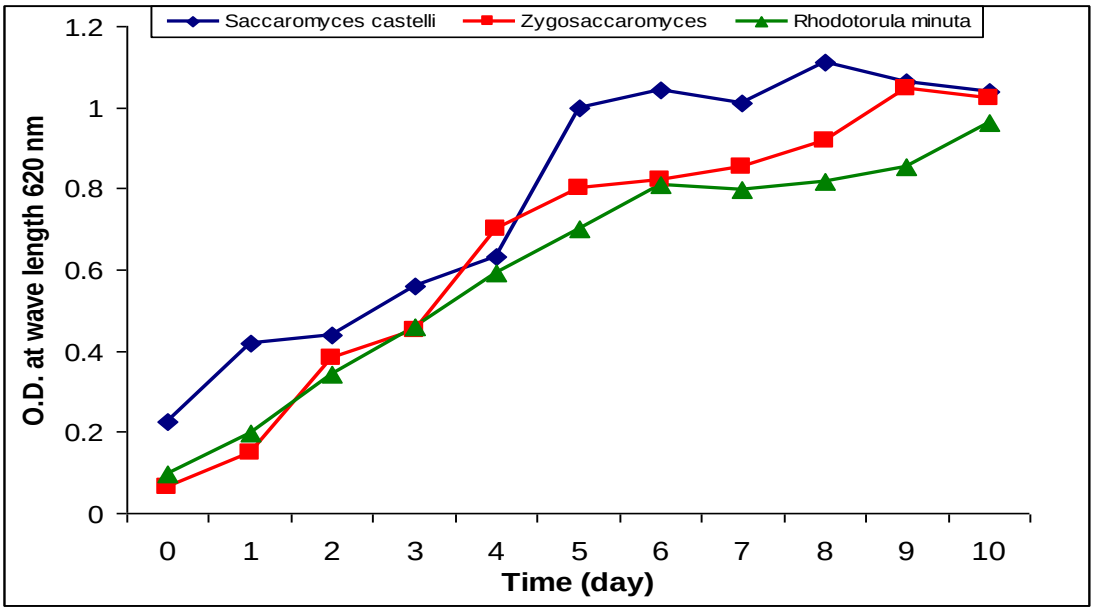

Fig (13): showed the growth curve of yeasts per day using spectroFigmeter.

Safety of yeast isolates:

The infection of *O. niloticus* with *Saccharomyces castelli*, *Zygosaccharomyces* species *and Rhodotorula minuta* revealed that *Zygosaccharomyces and Rhodotorula minuta* were pathogenic to *O. niloticus* at 0.2 ml $\times 10^5$ cells/ml by intraperiteneal injection (I/P). The mortality rate were 57.1 and 42.8% respectively in comparing to the control and *Saccharomyces castelli* groups, which the mortalities were 30 and 15.8% respectively as shown in table (7) and fig. (14). Clinical signs of infected *O. niloticus* with *Zygosaccharomyces* species and *Rhodotorula minuta* were dark coloration especially at peduncle region and hemorrhages at different sites (caudal fin and under anal fin). The Postmortem finding showed hemorrhages in the internal organs with petechi in liver as in Fig (15, 16, 17 and 18) comprising to the control which had pale pink liver and kidney Fig (19, 20). The infected *O. niloticus* with *Saccharomyces castelli*

had not clinical signs as in Fig (21) and had pale liver as in Fig (22). The re-isolation of the experimentally injected yeasts was occurred with *Rhodotorula minuta* and *Zygosaccharomyces* species. They isolated from the all internal organs of the recently dead fish and moribund fish. While, *Saccharomyces castelli* did not isolate from the recently dead fish or moribund fish.

Table (7): Mortality rate of *O. niloticus* due to the experimental infection with 0.2×10^5 cells/ml of *Saccharomyces castelli, Zygosaccharomyces and Rhodotorula minuta* I/P.

items	*Saccharomyces castelli*	*Zygosaccharomyces* species	*Rhodotorula minuta*	control
No. of examined fish	30	30	30	30
No. of dead fish	5	١٨	13	9
Mortality(%)	16	60	43	30

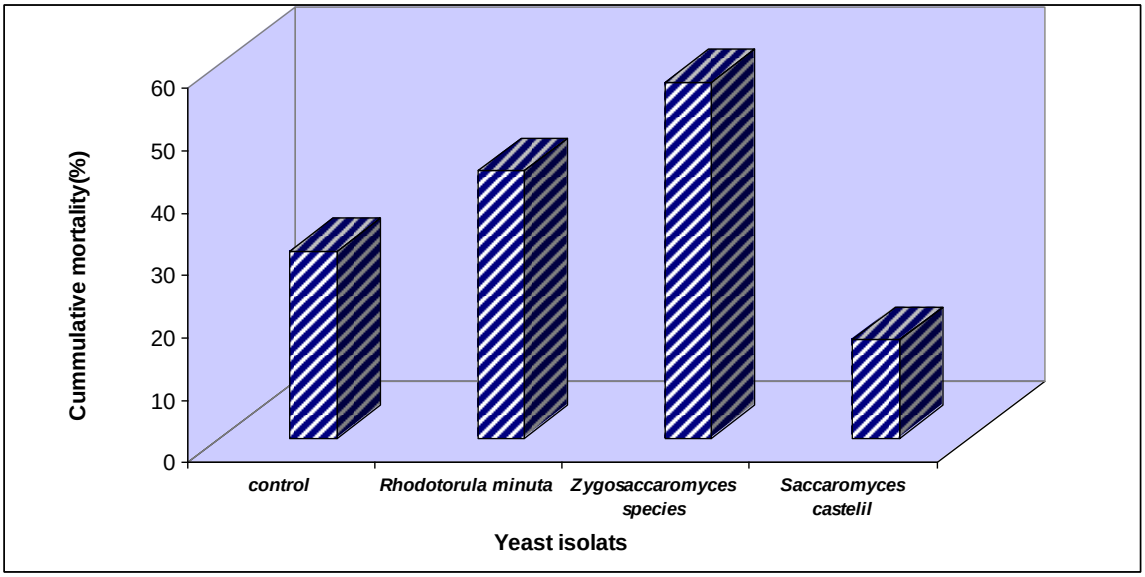

Fig (14): Cumulative mortality (%) of *O. niloticus* due to the experimental infection with 0.2×10^5 yeasts cells/ml I/P.

Fig (15): shows *O. niloticus* infected experimentally with *rhodoturulla minta* I/P suffered from fin rot and dark coloration with hemorrhage at the base of anal fin.

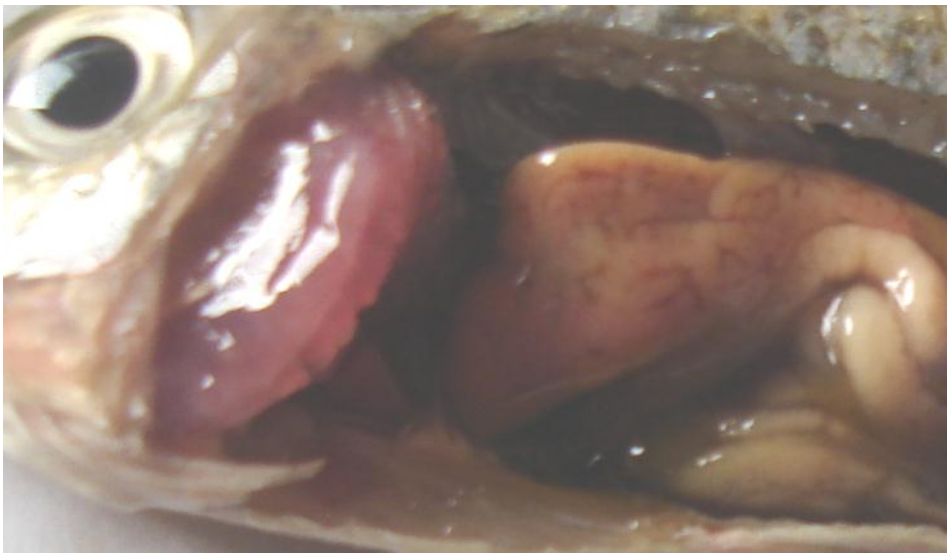

Fig (16): Shows *O. niloticus* infected experimentally with *rhodoturulla minta* I/P had hemorrhage in liver and congested kidney.

Fig (17): shows *O. niloticus* infected experimentally with *Zygosaccharomyces* I/P had dark coloration especially at peduncle region and hemorrhages at caudal fin.

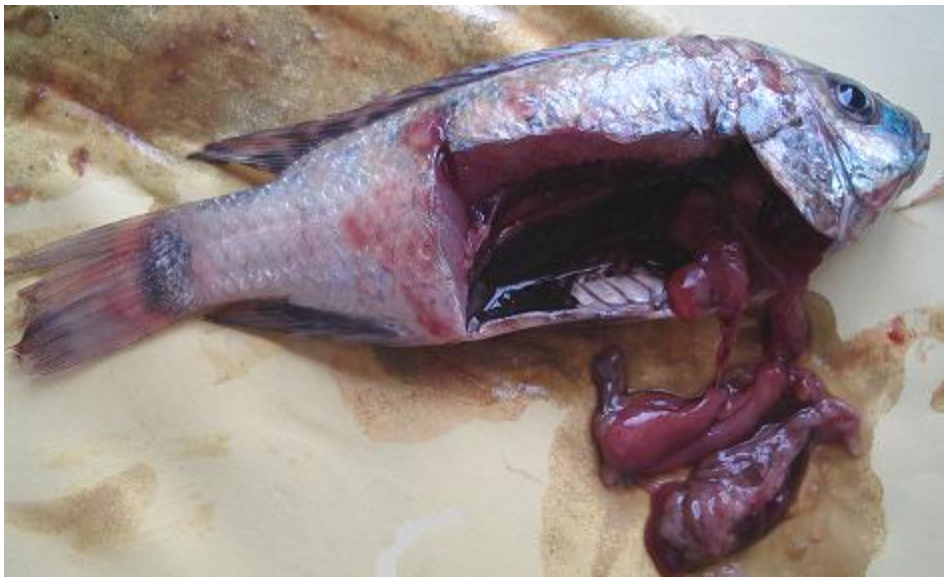

Fig (18): shows *O. niloticus* infected experimentally with *Zygosaccharomyces* I/P had hemorrhages in the internal organs with petechi in liver.

Fig (19): shows *O. niloticus* infected experimentally with *saccharomyces castlii* I/P had not clinical signs.

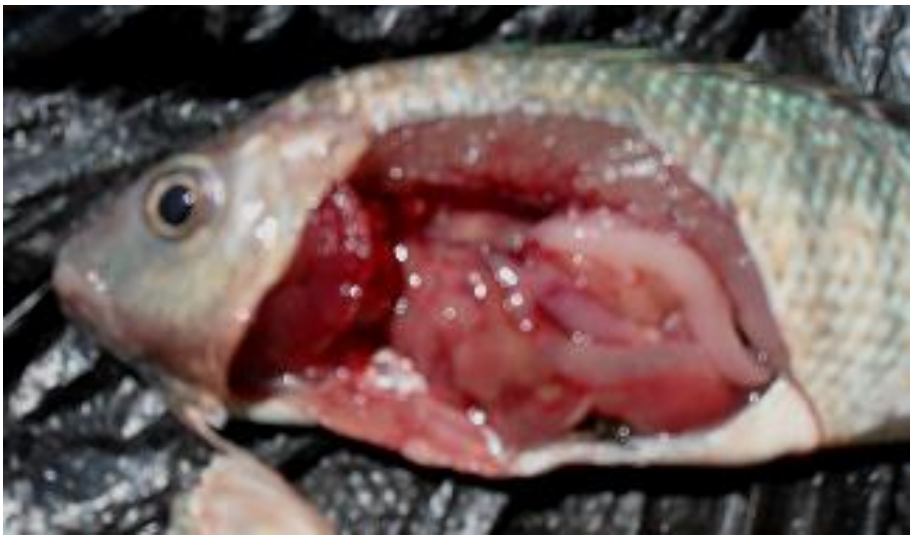

Fig (20) shows *O.niloticus* infected experimentally with *saccharomyces castlii* I/P had pale liver.

Fig (21): shows *O. niloticus* not infected experimentally (control) had hemorrhages on lateral fin.

Fig (22): shows *O. niloticus* not infected experimentally (control) had the internal organs with pale liver and kidney.

Viability of living yeast:

The colony count of *Saccharomyces castlii* supplemented feed at 5 g yeast/kg diet and stored in refrigerator (4ºC) and room temperature (25ºC) were 3.2 $x10^6$±2.6 cfu/g as in table (8) which decreased gradually significantly throughout storage period at 4°C and at 25°C reached to 2.6 $x10^5$ ±0.17 and 2 $x10^3$ ±0.0005 respectively at 2nd months. At 4°C showed higher number of viable cells in comparison with feed stored at 25°C at 3rd week in which the yeast disappeared after the 2nd month in feed stored at 25°C while the number of viable cells of the feed stored at 4°C not decreased and reached to 3.4 $x10^4$±0.2 at the 5th month.

Table (8): Viability of the *Saccharomyces castelli* after add to the commercial feed and storage in refrigerator (4ºC) and room temperature (25 ºC).

period	*Saccharomyces casteli*	
	4ºC	**25ºC**
zero time	$3.2 \times 10^6 \pm 2.6_a$	$3.2 \times 10^6 \pm 2.6_a$
1st week	$1.6 \times 10^6 \pm 11.5_b$	$3 \times 10^6 \pm 2.02_a$
2nd week	$1.2 \times 10^5 \pm 0.26_c$	$1.5 \times 10^5 \pm 0.14_b$
3rd week	$2.6 \times 10^5 \pm 0.17_c$	$6 \times 10^4 \pm 0.34_b$
4th week	$1.7 \times 10^5 \pm 0.11_c$	$4 \times 10^4 \pm 0.17_b$
2nd month	$2.6 \times 10^5 \pm 0.17_c$	$2 \times 10^3 \pm 0.0005_b$
3rd month	$3.8 \times 10^4 \pm 0.13_c$	0
4th month	$1.1 \times 10^4 \pm 0.01_c$	0
5th month	$3.4 \times 10^4 \pm 0.2_c$	0

Means carrying different superscripts are significant at ($p \leq 0.05$).
(a- c) increasing significant in the same column.

Growth performance:

The growth rate of *O. niloticus* due to feed supplemented yeast was significantly increased with T_2, T_3, T_4 and T_5 when compared with T_1 as showed in (Table, 9 and Fig, 23) while no significant difference between T_2 and T_3. The average weight gains (AWG) have significant increased with T_2, T_3, T_4 and T_5 reached to 0.241 ±0.021, 0.26 ±0.038, 0.20 ±0.095 and0.355

±0.034 respectively compared with T_1 (0.133 ±0.017) (Fig, 4). Specific growth rate (SGR) have significant increased with T_2, T_3, T_4 and T_5 reached to1.084 ±0.10, 1.110 ±0.14, 1.02 ±0.052 and 1.438 ±0.10 respectively compared with T_1 (0.646 ±0.078). Feed efficiency ratio (FER) have significant increased with T_2, T_3, T_4 and T_5 reached to 0.873 ±0.071, 0.887 ±0.070, 0.6132 ±0.049 and 1.096 ±0.082 respectively compared with T_1 (0.508±0.073). On the other hand, the best feed conversion ratio (FCR) was obtained with T_5 (0.911). *Rhodotorula minuta* (10g/Kg) supplemented diet was the best treatment for growth parameters.

Table (9): The effect of *Saccharomyces castelli* and *Rhodotorula minuta* supplemented diet on growth parameters in *O. niloticus* fed for 28 day.

Parameter	Treatments				
	T_1	T_2	T_3	T_4	T_5
AWG	$0.133\pm0.017_{c}$	$0.241\pm0.021_{b}$	$0.26\pm0.038_{b}$	$0.20\pm0.095_{bc}$	$0.355\pm0.034_{a}$
SGR	$0.646\pm0.078_{c}$	$1.084\pm0.10_{b}$	$1.110\pm0.14_{b}$	$1.02\pm0.052_{b}$	$1.438\pm0.10_{a}$
FCR	$1.965\pm0.011_{a}$	$1.145\pm0.011_{ab}$	$1.126\pm0.011_{ab}$	$1.63\pm0.009_{bc}$	$0.911\pm0.018_{c}$
FER	$0.508\pm0.073_{c}$	$0.873\pm0.071_{bc}$	$0.887\pm0.070_{bc}$	$0.6132\pm0.049_{ab}$	$1.096\pm0.082_{a}$

Means carrying different superscripts are at ($p\leq 0.05$). (a- c) increasing significant in the same row. T_1= basal diet free from yeast cells. T_2= Fish fed basal diet supplemented with 5 g *Saccharomyces castelli* /kg.T_3= Fish fed basal diet supplemented with 10 g *Saccharomyces castelli* /kg. T_4= Fish fed basal diet supplemented with 5 g *Rhodotorulla minuta* /kg. T_5= Fish fed basal diet supplemented with 10 g *Rhodotorulla minuta* /kg. AWG= Average weight Gains, SGR= Specific Growth Rate, FCR= Feed Conversion Ratio, FER= Feed Efficiency Ratio.

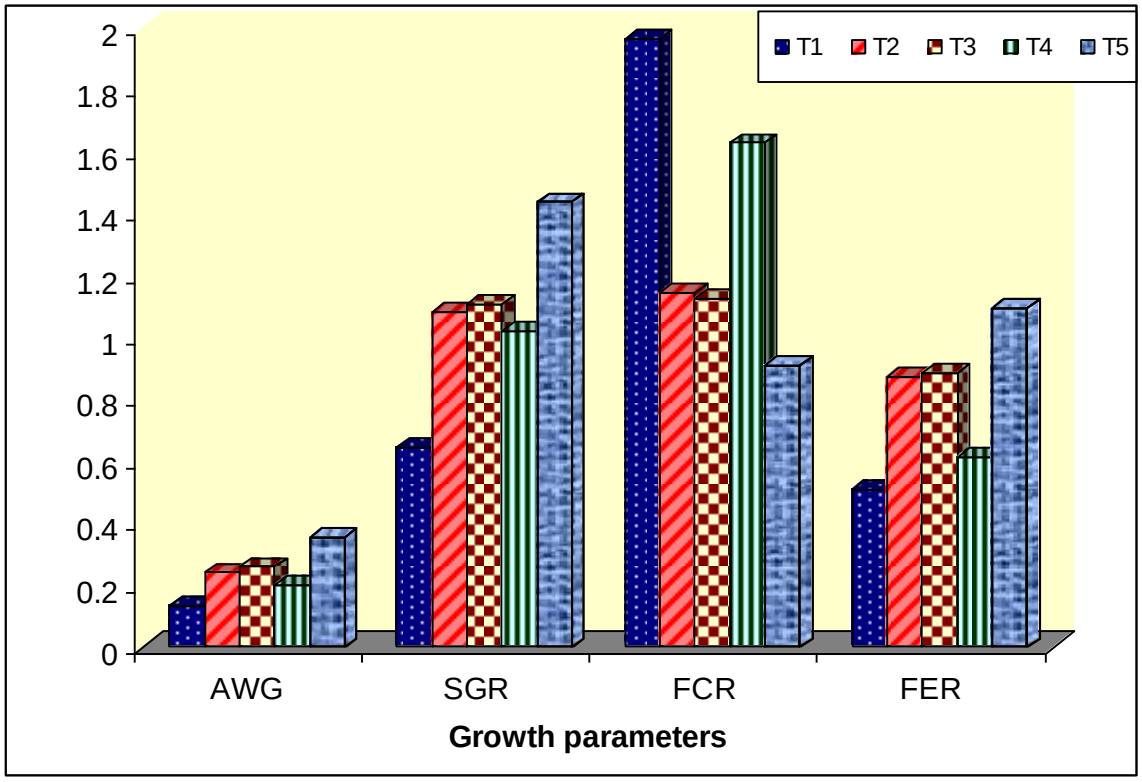

Fig (23): Shows the effect of *Saccharomyces castelli* and *Rhodotorula minuta* supplemented diet on growth parameters in *O. niloticus* fed for 28 day. T_1= basal diet free from yeast cells. T_2= Fish fed basal diet supplemented with 5 g *Saccharomyces castelli* /kg.T_3= Fish fed basal diet supplemented with 10 g *Saccharomyces castelli* /kg. T_4= Fish fed basal diet supplemented with 5 g *Rhodotorulla minta* /kg. T_5= Fish fed basal diet supplemented with 10 g *Rhodotorulla minta* /kg. AWG= Average weight Gains, SGR= Specific Growth Rate, FCR= Feed Conversion Ratio, FER= Feed Efficiency Ratio.

Organ-somatic index:

The hepatosomatic (Table, 10 and Fig., 24) and splenosomatic (Table, 9 and Fig., 25) indices were not increased significantly in T_2, T_3, T_4 and T_5.

Table (10): The hepatosomatic and splenosomatic indecies among fish fed yeasts supplemented diet groups.

Organ	Treatments				
	T_1	T_2	T_3	T_4	T_5
Spleen	$0.084\pm0.006_a$	$0.051\pm0.006_a$	$0.095\pm0.022_a$	$0.079\pm0.02_a$	$0.077\pm0.021_a$
Liver	$1.57\pm0.29_a$	$1.80\pm0.42_a$	$2.13\pm0.23_a$	$2.48\pm0.22_a$	$2.52\pm0.36_a$

Means carrying different superscripts are significant at ($p\leq 0.05$). T_1= basal diet free from yeast cells. T_2= Fish fed basal diet supplemented with 5 g *Saccharomyces castelli* /kg.T_3= Fish fed basal diet supplemented with 10 g *Saccharomyces castelli* /kg. T_4= Fish fed basal diet supplemented with 5 g *Rhodotorulla minta* /kg. T_5= Fish fed basal diet supplemented with 10 g *Rhodotorulla minta* /kg.

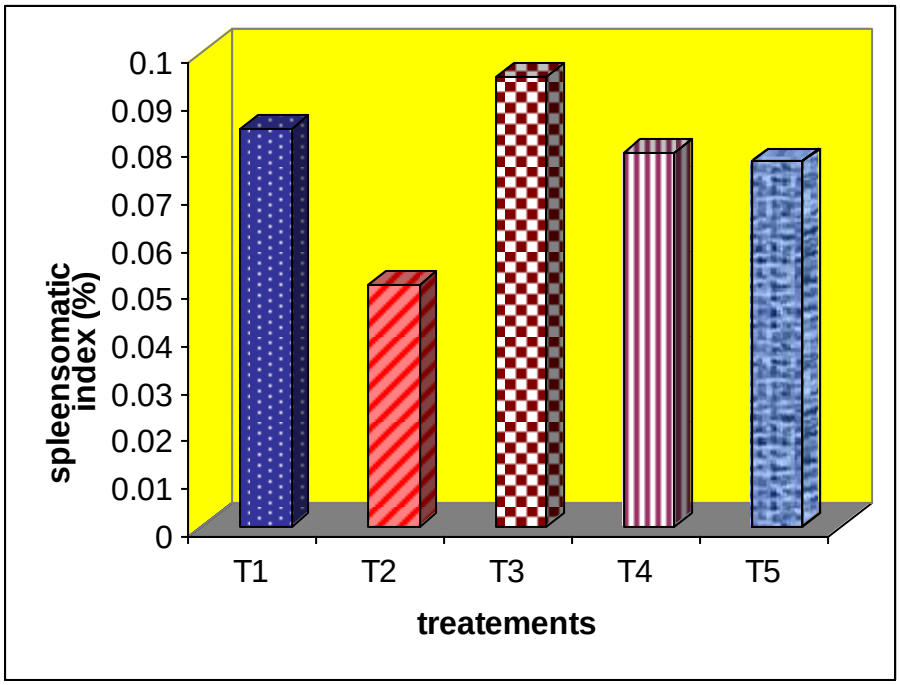

Fig (24): Shows the splenosomatic index among fish fed yeast supplemented groups. T_1= basal diet free from yeast cells. T_2= Fish fed basal diet supplemented with 5 g *Saccharomyces castelli* /kg. T_3= Fish fed basal diet supplemented with 10 g *Saccharomyces castelli* /kg. T_4= Fish fed basal diet supplemented with 5 g *Rhodotorulla minta* /kg. T_5= Fish fed basal diet supplemented with 10 g *Rhodotorulla minta* /kg.

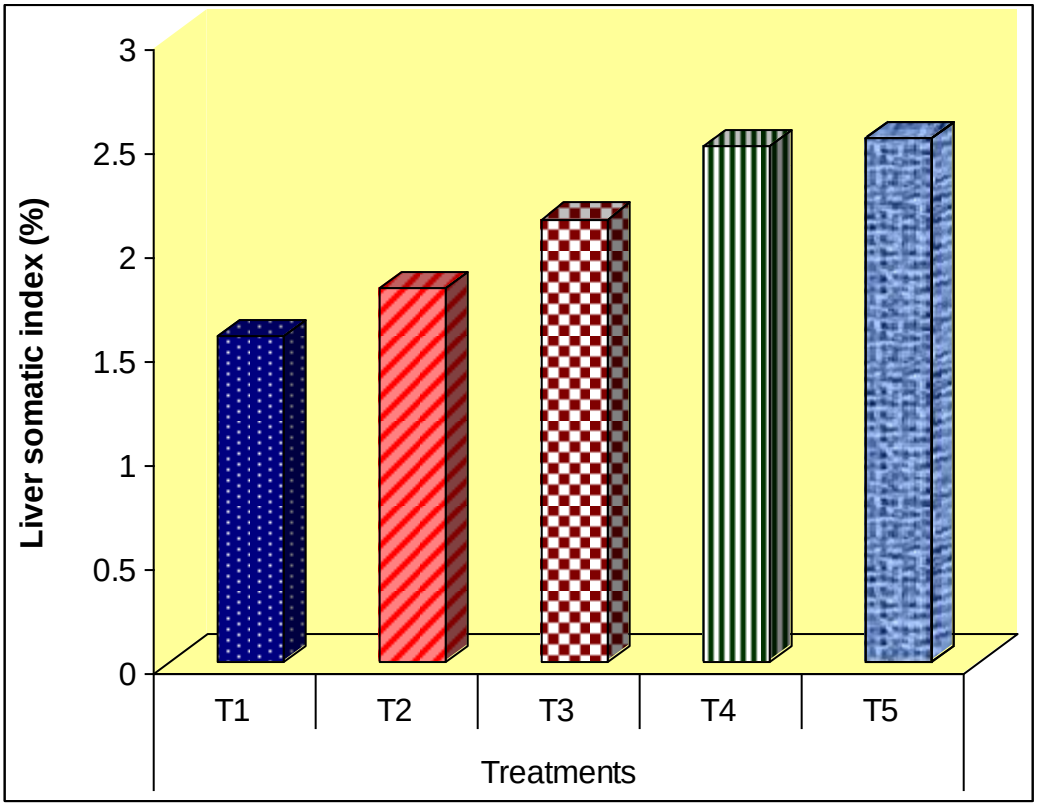

Fig (25): Shows the hepatosomatic index among fish fed yeast supplemented groups. T_1= basal diet free from yeast cells. T_2= Fish fed basal diet supplemented with 5 g *Saccharomyces castelli* /kg. T_3= Fish fed basal diet supplemented with 10 g *Saccharomyces castelli* /kg. T_4= Fish fed basal diet supplemented with 5 g *Rhodotorulla minta* /kg. T_5= Fish fed basal diet supplemented with 10 g *Rhodotorulla minta* /kg.

Heamatocrite value:

The results in table (11) and fig (26) indicated that the initial heamatocrite was 22.7±6.08 at the beginning of experiment and increased significantly at the second week of feeding and reached to 34.7±0.47, 43.1±2.12, 40.1±3.16 2 and 45.7±1.36 for T_2, T_3, T_4 and T_5 respectively in comparison with T_1. It could be notice that there was significant difference between treated groups.

At the fourth week of feeding experiment, hematocrite value showed significant increased in T3 reached to 49±4.3and non significant increased in

T_2, T_4 and T_5 reached to 45.5±2.63, 42±3.4 and 48±0.7 respectively in comparison with T_1.

There was asignificance differece was detected between the heamatocrite findings after second and fourth week of feeding experiment in all treatments.

Table (11): Effect of yeasts supplemented diet on heamatocrte value in *O. niloticus* at second and fourth week of feeding experiment.

Time	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**
Zero time	$22.7\pm6.08_{Aa}$	$22.7\pm6.08_{Ab}$	$22.7\pm6.08_{Ab}$	$22.7\pm6.08_{Ab}$	$22.7\pm6.08_{Ab}$
Second week	$22.7\pm6.08_{Ca}$	$34.7\pm0.47_{Bab}$	$43.1\pm2.12_{ABa}$	$40.12\pm3.16_{ABa}$	$45.7\pm1.36_{Aa}$
Fourth week	$22.8\pm6.08_{Ba}$	$45.5\pm2.63_{Aa}$	$49\pm4.3_{Aa}$	$42\pm3.4_{Aa}$	$48\pm0.7_{Aa}$

Means carrying different superscripts are significant at ($p\leq 0.05$). A-C was increasing with row and a-b increasing by column. Shows the splenosomatic indices among fish fed yeast supplemented groups. T_1= basal diet free from yeast cells. T_2= Fish fed basal diet supplemented with 5 g *Saccharomyces castelli* /kg. T_3= Fish fed basal diet supplemented with 10 g *Saccharomyces castelli* /kg. T_4= Fish fed basal diet supplemented with 5 g *Rhodotorulla minta* /kg. T_5= Fish fed basal diet supplemented with 10 g *Rhodotorulla minta* /kg.

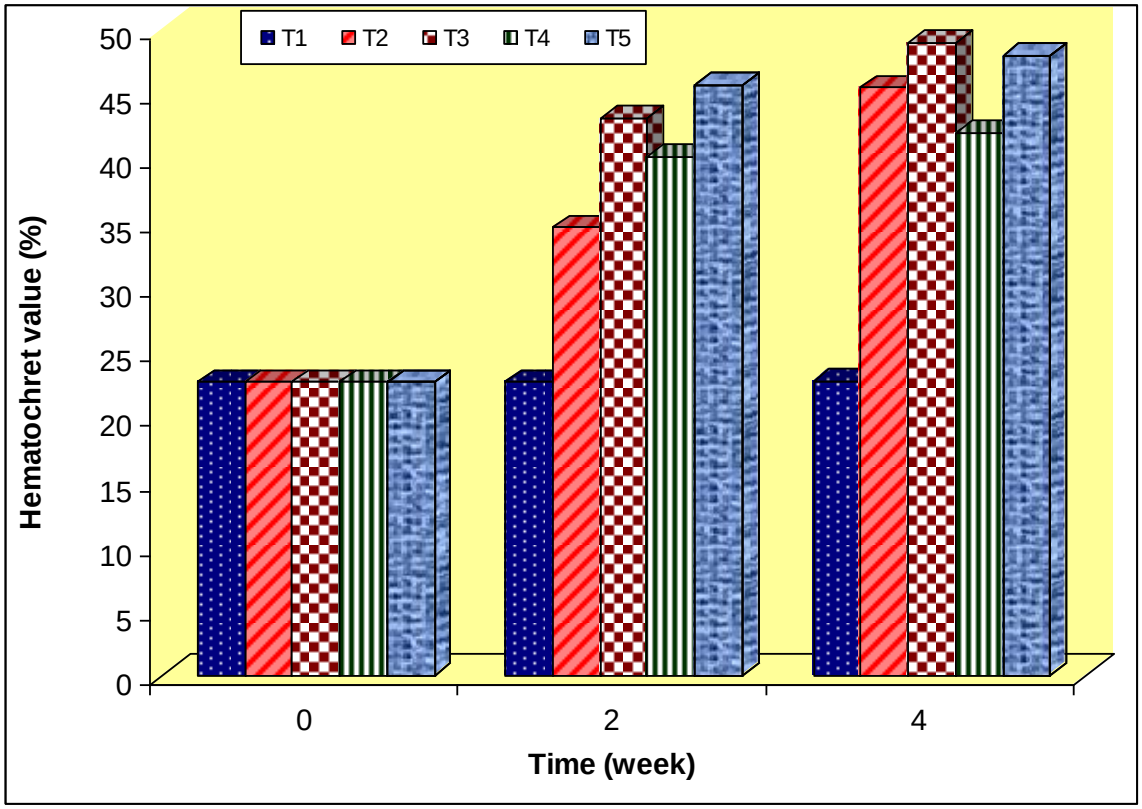

Fig (26): Shows the effect of yeasts supplemented diet on heamatocrite value in *O. niloticus* at second and fourth week of feeding experiment. T_1= basal diet free from yeast cells. T_2= Fish fed basal diet supplemented with 5 g *Saccharomyces castelli* /kg. T_3= Fish fed basal diet supplemented with 10 g *Saccharomyces castelli* /kg. T_4= Fish fed basal diet supplemented with 5 g *Rhodotorulla minta* /kg. T_5= Fish fed basal diet supplemented with 10 g *Rhodotorulla minta* /kg.

Respiratory burst activity by measuring nitroblue titrazolium activity (NBT):

The result in table (12) revealed that and fig (27) illustrated that the initial value of NBT was 1.47±0.02 mg/0.1ml blood at zero time and increased significantly at the second week of feeding experiment to 1.85±0.05, 1.96±0.02 and 1.81±0.04 mg/0.1ml blood for T_2, T_3 and T_5

respectively and non significant increased in T_4 reached to 1.55±0.15 mg/0.1ml in comparison with T_1.

At the fourth week of feeding experiment, NBT assay was significantly increased in all groups received yeast supplemented diet to 1.96±0.05, 2.58±0.1, 1.74±0.02 and 3.3±0.11 mg/0.1ml blood for T_2, T_3, T_4 and T_5 respectively in comparison with T_1.

There was significant difference was noticed between result of NBT at second and fourth week of feeding experiment in all treatments.

Table (12): Effect of yeasts supplemented diet on respiratory burst by using Nitro Blue Tetrazolium activity (NBT) mg/ml in *O. niloticus* at second and fourth week feeding experiment.

Time	T_1	T_2	T_3	T_4	T_5
Zero time	$1.47\pm0.02_{Aa}$	$1.47\pm0.02_{Ab}$	$1.47\pm0.02_{Ac}$	$1.47\pm0.02_{Aa}$	$1.47\pm0.02_{Ac}$
Second week	$1.47\pm0.02_{Ba}$	$1.85\pm0.05_{Aa}$	$1.92\pm0.02_{Ab}$	$1.55\pm0.15_{Ba}$	$1.81\pm0.04_{Ab}$
Fourth week	$1.47\pm0.02_{Da}$	$1.96\pm0.05_{Ca}$	$2.58\pm0.1_{Ba}$	$1.74\pm0.02_{BCa}$	$3.3\pm0.11_{Aa}$

Means carrying different superscripts are significant at ($p\leq 0.05$). A-C means increasing significant in the same row and a-b increasing significant in the same column. T_1= basal diet free from yeast cells. T_2= Fish fed basal diet supplemented with 5 g *Saccharomyces castelli* /kg. T_3= Fish fed basal diet supplemented with 10 g *Saccharomyces castelli* /kg. T_4= Fish fed basal diet supplemented with 5 g *Rhodotorulla minta* /kg. T_5= Fish fed basal diet supplemented with 10 g *Rhodotorulla minta* /kg.

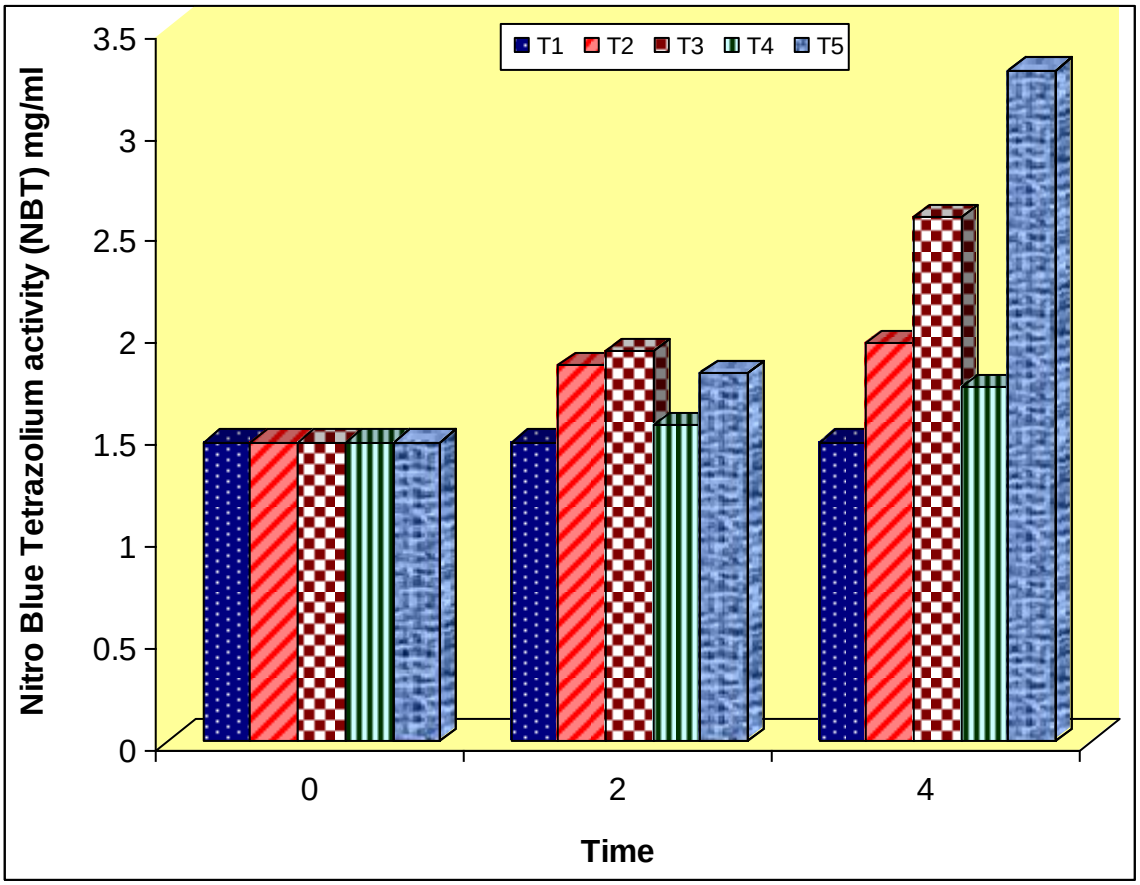

Fig (27): Shows the effect of yeasts supplemented diet on respiratory burst by using Nitro Blue Tetrazolium activity (NBT) mg/ml in *O. niloticus* at second and fourth week feeding experiment. T_1= basal diet free from yeast cells. T_2= Fish fed basal diet supplemented with 5 g *Saccharomyces castelli* /kg. T_3= Fish fed basal diet supplemented with 10 g *Saccharomyces castelli* /kg. T_4= Fish fed basal diet supplemented with 5 g *Rhodotorulla minta* /kg. T_5= Fish fed basal diet supplemented with 10 g *Rhodotorulla minta* /kg.

Lysozyme activity:

The result in table (13) indicated that and fig (28) illustrated that the initial value of lysozyme activity was 1.29± 0.01 µg/ml serum at zero time and increased significantly at the second week of feeding experiment, reached to 1.73± 0.04, 1.79±0.07, 1.62±0.09 and 2.1±0.02 µg /ml for T_2, T_3, T_4 and T_5 respectively in comparison with untreated control group T_1.

At the fourth week of feeding experiment, lysozyme activity was significantly increased in T_3 reached to 2.08± 0.11 µg /ml and decreased significantly in T_2 and T_4 reached to 1.50± 0.07 and 1.44± 0.08 µg /ml respectively but decreased not significant for T_5 reached to 1.8± 0.18 µg /ml in comparison with T_1.

There was asignificante difference was detected between the Lysozyme activity results at the second and fourth week of feeding experiment in all treatments.

Table (13): Serum lysozym activity (µg/ml) in *O. niloticus* due to feeding by yeasts supplemented diet at second and fourth week of experiment.

Time	T_1	T_2	T_3	T_4	T_5
Zero time	$1.29\pm0.01_{Aa}$	$1.29\pm0.01_{Ac}$	$1.29\pm0.01_{Ac}$	$1.29\pm0.01_{Ab}$	$1.29\pm0.01_{Ab}$
Second week	$1.29\pm0.01_{Ca}$	$1.73\pm0.04_{Ba}$	$1.79\pm0.07_{Bb}$	$1.62\pm0.09_{Ba}$	$2.1\pm0.02_{Aa}$
Fourth week	$1.29\pm0.01_{Ca}$	$1.50\pm0.07_{BCb}$	$2.08\pm0.11_{Aa}$	$1.44\pm0.08_{Cab}$	$1.8\pm0.18_{ABa}$

Means carrying different superscripts are significant at ($p\leq 0.05$). A-C means increasing significant in the same row and a-b increasing significant in the same column. T_1= basal diet free from yeast cells. T_2= Fish fed basal diet supplemented with 5 g *Saccharomyces castelli* /kg. T_3= Fish fed basal diet supplemented with 10 g *Saccharomyces castelli* /kg. T_4= Fish fed basal diet supplemented with 5 g *Rhodotorulla minta* /kg. T_5= Fish fed basal diet supplemented with 10 g *Rhodotorulla minta* /kg.

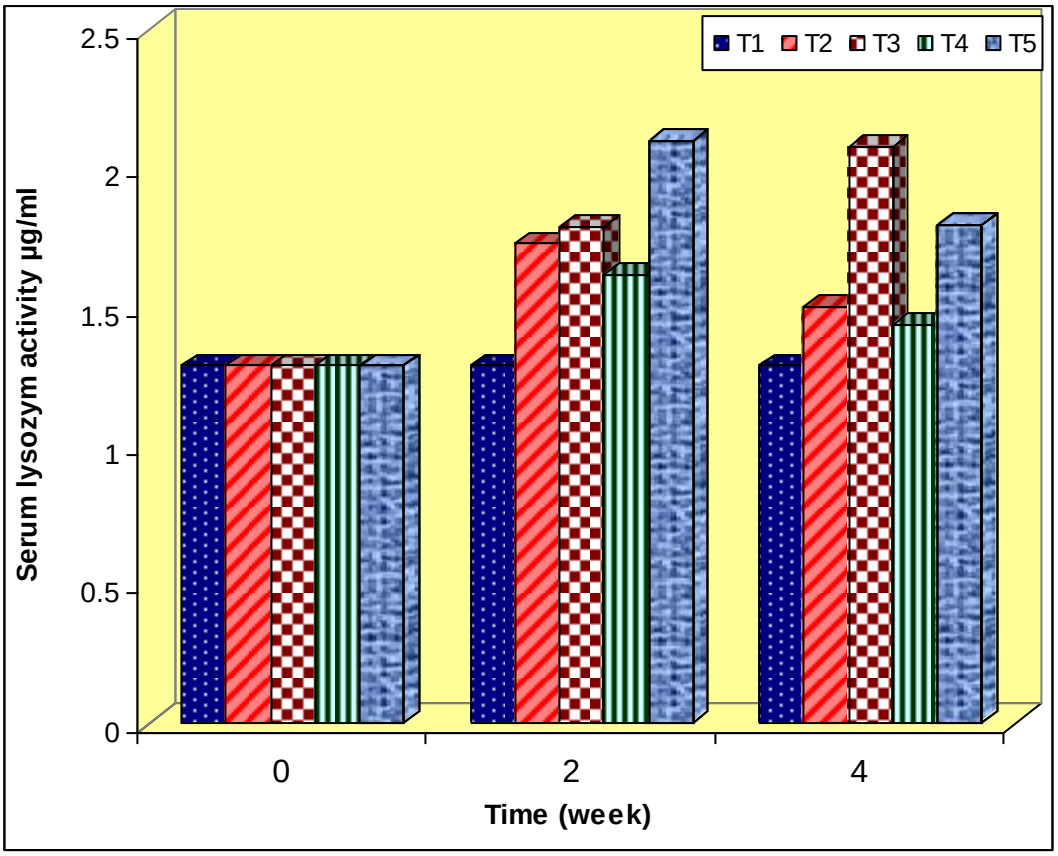

Fig (28): Shows serum lysozym activity (µg/ml) in *O. niloticus* due to feeding with yeasts supplemented diet at second and fourth week of experiment. T_1= basal diet free from yeast cells. T_2= Fish fed basal diet supplemented with 5 g *Saccharomyces castelli* /kg. T_3= Fish fed basal diet supplemented with 10 g *Saccharomyces castelli* /kg. T_4= Fish fed basal diet supplemented with 5 g *Rhodotorulla minta* /kg. T_5= Fish fed basal diet supplemented with 10 g *Rhodotorulla minta* /kg.

Serum bactericidal activity (SBT):

1- **Serum bactericidal activity against *Aeromonas sobria* :**

The result in table (14) and fig (29) indicated that at the second week of feeding experiment, serum bactericidal activity against *Aeromonas sobria* was lowest in T_1 group and highest in T_5. The viable bacterial counts were significantly lower in all treatments groups with values $5.8 \times 10^5 \pm 0.08$, $5.4 \times 10^5 \pm 0.03$, $5.3 \times 10^5 \pm 0.05$ and $3 \times 10^5 \pm 0.05$ cfu/ml for T_2, T_3, T_4 and T_5 respectively when compared with control group ($6 \times 10^6 \pm 0.57$ cfu/ml).

At the fourth week of feeding with yeast supplemented diet, the serum bactericidal activity against *Aeromonas sobria* was lowest in T_1 group and reached to the highest value with T_5. The viable bacterial counts were significantly lower in all treatment groups reached to $4 \times 10^5 \pm 0.03$, $2.9 \times 10^5 \pm 0.01$, $4 \times 10^5 \pm 0.2$ and $2.1 \times 10^5 \pm 0.03$ cfu/ml for T2, T3, T4 and T5 respectively when compared with control group was $3.9 \times 10^6 \pm 0.5$ cfu/ml.

Table (14): Effect of yeasts supplemented diet on serum bactericidal activity against *Aeromonas sobria* at second and fourth week of feeding experiment of *O. niloticus*.

Time	T_1	T_2	T_3	T_4	T_5
Second week	$6 \times 10^6 \pm 0.57_{Aa}$	$5.8 \times 10^5 \pm 0.08_{Ba}$	$5.4 \times 10^5 \pm 0.03_{Ba}$	$5.3 \times 10^5 \pm 0.05_{Ba}$	$3 \times 10^5 \pm 0.05_{Ca}$
Fourth week	$3.9 \times 10^6 \pm 0.5_{Ab}$	$4 \times 10^5 \pm 0.03_{Bb}$	$2.9 \times 10^5 \pm 0.01_{Cb}$	$4 \times 10^5 \pm 0.2_{Bb}$	$2.1 \times 10^5 \pm 0.03_{Cb}$

Means carrying different superscripts are significant at ($p \leq 0.05$). A-C means increasing significant in the same row and a-b increasing significant in the same column. T_1= basal diet free from yeast cells. T_2= Fish fed basal diet supplemented with 5 g *Saccharomyces castelli* /kg. T_3= Fish fed basal diet supplemented with 10 g *Saccharomyces castelli* /kg. T_4= Fish fed basal diet supplemented with 5 g *Rhodotorulla minta* /kg. T_5= Fish fed basal diet supplemented with 10 g *Rhodotorulla minta* /kg.

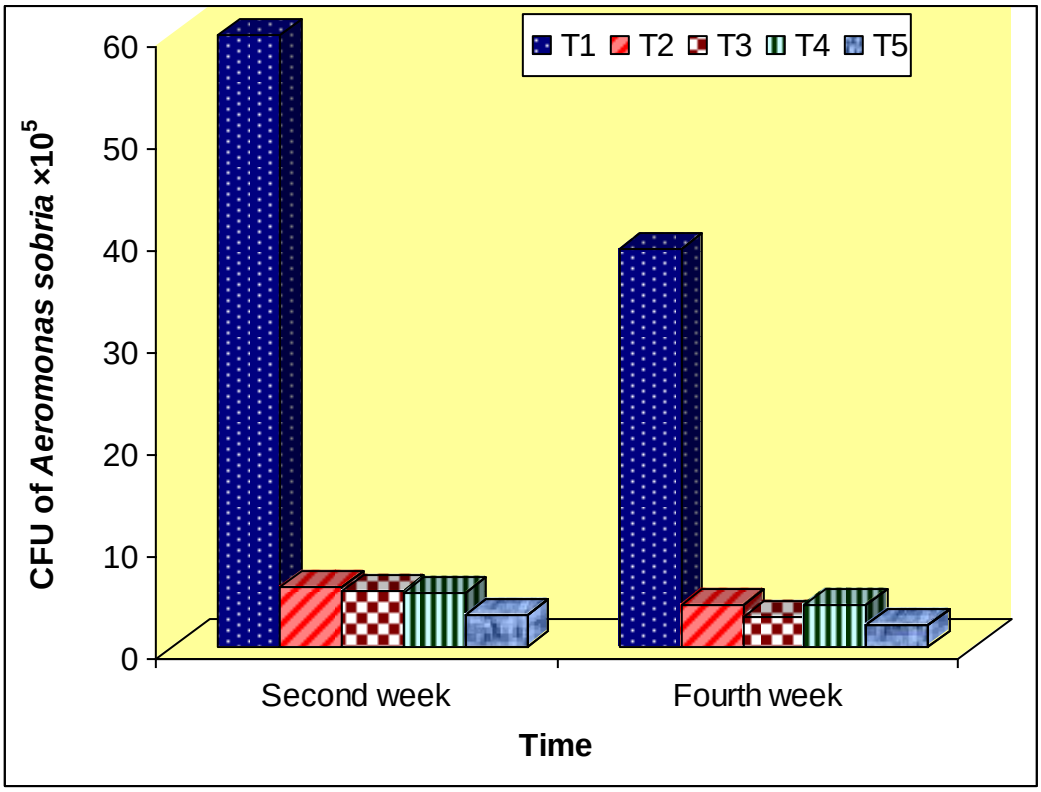

Fig (29): shows the effect of yeasts supplemented diet on serum bactericidal activity of *O. niloticus* against *Aeromonas sobria* at second and fourth week of experiment. T_1= basal diet free from yeast cells. T_2= Fish fed basal diet supplemented with 5 g *Saccharomyces castelli* /kg. T_3= Fish fed basal diet supplemented with 10 g *Saccharomyces castelli* /kg. T_4= Fish fed basal diet supplemented with 5 g *Rhodotorulla minta* /kg. T_5= Fish fed basal diet supplemented with 10 g *Rhodotorulla minuta* /kg.

2- **Serum bactericidal activity against *Pseudomonase fluorecence* :**

After the second week of feeding experiment, serum bactericidal activity against *Pseudomonase fluorecence* was lowest in T_1 group and the highest value with T_5. The viable bacterial counts were significantly lower in all treatment groups with values 2.5 x 10^6±0.5, 6 x 10^4±0.05, 7 x 10^5±0.05 and 4.2 x 10^5±0.11 cfu/ml for T_2, T_3, T_4 and T_5 respectively compared with control group was 6.5 x 106±0.5 cfu/ml (Table, 15 and Fig, 30).

After the fourth week, serum bactericidal activity against *Pseudomonase fluorecence* was the lowest in T_1 group and the highest value with T_5. The viable bacterial counts were significantly lower in all treatment groups reached to 5.6 x 10^5±0.2, 2.7 x 10^4±0.008, 4.5 x 10^5±0.2 and 1.5 x 10^5 cfu/ml for T_2, T_3, T_4 and T_5 respectively compared with control group (5.2 x 10^6±0.5 cfu/ml).

Table (15): Effect of yeasts supplemented diet on serum bactericidal activity of *O. niloticus* against *Pseudomonas fluorescens* at second and fourth week of experiment (cfu × 10^4).

Time	T_1	T_2	T_3	T_4	T_5
Second week	$650\pm0.5_{Aa}$	$250\pm0.5_{Ba}$	$6\pm0.05_{Ea}$	$70\pm0.05_{Ca}$	$42\pm0.11_{Da}$
Fourth week	$520\pm0.5_{Ab}$	$56\pm0.2_{Bb}$	$2.7\pm0.008_{Eb}$	$45\pm0.2_{Cb}$	$15\pm0.05_{Db}$

Means carrying different superscripts are significant at ($p\leq 0.05$). A-B means increasing significant in the same row and a-b increasing significant in the same column. T_1= basal diet free from yeast cells. T_2= Fish fed basal diet supplemented with 5 g *Saccharomyces castelli* /kg. T_3= Fish fed basal diet supplemented with 10 g *Saccharomyces castelli* /kg. T_4= Fish fed basal diet supplemented with 5 g *Rhodotorulla minuta* /kg. T_5= Fish fed basal diet supplemented with 10 g *Rhodotorulla minuta* /kg.

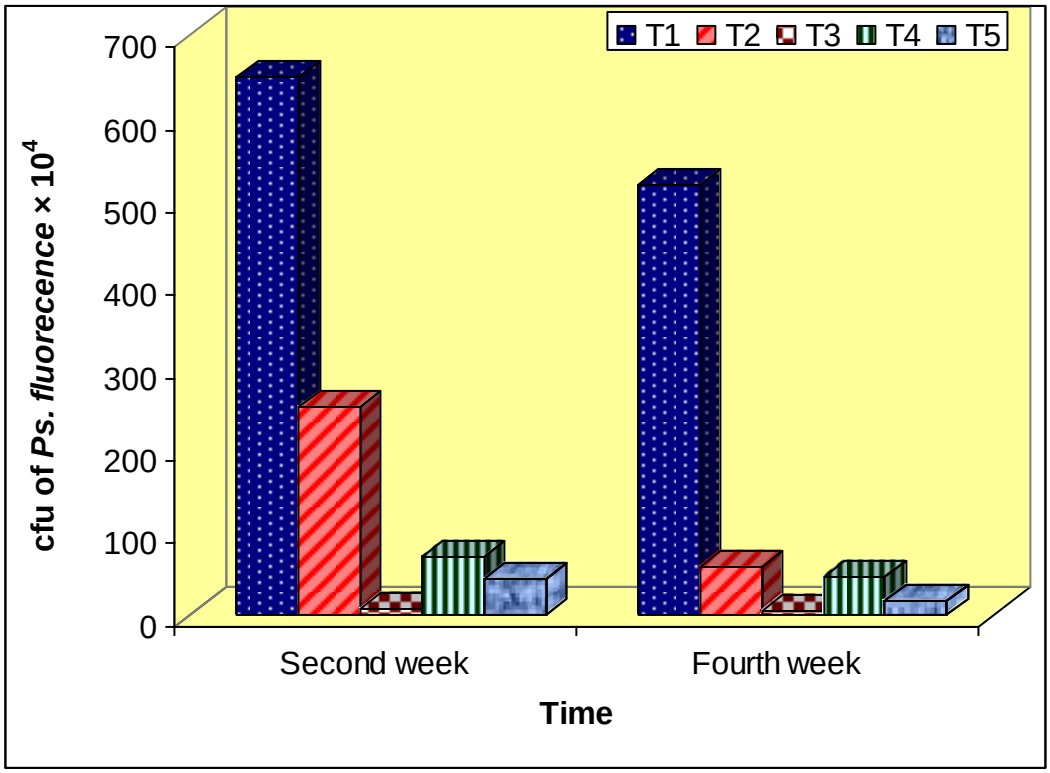

Fig (30): Shows the effect of yeasts supplemented diet on Serum bactericidal activity of *O. niloticus* against *Pseudomonas fluorescens* at second and fourth week of experiment. T_1= basal diet free from yeast cells. T_2= Fish fed basal diet supplemented with 5 g *Saccharomyces castelli* /kg. T_3= Fish fed basal diet supplemented with 10 g *Saccharomyces castelli* /kg. T_4= Fish fed basal diet supplemented with 5 g *Rhodotorulla minta* /kg. T_5= Fish fed basal diet supplemented with 10 g *Rhodotorulla minuta* /kg.

Total bacterial count of fish intestine

Data in table (16) and fig (31) indicated that the total bacterial count of fish intestine at the second week of feeding experimental were 3.2 x 10^6±37.11, 1.6x 10^6±185, 3.8 x 10^5±217, 1 x 10^5±6.6 and 1.4 x 10^5±29.05 cfu/g in T_1, T_2, T_3, T_4 and T_5 respectively and decreased significantly reached to 6.6 x 10^4 ±12.01, 2.8 x 10^4±16.1 and 5 x 10^3±0.57 cfu/g for T_2, T_4 and T_5 and decreased in T_3 to 8.6 x 10^3±2.4 cfu/g. there were significant between T_1 and the other treatments.

Table (16): Effect of yeasts supplemented diet on total bacterial count of *O. niloticus* intestine after second and fourth week of feeding experiment (cfu ×10^3).

Time	T_1	T_2	T_3	T_4	T_5
Second weeks	$3200±37.11_{Aa}$	$1600±185_{Ba}$	$380±217_{Ca}$	$100±6.6_{Ca}$	$140±29.05_{Ca}$
Fourth weeks	$2200±95.39_{Ab}$	$66±12.01_{Bb}$	$8.6 ±2.4_{Ba}$	$28 ±16.1_{Bb}$	$5 ±0.57_{Bb}$

Means carrying different superscripts are significant at ($p\leq 0.05$). A-C means increasing significant in the same row and a-b increasing significant in the same column. T_1= basal diet free from yeast cells. T_2= Fish fed basal diet supplemented with 5 g *Saccharomyces castelli* /kg. T_3= Fish fed basal diet supplemented with 10 g *Saccharomyces castelli* /kg. T_4= Fish fed basal diet supplemented with 5 g *Rhodotorulla minuta* /kg. T_5= Fish fed basal diet supplemented with 10 g *Rhodotorulla minuta* /kg.

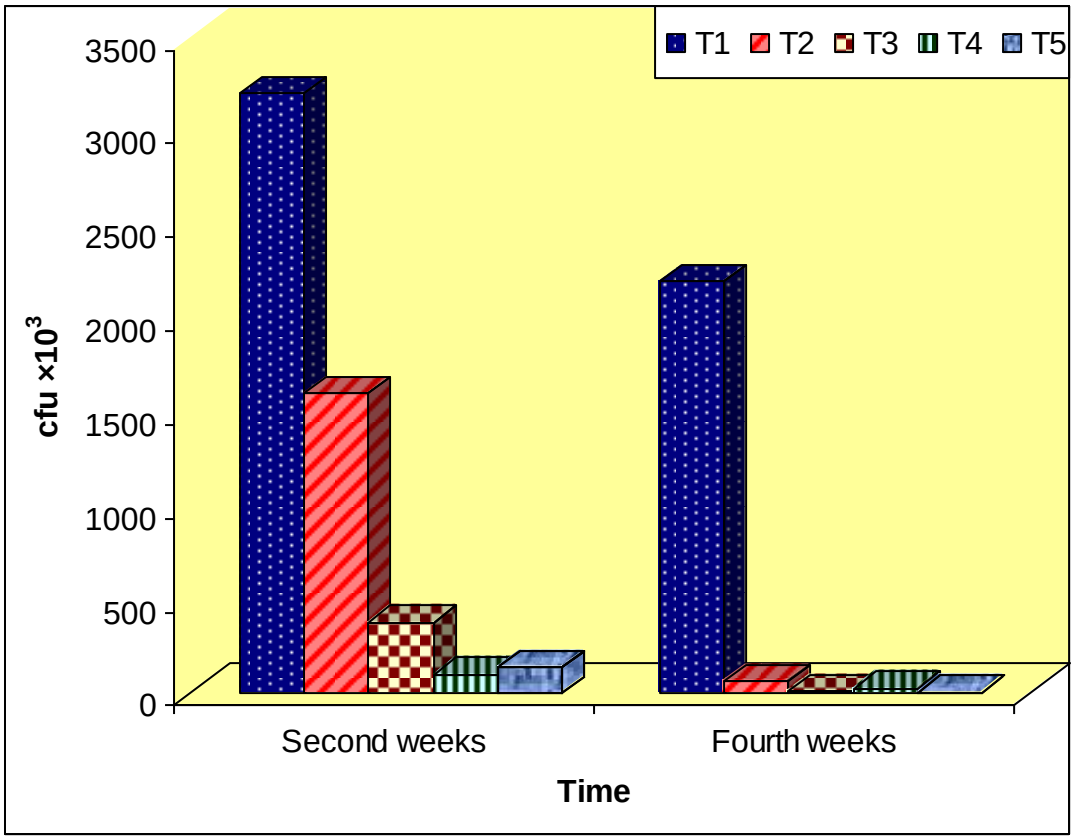

Fig (31): Shows the effect of yeasts supplemented diet on total bacterial count of *O. niloticus* intestine at second and fourth week of feeding experiment. T_1= basal diet free from yeast cells. T_2= Fish fed basal diet supplemented with 5 g *Saccharomyces castelli* /kg. T_3= Fish fed basal diet supplemented with 10 g *Saccharomyces castelli* /kg. T_4= Fish fed basal diet supplemented with 5 g *Rhodotorulla minuta* /kg. T_5= Fish fed basal diet supplemented with 10 g *Rhodotorulla minuta* /kg.

Challenge test:

At the end of feeding experiment, *O. niloticus* challenged with pathogenic strain of *Aeromonas sobria* and *Pseudomonase fluorescence* (0.5 ml 10^7 bacterial cell suspensions).

a- Results of fish challenged with *Aeromonas sobria* :

Table (17) and fig (32) illustrated that *O. niloticus* challenged with pathogenic *Aeromonas sobria* had higher mortality with T_1 (61.5%) than other treatments that received yeasts supplemented diet. The mortality rates were 10, 10 and 30% in T_2, T_3 and T_4 respectively and there was not any mortality with T_5. Relative level of protection against *Aeromonas sobria* in T_5 was higher than in other treatments (100%) and decreased in the other treatments to 0, 83, 83and 51.22% for T_1, T_2, T_3 and T_4 respectively.

b- Results of fish challenged with *Pseudomonase fluorescence*:

Table (17) and fig (33) indicated that *O. niloticus* challenged with pathogenic *Pseudomonase fluorescence* had higher mortality with T_1 (53.46%) than other treatments that received the yeasts supplemented diet (10, 30 and 10%) in T_2, T_4 and T_5 respectively. There was no mortality with T_3. Relative level of protection against *Pseudomonase fluorescence* with T_3 was higher than in other treatments (100%) and decreased in the other treatments to 0, 81.3, 43.89 and 81.3% in T_1, T_2, T_4 and T_5 respectively.

Table (17): Mortality rate and Relative level of protection in treated *O. niloticus* due to challenge with *Aeromonas sobria* and *Pseudomonase fluorecence*.

treatments	*Aeromonas sabia*		*Pseudomonase fluorecence*	
	Mortality%	RLP	Mortality%	RLP
T_1	61.5	0	53.46	0
T_2	10	83	10	81.3
T_3	10	83	0	100
T_4	30	51.22	30	43.89
T_5	0	100	10	81.3

T_1= basal diet free from yeast cells. T_2= Fish fed basal diet supplemented with 5 g *Saccharomyces castelli* /kg. T_3= Fish fed basal diet supplemented with 10 g *Saccharomyces castelli* /kg. T_4= Fish fed basal diet supplemented with 5 g *Rhodotorulla minuta* /kg. T_5= Fish fed basal diet supplemented with 10 g *Rhodotorulla minuta* /kg.

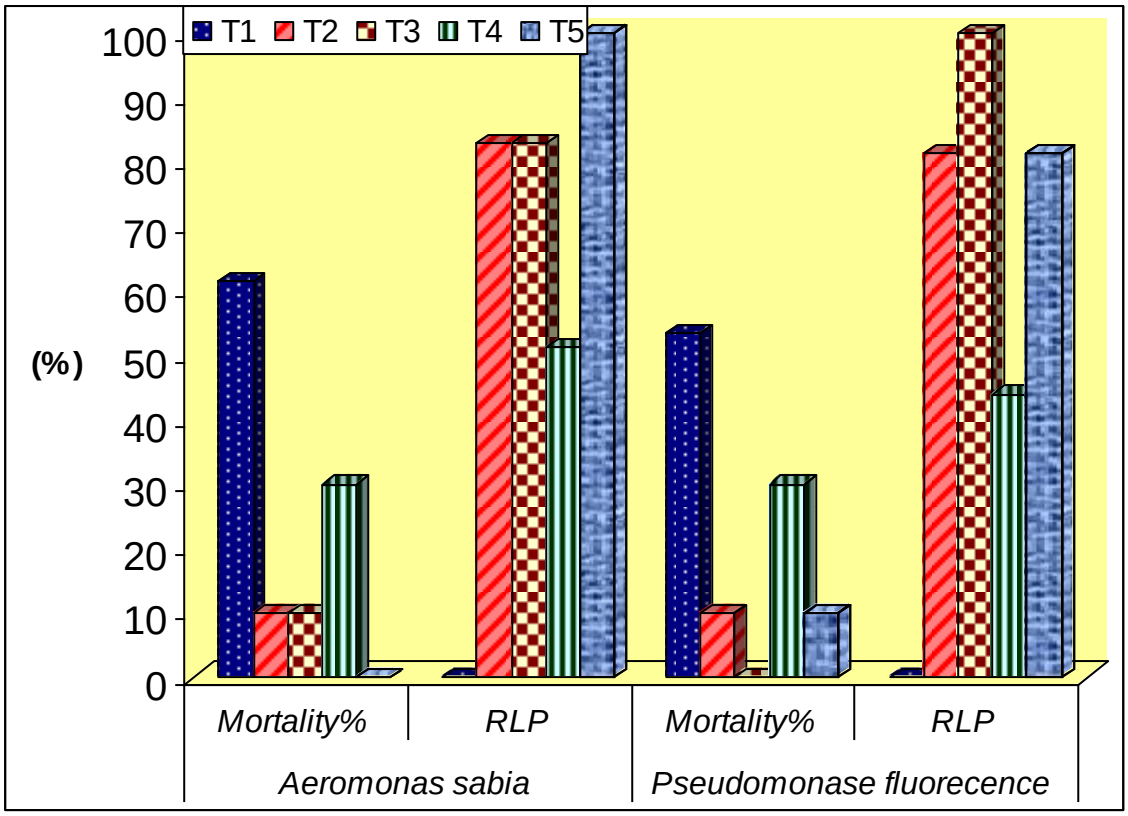

Fig (33): Shows the mortality rate and relative level of protection (RLP) in treated *O. niloticus* due to challenge test with *Aeromonas sobria* and *Pseudomonase fluorecence*. T_1= basal diet free from yeast cells. T_2= Fish fed basal diet supplemented with 5 g *Saccharomyces castelli* /kg. T_3= Fish fed basal diet supplemented with 10 g *Saccharomyces castelli* /kg. T_4= Fish fed basal diet supplemented with 5 g *Rhodotorulla minta* /kg. T_5= Fish fed basal diet supplemented with 10 g *Rhodotorulla minta* /kg.

Antibody titer:

Results of table (18) and Fig (34) showed that the highest level of the antibody titre to *A. sobria* and *Ps. fluorescens* infection was obtained with T_2 (8 log10). T_3, T_4 and T_5 increased antibody titre with *A. sobria* infection than *Ps. fluorescens* and the lowest value with T_1 (1 log10 and 3 log1o) with *A. sobria* and *Ps. fluorescens* respectively.

Table (18): Effect of yeasts supplemented diet on the antibody titre level due to *A. sobria* and *Ps. Fluorescens* infection.

Antigen	Antibody titre (log_{10})				
	T_1	**T_2**	**T_3**	**T_4**	**T_5**
Ps. fluorescens	3	8	4	4	5
A. sobria	1	8	6	5	6

T_1= basal diet free from yeast cells. T_2= Fish fed basal diet supplemented with 5 g *Saccharomyces castelli* /kg. T_3= Fish fed basal diet supplemented with 10 g *Saccharomyces castelli* /kg. T_4= Fish fed basal diet supplemented with 5 g *Rhodotorulla minuta* /kg. T_5= Fish fed basal diet supplemented with 10 g *Rhodotorulla minuta* /kg.

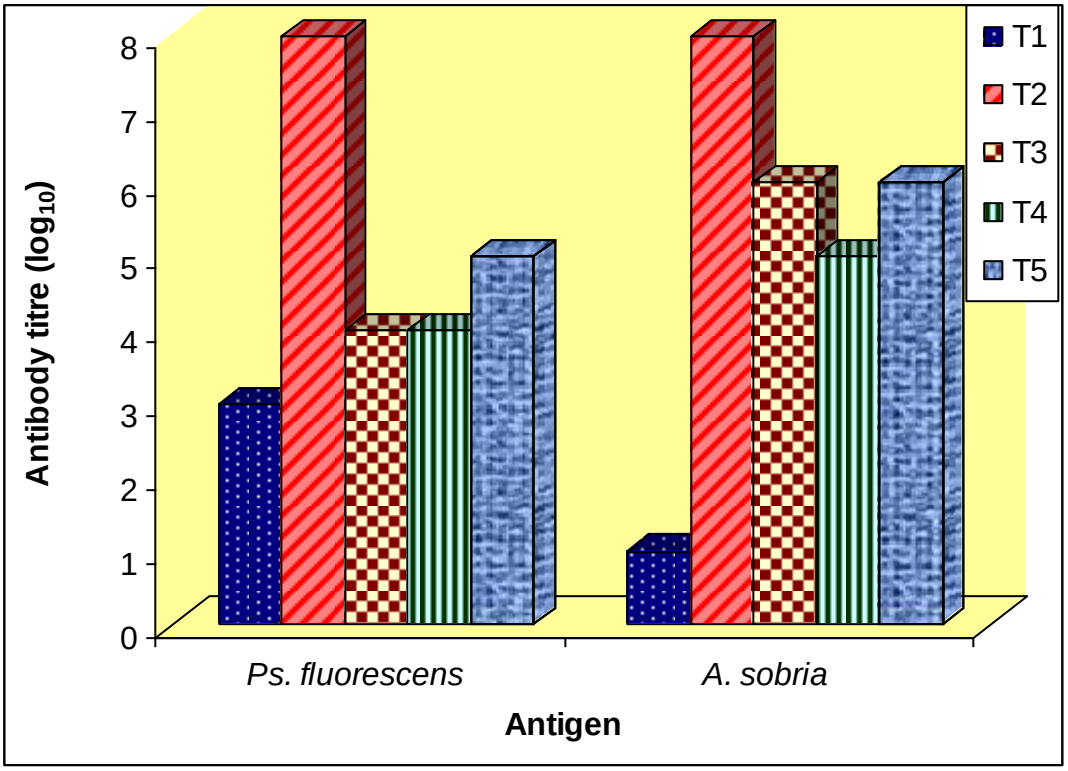

Fig (34): Shows the effect of yeasts supplemented diet on the antibody titre due to *A. sobria* and *Ps. fluorescens* infection. T_1= basal diet free from yeast cells. T_2= Fish fed basal diet supplemented with 5 g *Saccharomyces castelli* /kg. T_3= Fish fed basal diet supplemented with 10 g *Saccharomyces castelli* /kg. T_4= Fish fed basal diet supplemented with 5 g *Rhodotorulla minuta* /kg. T_5= Fish fed basal diet supplemented with 10 g *Rhodotorulla minuta* /kg.

Discussion

Fish cultures are increasing to compensate for the shortage of animal protein all over the world. Fish under intensive culture conditions will be badly affected and often fall prey to different microbial pathogens that have been treated with chemotherapeutic substances of which antibiotics were intensively used. These curative substances produce the problem of bacterial drug fastness on one hand and the public health hazards on the other hands (**Robertson *et al.*, 2000**).

This study was planed to isolate yeasts from cultured fresh water fishes and used it as a probiotic in the cultured *O. niloticus* for the growth performance point of view as well as their effect on the immune response and increasing fish resistance to pathogenic bacteria. The β-1,3-glucans of certain fungi and yeasts have been successfully used as immunostimulants to enhance the defense potential of fish and shellfish against bacterial and viral infection (**Sung et al., 1994; Song et al., 1997**).

Our study revealed that *Rhodotorula minuta* was isolated from *Oreochromis niloticus* intestine; *Saccharomyces castelli* from *Mylopharyngodon piceus* intestine; *Zygosaccharomyces* spp from *Hypophthalmichthys molitrix* intestine and three different species of *Candida* were isolated from *Clarias Garipeneaus* muscles. That is mean yeasts have been commonly isolated in the gastrointestinal tract, and high population densities were sometimes noted in healthy fish as mentioned by **Gatesoupe (2007)**. *Rhodotorula* spp. seemed relatively frequent in both marine and freshwater fish. **Patterson and McGinnis (2006)** reported that yeasts occurring in fish microbiota may be classified in two distinct phyla of the fungal kingdom: *Ascomycota*, among which *Saccharomycetaceae* are

probably the most important family, but also *Basidiomycota*, which include the genus *Rhodotorula* – these red yeasts are commonly observed in fish microbiota. **Vazquez-Juarez et al., (1993)** isolated yeast from the intestines of wild rainbow trout and introduced it with feed into the digestive tracts of domestic rainbow trout.

Saccharomyces castelli vegetative reproduction occurred` by budding, no filaments formation, persistent asci containing 1 or 2 rough, round ascospores, These results in agreement with **Larone** (**1995)**; **Sutton (1998) and Barnett et al. (2000)**. The isolate fermented glucose and failed to ferment galactose, sucrose, maltose, salicine, xylose, arabinose, lactose, trehalose and manitol. It was able to assimilate the nitrate, These results in agreement with **De Hoog et al**. **(2000)**. It could not form starch. **Barnett et al. (1990)** reported that most of the yeasts, except *Saccharomyces* spp., can grow on cellulosic materials, however; only few genera are able to degrade starch. Among disaccharides, lactose is one of the most refractory carbon substrate to most of the yeasts.

Zygosaccharomyces species vegetative reproduction occurred by budding, These results agree with **Barnett et al. (2000)**. The persistent asci contained 1 or 2 rough and round ascospores. It fermented glucose and failed to ferment galactose, sucrose, maltose, salicine, xylose, arabinose, lactose, trehalose and manitol.

Rhodotorula minuta was pink color, mucoid to butyrous, raised, glysting, circular, entire edges and 3 - 4 mm in diameter**.** These results are in agreement with **De Hoog et al. (2000)** and it *contains* carotenoid pigments **(Yurkov et al., 2008).** The vegetative reproduction occurred by budding, no filaments growth and no sexual production. These results agree with **Barnett et al. (2000)**. The isolate failed to ferment glucose, galactose,

sucrose, maltose, salicine, xylose, arabinose, lactose, trehalose and manitol. These results in agreement with **Kappe and Schulze-Berge (1993)**.

Candida species vegetative reproduction was by budding, have filaments, elaborate pseudo hyphae. The isolate fermented glucose, galactose, sucrose, maltose, trehalose and failed to ferment salicine, xylose, arabinose, lactose and manitol. It assimilated the nitrate and did not excreted starch. These characters of Candida are as described by **Larone (1995) and Barnett et al. (2000).**

Antimicrobial activity in our results revealed that *Rhodotorula minuta*, *Zygosaccharomyces* species and *Saccharomyces castelli* had antibacterial activity against *Aeromonas hydrophila, Aeromonas veronii, Aeromonas sobria, Aeromonas jandaei, Pseudomonase anguilliseptica and Pseudomonas fluorescence.* The three yeasts isolates had antibacterial activity because of the producing the ethanol which have power antimicrobial activity. This result was in agreement with **Gedek (1999)** and **Castagliuolo et al. (1999)** who reported that the yeast may be antagonistic to entero-pathogenic bacteria, due to adhesion of bacterial cells or by secreting proteases which inhibit bacterial toxins. Also yeasts produced extracellular proteases and siderophores, and they bound lactoferrin **(Gatesoupe et al., 1997).** (**Edskes et al., 2009**) mentioned that *S. castellii* has URE2 gene in which it was toxic to *Escherichia coli*. **Peter et al. (1994**) reported that some yeasts as *Rhodotorula glutinis*, and *Sporobolomyces roseus* produced antibacterial compounds inhibitory to both *Pseudomonas fluorescens* and *Staphylococcus aureu.*

The occurrence of antibiotics resistant strains in aquacultural environments has apparently increased over the past few years. The results of this study demonstrated that *Aeromonas veronii, Aeromonas Sorbria* and *A.*

jandaei were resistance to Ampicllin, Erthromycine, Tetracycline, Vancomycin, Pencilin G and Streptomycin. *Pseudomonase anguilliseptica* was resistance to Ampicllin, Ciprofloxacin, Kanamycin, Tetracycline, Vancomycin and Pencilin G. *Pseudomonase fluorescence* was resistance to Ampicllin, Tetracycline, Vancomycin and Pencilin G. The report percentages of bacteria resistant to Tetracycline have increased from 14.5% (**Ruangpan and Kitao, 1992**) to 20% (**Sangrungruang et al., 1993**) and finally to 45.9%. Similar findings have been reported by **Austin and Austin (1985)** who found an increase in antibiotic resistance of bacteria effluent water from fish farms and a change in the composition of the bacterial flora in the farm. **Bjorklund et al. (1991)** found a higher degree of tetracycline resistant bacteria in farm sediment than in fish. It was suggested that some portion of the chemotherapeutic mixed in the feed passed through the fish unabsorbed and reached the environment in antimicrobial active form (**Bjorklund and Bylund, 1990**). **Lewin et al. (1992**) decided that bacterial resistance to antimicrobial drugs has become widespread in aquaculture. Cultured fish have been reported to be infected with resistant strains of *Aeromonas hydrophila, Aeromonas salmonicida, Edwardsiella ictaluri, Pasteurella piscicida, Vibrio anguillarum, Yersinia ruckeri,* and *streptococci* resistant to many drugs used in aquaculture, including nalidixic acid, oxolinic acid, tetracycline, sulfa drugs and chloramphenicol.

The minimum inhibitory concentration (MIC) of *Saccharomyces castelli* ranged from 0.008 - 0.8 µg against *A. veronii*, *A. Sobria*, *Ps. Anguilliseptica* and *Ps. fluorecence* respectively and the MIC of *Rhodotorula minuta* 0.8 - 1 µg with *A. veronii*, *A. Sobria*, *A. jandaei*, *Ps. anguilliseptica* and *Ps. fluorecence* respectively. From the antibacterial experimental results, it is evident that the active metabolite of the isolates showed significant antibacterial activity especially against *A. veronii, A.*

Sobria, Ps. anguilliseptica, *Ps. fluorecence* but were less potent than that of standard antibiotics. The results of this study strongly support that the isolated metabolite may be used in the management of microbial infection. In recent years the pathogenic organisms are gaining resistance to existing antibiotics hence the search for new, safe and more effective antimicrobial agents is a pressing need. **Robert (2000)** recorded that the MIC is used as a comparative index for other antimicrobial agents. **Wen et al., 1996** found that the Aeromonas strains were more resistant to tetracycline, trimethoprim-sulfamethoxazole, some extendedspectrum cephalosporins, and aminoglycosides. Also they found that the MICs of ciprofloxacin, the most potent antibiotic, the MIC at which 90% of isolates are inhibited (MIC90) was 0.25 mg/ml. **Chandra Mohan et al., 2010** evaluated the interaction between antibiotics and microbial activity during the sensitivity test. They found that MIC's 0.5 µg/ml (E.coli) and 0.25 µg/ml (S. aureus) of ciprofloxacin showed less sensitivity, partial regrowth was observed in E.coli.

The yeast growth was stopped at the 5^{th} and 6^{th} day with *Saccharomyces castelli* and *Rhodotorula minuta* while *Zygosaccharomyces* at 7^{th} and 8^{th} day. **Emilina et al. (2003)** established that *Rh. rubra* carotenoid production continued and reached a maximum concentration on the 6th day.**Ginka et al. (2004)** reported that the stationary phase of the growth cycle of *Rh. Rubra* reached on the 5th day.

In our results *Zygosaccharomyces and Rhodotorula minuta* were pathogenic to *O. niloticus* while *saccharomyces castilli* was non pathogenic. **Moore and Strom (2003)** said that few types of yeast have been reported as responsible for disease in fish. *Metschnikowia bicuspidata* vA. *bicuspidata* caused mortality in chinook salmon fry fed infected *Artemia franciscana*.

They succeeded to reproduce the disease by intraperitoneal injection of the pathogen. Clinical signs of infected *O. niloticus* with *Zygosaccharomyces* species and *Rhodotorula minuta* were dark coloration especially at peduncle region and hemorrhages at different sites (caudal fin and under anal fin). The Postmortem finding showed hemorrhages in the internal organs with petechi in liver. **Alliot et al. (2000**) and **Groll and Walsh (2001)** reported that *Rhodotorula spp.* was rarely isolated as causative agents of opportunistic mycoses, while; **Shaheen (1991)** reported that *Rhodotorula glutinis* caused desquamation of scales and superficial erosion of the skin especially at the caudal peduncle. Necrosis and hyperemia of the tail fins, hyperemic and slightly enlarged spleen with pale liver in tilapia fish by intraperitoneal injection of the pathogen. Few types of yeast have been reported as responsible for disease in fish such as Candida sp. caused internal lesions in *Oncorhynchus Tshawytscha* (**Mueller and Whisler, 1994**); swim bladder swollen with dense material in *Sparus aurata* (**Galuppi et al., 2001**), *Cryptococcus* sp. caused surface and internal lesions in *Oncorhynchus tshawytscha* (**Mueller and Whisler, 1994**), *Sporobolomyces salmonicolo* caused ascites, visceral mycosis in *Oncorhynchus tshawytscha* (**Muench et al., 1996**) and *Trichosporon* sp. caused internal lesions in *Oncorhynchus tshawytscha* (**Mueller and Whisler, 1994**). The pathogencity of yeasts may be due to some killer toxins. The killer phenotype is very common in occurrence and can be found both in natural yeast isolates and in laboratory yeast strain collections. Up to now, toxin-producing killer yeasts have been identified in genera *Candida, Cryptococcus, Debaryomyces, Hanseniaspora, Hansenula, Kluyveromyces, Metschnikowia, Pichia, Saccharomyces, Ustilago, Torulopsis, Williopsis and Zygosaccharomyces* indicating that the killer phenomenon is indeed widespread among yeasts (**Magliani et al.,**

2004).Also hydrolytic enzyme production is known to play a central role in the pathogenic yeasts and proteinases are the most commonly associated with virulence of microorganism **(Ogrydziak, 1993)**. Clinical signs of non infected *O. niloticus* were hemorrhages at lateral fin and the internal organs with pale pink liver and kidney may possibly be due to the low temperature in which the safety experiment take place in winter season (under stress) and the presence of the antibiotic act as antibacterial and may be as immune depressor. **Shaheen**, **1991** used oxtetracycline as immune depressor. **Rijkers et al., 1981** studied the effect of Oxytetracycline upon the regulation of humeral immunity in fish they found that serum immunoglobulin levels were reduced by 40%-75% after oxytetracycline treatment. Whereas the antibiotic had no effect on the secondary anti-sheep red blood cell response.

The higher viable yeast cells were recorded with 4^{o}C storage and the number were declined by increasing storage time while the number of viable yeast of stored feed at 25^{o}C declined and disappeared at the second month. **Irianto and Austin (2002)** found that the probiotics declined in activity when incorporated into diets over an eight week period.

The growth rate of fish was significantly increased with yeast supplemented diet. Probiotics are expected to have a direct growth promoting effect on fish either by a direct involvement in nutrient uptake, or by providing nutrients or vitamins (**Noh et al., 1994; Ringo and Gatesoupe, 1998 and Bogut et al., 1998**). In this study live and dead yeast supplemented diet had good effect on the growth parameters in *O. niloticus*. Also treated *Saccharomyces cerevisiae* increased growth in rainbow trout (**Tukmechi et al., 2011**). The improved fish growth and feed utilization may possibly be due to improved nutrient digestibility. In this regard, **Tovar et al. (2002), Lara-Flores et al. (2003),** and **Waché et al. (2006)** found that

the addition of live yeast improved diet and protein digestibility, which may explain the better growth and feed efficiency recorded with yeast supplements. **Vazquez-Juarez et al. (1993)** isolated yeast from the intestine of wild rainbow trout and introduced it with feed into the digestive tracts of domestic rainbow trout. They recorded that a significant increasing in the growth of the cultured trout. **Mohsen et al. (2008)** reported that the growth parameters increased significantly with the increase in dietary yeast level. The optimum growth was obtained at 1.0–5.0 g yeast/kg diet. **Marzouk et al. (2008b)** pointed out that the diets supplemented with living *Bacillus subtillis*, dead or living and *saccharomyces cerevisiae* frequently used probiotics that are able to improve the growth parameter. The β-1,3-glucans of certain yeasts have been successfully used as immunostimulants to enhance the defence potential of fish and shellfish against bacterial and viral infection (**Sung et al., 1994; Song et al., 1997** and **Sahoo and Mukherjee, 2002**) chitin (**Vecchiarelli 2000**), nucleic acids as well as mannan oligosaccharides (Li et al., 2004) and acts as well as growth promoters (**Lara-Flores et al., 2003; Li and Gatlin, 2003, 2004 and 2005**) of various fish species. Probiotics may improve digestive activity by synthesis of vitamins, cofactors or improve enzymatic activity (**Gatesoupe, 1999; Jory, 1998** and **Ziemer and Gibson, 1998**). *Rhodotorula minuta* contained unsaturated fatty acids, mainly oleic and linoleic, predominated in triacylglycerols, also contains Sterols and tocopherols (mainly ɣ- and 6-tocopherol) **(Zlatanov et al., 2001)** and yeast lipids has biological activity (**Celligoi et al. 1997).** *Rhodotorula minuta contain* carotenoid pigments (β-carotene) and ubiquinone Q_{10} which, being strong antioxidants (**Yurkov et al., 2008)**.

The hepatosomatic and splenosomatic indices had not significantly differences between the treatments. The organosomatic indices are indicators of health (hepatosomatic index and splenosomatic index) (**Goede and Barton, 1990**). **Marzouk et al. (2008b)** who worked on dead *Saccharomyces cerevisea* and live *Bacillus subtilis* and reported the similar results.

The present volume of erthrocytes in fish blood gives clues to the health status of the animal and can be helpful in determining any abnormalities arising from the use of immunostimulants. Reduced hematocrite may indicate that, fish are not eating or suffering from infections (**Blaxhall, 1972**).Yeast supplemented diet had significantly increasing of hematocrit-level in all treatments. The elevated hematocrit-value could explain the efficiency of the used yeast on the health of the fish status (**Abd El-Rhman, 2009**).

Respiratory burst is one of the most important bactericidal mechanisms in fish (**Ellis, 1999 and 2001**) and a very good indicator of their health status (**Anderson, 1992**). This process generates reactive oxygen species (ROS) that are toxic to many microorganisms (**Hardie et al., 1996**). The antioxidant defenses involve several enzymes, including superoxide dismutase and catalase which they protect against certain pathogenic bacteria (**Barnes et al., 1999**). NBT assay used to determine the activity of phagocytes especially neutrophils and monocytes. In the present study, NBT assay showed significant increase in all treatments received yeasts. This could explain the efficiency of the used yeasts on the non specific immune response. **Ortuno et al (2002)** did not detect NBT value in sea bream due to feeding with whole yeast *S. cervisiae* while, (**He et al., 2011**)

recorded an increasing in NBT value with *S. cervisiae* fermented product in hybrid tilapia.

As a first line of defence, various peptides, such as lysozymes are present in serum where they prevent adherence and colonization by micro-organisms (**Alexander and Ingram, 1992**). Lysozyme is a fish defence element, which causes lysis of bacteria and activation of the complement system and phagocytes by acting as an opsonin (**Magnadottir, 2006**). In the present study, a significant increase in the lysozyme activities was observed in the high dose (10 mg yeat/ kg diet), while it increased with live yeast at the fourth week than second week but it decreased with dead yeast at the fourth week than second week. The increased lysozyme activity has been reported after supplementing the fish-feed, with probiotic (**Panigrahi et al., 2004** and **Taoka et al., 2006**) and β-glucan (**Misra et al., 2005; Misra et al., 2006; Bonaldo *et al*., 2007 and El-Boshy et al., 2010**), Herbal **(Harikrishnan et al., 2010)** and polysaccharides (**Yuan *et al*., 2008**).

Serum *Aeromonas sobria* and *Pseudomonase fluorecence* bactericidal activity was highest in T_5. The viable bacterial counts were significantly lower in all treatments groups when compared with untreated control group. These results are triggered by an increased lysozyme-activity. **Misra et al. (2005)** and **El-Boshy et al. (2010)** mentioned that, serum bactericidal activity in the fish injected with different dosages of β -glucan was always significantly higher than in control. The increased serum bactericidal activity in Achyranthes treated groups indicates that various humeral factors are involved in the innate and/or acquired immunities (**Rao et al., 2006**).

There were significant decreased in total bacterial count of fish intestine of yeast supplemented groups compared with the control. This result was in agreement with **Salminen et al., (1999)** and **Andlid et al.,**

(1995) who defined a probiotic as “a live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance”. The yeasts have to compete with other microorganisms and produced extracellular proteases and siderophores, and they bound lactoferrin. **Marzouk et al, (2008b)** pointed out that the diets supplemented with *Bacillus subtillis*, and dead or living *Saccharomyces cerevisiae* decreased the number of some pathogenic bacteria, while dead *saccharomyces cerevisiae* supplemented diet has no effect.

It is important to estimate the relative level of protection in the treated fish to determine the efficacy of an immunostimulant. The yeast supplemented diet groups reduced mortality which induced by *A. sobria* and *Ps. fluorecence* when compared with the control group. These results indicate that the yeast activated the immune system of the Nile tilapia and it became resistance to pathogenic bacteria that due to yeast or structural polysaccharides which improved disease resistance in fish showed their capacity to reduce mortalities associated with infection by pathogens. These results suggest the yeast supplementation could increase the nonspecific immune system of Nile tilapia resulting in a fish resistance to *A. sabia* and *Ps. fluorecence* infection. Our results in agreement with **Irianto and Austin** (**2002**) **Kumari and Sahoo (2006); Villamil et al. (2002)** and **Mohsen et al., (2008)** reported that yeast or structural polysaccharides improved disease resistance in fish against *Aeromonas* and *Vibrio anguillarum*; **Taoka et al. (2006)** live and dead probiotic against *Edwardsiella tarda* infection.

Also yeast (live or dead) had positive effect on antibody titre, but the live yeast enhanced antibody titre than dead one. **Ainsworth et al. (1994)** observed increased antibody titres to *E. ictaluri* in channel catfish fed 0.1% β -glucan. **Siwicki et al. (1994)** tested several immunostimulants on rainbow

trout as lyophilised *Candida utilis*, lyophilized *S. cerevisiae*, β-glucans, deacylated chitin, a premix of selenium and vitamins C and E, and a premix of butane and amino acids. These additives increased cellular immune response and immunoglobulin serum titer and the most significant stimulations were generally observed with the two yeasts.

Summary

Fish are an important source of nutrients such as proteins of high biological value as well as other important nutrient for human. Tilapia fish have high commercial value because it is widely spread and preferred in Egypt on the view of its special flavor.

The purpose of this study was isolation and identification of some yeast, it antibacterial effect, its growth curve and studied its safety for *Oreochromis niloticus*. Yeast *(Rhodotorula minuta* and *Saccharomyces castelli*) were add to commercial feed (crude protein 30%) in two different dosages (5 and 10 g of yeast / kg diet) and fed Nile tilapia to evaluate its effect as immunostimulant and growth promoter. Fish were fed twice daily until satiation for 28 days. At the end of experimental period, fish was challenged i.p. with *Aeromonas sobria* and *Pseudomonase fluorescens* and kept under observation for 14 days.

The results can be summarized as follows:

1- *Rhodotorula minuta* was isolated from *Oreochromis niloticus* intestine; *Saccharomyces castelli* from *Mylopharyngodon piceus* intestine; *Zygosaccharomyces* sp from *Hypophthalmichthys molitrix* intestine and three different species of *Candida* were isolated from *Clarias Garipeneaus* muscles.

2- *Rhodotorula minuta*, *Zygosaccharomyces* species and *Saccharomyces castelli* had antibacterial activity against *Aeromonas hydrophila, Aeromonas veronii, Aeromonas sobria, Aeromonas*

jandaei, Pseudomonase anguilliseptica and Pseudomonas fluorescence.

3- The experimental infection of *O. niloticus* with *Saccharomyces castelli, Zygosaccharomyces* species *and Rhodotorula minuta* revealed that *Zygosaccharomyces* sp *and Rhodotorula minuta* were pathogenic for *O. niloticus* at 0.2 ml $\times 10^5$ cells/ml by intraperiteneal injection (I/P). The mortality rate was 57.1 and 42.8% respectively in comprising to the control and *Saccharomyces castelli* groups, which the mortalities were 30 and 15.8% respectively.

4- From the growth curve of three yeast species found that the growth of yeast was stopped at the 5^{th} and 6^{th} day of growth with *Saccharomyces castelli* and *Rhodotorula minuta* while *Zygosaccharomyces* at 7^{th} and 8^{th} day .

5- The higher viable cells were recorded with 4^oC storage and the number was declined by increasing storage time while the number of food stored at 25^oC declined and decreased monthly.

6- live yeast *Saccharomyces castelli* and *Rhodotorula minuta* significantly increased the growth parameters.

7- Yeast supplemented diet had significantly increasing of hematocrit-level.

8- Also yeasts significant increased NBT assay and lysozyme activity so they had immunostimulant effect.

9- Live and dead yeasts supplemented diet had Serum bacterialicidal activity against *Aeromonas sobria* and *Pseudomonas fluorescence*. The viable bacterial counts were significantly lower in all treatment groups when compared with untreated group.

10- Also yeasts were significant decreased total bacterial count of fish intestine.

11- Yeasts increased the relative level of protection against *Aeromonas sobria* and *Pseudomonas fluorescence* in Nile tilapia.

12- Live yeast more effective on the level of antibody titre than dead yeast.

It could be concluded that the live *Saccharomyces castelli* and dead *Rhodotorula minuta* had probiotic effect on Nile tilapia by enhancing growth performance, feed utilization, immune responses as well as the resistance to *Aeromonas sobria* and *Pseudomonase fluorecence* infection.