

INTRODUCTION AND REVIEW

Algae are important components of aquatic ecosystems and are used as primary source for fish feeding. Especially the micro-algae are often as a first food for rotifers and artemia. Where phytoplankton serves as feed for the fish larvae.

Marine or fresh water algae are one of the richest sources of bioactive compounds. These compounds may be used as antimicrobial agents. These compounds are simply refer to all types of natural, semi synthetic or synthetic substances which are capable of killing or inhibiting the growth of microorganisms. These antimicrobial agents include antibiotics, antiviral, antifungal, probiotics, and feed preservatives.

Antimicrobial agents are widely used in human, veterinary medicine and in plant agriculture for the treatment of microbial infections. Also it can be used as sub-therapeutically in agri-food and aquaculture industries. Sub-therapeutic uses of antimicrobial agents in animal husbandry practices have been shown preventing bacterial infections and growth promoter.

Fish diseases are major problems for the fish farming industry. Bacterial infections are considered the major causes of mortality in fish hatcheries (**Grisez and Ollevier, 1995**).

The use of antibiotics in aquaculture may introduce potential hazard to public health and environment by the emergence of drug-resistant microorganisms and antibiotic residues. Furthermore, the normal microbial flora in digestive tract, which is beneficial to fish, may also killed or inhibited by oral chemotherapy (**Sugita *et al.*, 1991**).

The antimicrobial substances involved may target various kinds of micro-organisms, prokaryotes as well as eukaryotes. Recently, there has been an increasing interest in Cyanobacteria as a potential source for new drugs (**Glombitza and Koch 1989 and Schwartz *et al.*, 1990**).

Secondary metabolites from algae (Cyanobacteria) are associated with toxic, hormonal, antineoplastic and antimicrobial effects (**Carmichael 1992 and Patterson *et al.*, 1994**).

Cyanobacteria have proved to produce antifungal and antibacterial substances. Bioactive substances from Cyanobacteria can be extracted from the biomass using organic solvents. The antimicrobial effects are visualized in bioassay using selected microorganisms (**Kellam *et al.*, 1988 and Frakmölle *et al.*, 1992**).

The aim of present work is concerning with the antibacterial and antifungal production from the isolated algae in Abbassa Fish Farm. The extracts from these algae used as natural source for treatment the pathogenic bacteria and fungi infecting fish.

The reasearch program including the following:-

- 1- Collection of water samples from Abassa-Fish-Farm.
- 2- Study the physiochemical properties of water samples.
- 3- Isolation and identification of some phytoplankton species.
- 4- Isolation and identification of bacteria and fungus from diseased fish.
- 5- Study the ability of algal extracts to inhibit microbial growth.
- 6- Study the role of algal extracts as treatment of infected fish with the pathogenic bacteria.

Phytoplankton studies:-

El-Ayouty and Ibrahim (1980) surveying the phytoplankton distribution along the River Nile from Assiut to the end of Damietta and Rosetta branches revealed that the dominance of phytoplankton can be arranged descend as follow, Bacillariophyta, Cyanophyta and Chlorophyta. **Soliman (1983)** pointed out that in Lake Edku the maximum frequency of Chlorophyta recorded in July, where the community was mainly represented by *Pediastrum* and *Scendesmus*.

John (1986) noticed that the species numbers of blue green and green algae represent 5 to 15% respectively of total phytoplankton of fresh water.

Cannell and Walker (1989) identified the biological activity of four fresh water members *Spirogyra varians*, *Zygnema cylindricum*, *Mesotaenium caldariorum* and *Mougeotia* sp. They found that these algae inhibit bacterial activity.

Abd El-Tawwab (1994) isolated and purified two kinds of Cyanobacteria, *Anabeana variabilis* and *Oscillatoria* sp. from water of fish ponds of (CLAR) at Abbassa, Sharkia.

Dawah (1998) isolated and formed mass culture from green alga *Chlorella vulgaris* from water of Abbassa fish ponds where it used as a source of dietary protein and used as natural food for fish.

El-Gammal (2005) isolated *Spirulina platensis* from water of fishponds of International Center for Living Aquatic Resources Management (ICLARM) at Abbassa, Sharkia, Egypt. He indicated that *Spirulina platensis* can be used to replace fishmeal protein up to 30%

replacement and also it enhanced the immunity of fish for resistance of pathogenic bacteria as *Aeromonas hydrophila*.

Water analysis:-

Water temperature in ponds is related to solar radiation and air temperature. Water temperatures closely follow air temperatures (**Morrissy, 1976**). Therefore, water temperatures generally are quite predictable by season and by location. It is important to remember that air temperatures at a given locality may deviate from normal for a particular period, and water temperature also will deviate (**Boyd, 1990**).

Water temperature and water chemistry are generally recognized as being important determinants of contaminant toxicity to fish (**Sprague, 1985**).

The temperature of water affects the activity, behavior, feeding, growth and reproduction of all fishes. Metabolic rates in fish double for each 18-°F rise in temperature. Fish are generally categorized into warm water, cool-water and cold-water species based on optimal growth temperature. Channel catfish and tilapia are examples of warm-water species. Their temperature range for growth is between 75-90°F. A temperature of 85°F for catfish and 87°F for tilapia is considered optimum (**Neill and Bryan, 1991**).

Although oxygen can diffuse between air and water, biological processes is regulating dissolved oxygen concentrations in pond water. Plants growing in ponds produce oxygen in photosynthesis. Factors controlling photosynthesis and the amount of oxygen evolved include temperature, light, nutrient concentrations, species and abundance of

plants, turbulence and many other factors of less importance. In surface water of fish ponds, the amount of oxygen produced by photosynthesis usually increases as function of phytoplankton abundance. Cloudy weather profoundly influences dissolved oxygen concentrations in ponds (**Romario and Boyd, 1979**). On clear days, there is sufficient light for high rates of photosynthesis, and dissolved oxygen concentrations often are low at dusk. On cloudy days, photosynthesis is limited by insufficient light, and dissolved oxygen concentrations often are low at dusk. The probability of dissolved oxygen depletion is much greater during nights following cloud days than during nights following clear days (**Boyd, 1990**).

Organic fertilizer act as energy for bacterial growth, but the aerobic decomposition of organic matter by bacteria is an important drain of oxygen supplies in ponds (**Boyd, 1981**). The dissolved oxygen (DO.) in fish ponds with organic fertilizer was lower than in those without organic fertilizer, and DO in enclosures decreased with the increase of organic fertilizer loading. Laboratory experiment also showed that oxygen consumption increased with increasing alfalfa inputs, but gross primary productivity changed little (**Qin *et al.*, 1995**).

The water used in fish cultivation is not pure chemically and contains, in solution, different substances that give it an acid, natural or alkaline reaction (**Huet, 1970**).

Swingle (1961) and Calabrese (1969) showed that the acid and alkaline death points for fish are approximately at pH 4 and pH 11. **King (1970)** reported that photosynthesis by aquatic plants remove carbon dioxide from water during daylight and causes a rise in pH.

In many ponds of fish and crustacean culture, plankton is the primary source of turbidity. Thus, the secchi disk visibility provides an index of plankton abundance (**Swingle, 1945**).

Secchi disk visibilities fluctuate over time and at lower levels, plankton blooms are so dense that low dissolved oxygen concentrations may be a problem. At higher secchi disk visibilities, there will be insufficient plankton for a good food base and the clear water will favor weed problems (**Boyd, 1990**). Secchi disk visibility is an appropriate index of plankton abundance only when plankton is the primary source of turbidity (**Almazan and Boyd, 1978**).

Alkalinity is important for fish and other aquatic life in freshwater systems because it buffers pH changes that occur naturally as a result of photosynthetic activity of the chlorophyll-bearing vegetation. Components of alkalinity such as carbonate and bicarbonate will complex some toxic heavy metals and reduce their toxicity markedly. For these reasons, **the National Technical Advisory Committee (1968)** recommended a minimum alkalinity of 20 mg/l.

Water is categorized according to degrees of hardness (**Sawyer and McCarty, 1967**), into soft, moderately hard, hard, and very hard water.

Whitfield (1974); Bower and Bidwell (1978); Boyd (1982), found that increasing pH one unit causes a ten fold increase in concentration percentage of unionized ammonia.

Higher concentration of nitrate was observed in ponds soon after application of fertilizers (**Zeller, 1952**). Ammonia and ammonium may be used by aquatic plants or nitrified to nitrate which also can be absorbed by aquatic plants. Oxidation of ammonia nitrogen to nitrate

occurs by chemoautotrophic bacteria, primarily Nitrosamines and Nitrobacteria that use ammonium and nitrite, respectively, as energy source (**Boyd, 1990**).

Most fish species concentrate external nitrite in their blood plasma, presumably through active transport by the chloride/bicarbonate exchanger in the chloride cells of the gills (**Bath and Eddy 1980; Eddy *et al.*, 1983**).

Nitrite is a well known toxicant for fish and has been studied extensively (**Lewis and Morris 1986; Eddy and Williams, 1987**). Nitrite has an affinity for the active chloride uptake mechanism in gills of freshwater crayfish and teleosts (**Williams and Eddy, 1986; Harris and Coley, 1991**).

Phosphorus is essential for all living organisms; living matter contains about 0.3 percent dry weight phosphorus. It plays an irreplaceable role as a structural link in the genetic materials DNA and RNA. In adenosine triphosphate (ATP) phosphorus is involved, as short-term energy (currency) in biochemical reactions, and it is a component in the phospholipid membranes of all cell walls. Although phosphorus is not needed for growth in such large amounts as carbon, oxygen, hydrogen or nitrogen, it is perhaps the most common growth-limiting element in freshwater (**Goldman, 1983**).

Dissolved orthophosphate is readily available to plants (**Chamberlain and Shapiro, 1969**), but the availability of other forms has not been determined with certainty (**Lean, 1973; Herbes *et al.*, 1975 and Minear, 1975**). A variable proportion of both particulate and dissolved phosphorus can occur as a molybdate reactive form, which is

believed to be the most available to algae (**Flanagan 1992; Smith 1993**).

The effect of inorganic nutrient loading on chlorophyll "a" concentration is obvious, where 55% increase in amount of inorganic nutrients produced 20% increase of chlorophyll "a" concentration (**Zhu et al., 1990**). **Yusoff and McNabb (1989)** observed that chlorophyll "a" and phytoplankton production were found to be higher in ponds receiving combined phosphorus and nitrogen than in those receiving phosphorus alone.

(**Boyd, 1991**) mentioned that phytoplankton growth in aquaculture ponds is regulated in freshwater primarily by phosphorus concentration and blue green algae, which often abound freshwater aquaculture ponds can fix nitrogen, so phosphorus is more likely to limit phytoplankton growth than is nitrogen in freshwater.

Algal productivity is primarily a function of nutrients (N, P and C), light availability and temperature (**Knud-Hansen and Batterson, 1994**). Factors such as light, temperature and nutrients play an important role in determining phytoplankton productivity in aquatic systems (**Hutchinson, 1967; Talling, 1971 and Wetzel, 1983**). Nutrients may play a major role in controlling phytoplankton productivity since they are usually in short supply as stated by **Yaacob and Shamsuddin (1982)**.

Algal extract as antimicrobial agents:

Shelat (1981) tested the anti-fungal activity of some members of Rhodophyceae against 17 fungal species. He found that the methanol

extracts of *Gelidiella acerosa* caused the maximum growth inhibition of *Candida tropicalis* and *Gracilaria corticata*.

Lustigman (1988) demonstrated that the alcoholic extract of the marine algae *Dunaliella* showed a broad spectrum antibiotic activity against Gram positive and Gram negative bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, *P. vulgaris*, *Salmonella gallineum* and *S. epidermidis*.

Miura and Matsunaga (1989) stated that, organic solvents and hot water extracts of 100 marine micro algae were screened for antibiotic activity using paper disc method. Two strains produced antibiotics, against *Saccharomyces cerevisiae*, which was extracted by hot water, and organic solvent extracts of 33 strains had activity against *Mycobacterium phlei*. Extracts of many strains by organic solvent had activity against some *Bacillus species*.

DeCano et al. (1990) determined phenolic compounds in methanolic extract from the algal mass of *Nostoc muscorum* culture. These algal phenolic compounds evoked significantly growth inhibition for two human pathogens *Candida albicans* and *Staphylococcus aureus* (89.1% and 88.2% respectively).

Crosby (1991) and McGill and Hardy (1992) pointed out that antimicrobial effects are shown as visible zones of growth inhibition, where bacterial bioassays comprise different tests bacteria *Micrococcus luteus*, *Bacillus subtilis*, *B. cereus* and *Escherichia coli* are commonly used to detect antibiotic residues in food .

Uddin et al. (1991) isolated a new metabolite (pinnatifolide) from methanolic extract of red algae *Laurencia pinnatifida*; it showed significant activity against Gram positive bacteria.

Chen et al. (1994) found that, methanol toluene extracts of ten red and brown algae from Fujian coast had antibacterial and antifungal activity.

Kim et al. (1994) extracted *Hizikia fusiforme* by solvents (hexane, ethyl ether and ethanol), these extracts were found to be active against *E. coli* and *Bacillus subtilis*.

Sastry and Rao (1994) secured that, antibacterial substances from successive extractions of marine algae using benzene, chloroform and ethanol had activity against both Gram-positive and Gram-negative bacterial strains. The chloroform extract exhibited the greatest antibacterial activity.

Mahasneh et al. (1995) stated that various degrees of activity were presented in 18 algal extracts against multi-antibiotic resistant bacteria and found the best solvents for extractions were methanol and acetone for Rhodophyta, Phaeophyta and Chlorophyta.

De- Lara- Isassi et al. (1996) found that, ethanolic and acetonic extracts were effective against five pure strains of Gram positive and Gram negative bacteria. The highest activity was found in the acetonic extract of *Jania tenella*, with inhibition zones of 22 mm against *Staphylococcus aureus* and 18 mm against *S. pyogenes*.

Ostensvik et al. (1998) used aqueous and methanol extracts from five selected Cyanobacterial strains and recorded that they have antibacterial properties.

Hellio et al. (2000) secured that, aqueous, ethanolic and dichloromethane fractions from 16 marine algae had inhibitory effects against fungi, bacteria and yeasts.

Liu et al. (2000) reported that the phycocyanin extracted from *Spirulina platensis*, significantly inhibited the growth of myelogenous leukemia-blast crisis K562 cells.

Nagayama et al. (2002) recorded that, phlorotannins extract from thalli of the brown algae *Ecklonia kurome* have bactericidal activity against food-borne pathogenic bacteria.

Zineb et al. (2004) found that different extracts from marine algae (*Cystoseira tomaricifolia*) had antimicrobial, antifungal and antimycotoxins activities. They recorded that the minimal Inhibitory Concentration (MIC) with ethanolic crude extract on all the fungal species at concentration 10%. The aqueous extract showed an activity only on some strains used while no activity was observed on yeast moulds for both methanolic and aqueous extract. Mycotoxins formation in *Aspergillus flavus* was inhibited by the ethanolic extracts at the concentration of 5%.

Katircioglu, et al. (2006) found that, ether, acetone, ethanol and methanol extract of 10 microalgae strains (*Chroococcus* sp., *Oscillatoria* sp., *Anabaena* sp., *Synechocystis aquatilis* and *Chlorella vulgaris*) isolated from different freshwater reservoirs situated in Turkey, had antimicrobial activity against *Bacillus subtilis*, *Bacillus cereus*, *Bacillus megaterium*, *Escherichia coli*, *Staphylococcus aureus*, *Micrococcus flavous*, *Pseudomonas aeruginosa*, *Saccharomyces cerevisiae*, *Candida albicans* and *Candida tropicalis*.

Antimicrobial activities of algal extract:

Duff *et al.* (1966) and Lincoln *et al.* (1990) indicated that the presence of bioactive compounds in *Tetraselmis* have ability in the control of fish diseases.

Sims *et al.* (1975) found that five compounds extracted from marine algae had antibacterial activity against, *Staphylococcus aureus*, *Salmonella choleraesuis*, *Mycobacterium smegmatis*, *Candida albicans* and *Escherichia coli*.

Caccamese *et al.* (1980) tested the lipid extracts of 13 algae from eastern Sicily for antimicrobial activity and they found that *Zonardinia* (Phaeophyceae) and *Codium coralloides* (Chlorophyceae) showed the strongest inhibitory effect against *Bacillus subtilis*.

Findlay and Patil (1984) isolated antibacterial compounds from the diatoms *Navicula delognei*, which showed significant antibacterial activity against *Staphylococcus ureus*, *Staphylococcus epidermal*, *Salmonella typhimurium* and *Proteus vulgaris*.

Jones (1988) suggested that *Chlorella* produces more than one antibiotic substance and that one of these may be chlorophyllid, a precursor of chlorophyll.

Kellam *et al.* (1988) proved that antimicrobial effect from Cyanobacterial aqueous and organic solvent extracts are visualized in bioassay using selected micro-organisms.

Austin and Day (1990) reported that *Tetraselmis suecica* had inhibitory activity towards bacterial fish pathogens and suggests that the algal cells could make a valuable dietary supplement for Salmonids.

Hiroshi *et al.* (1990) tested twelve polyether compounds originating dinoflagellates. These compounds showed antifungal and antibacterial activities.

Bloor and England (1991) reported that nitrate and iron, were the factors significantly affecting antibiotic production by *Nostoc Muscorum* and the extra cellular metabolites produced inhibited the growth of *Bacillus Circulans*.

Tariq (1991) detected antifungal activity include extracts of marine red algae *Dilsea carnose*, *Laurencia pinnatifida*, *Odonthalia dentata* and *Polysiphonia lanosa* which reduced the rate of colony extention in *Microsporum conis* and *Trichophyton verrucosum* with seasonal variation in the level of inhibitory activity.

Frankmölle *et al.* (1992) pointed out that, the cyclic peptides of crude ethanolic extracts from the cultured blue green alga *Anabaena laxa* exhibited unusual biological synergisms when tested for antifungal effects specially against *Aspergillus oryzae*, *Candida albicans*, *Penicillium notatum*, *Saccharomyces cerevisiae*, and *Trichophyton mentagrophytes*.

Kamat *et al.* (1992) proved that, ethanolic extracts of Indian marine algae belonging to the Rhodophyceae, Phaeophyceae and Chlorophyceae had antibacterial and antiviral activity. The antiviral activity observed in *Codium elongatum* and two species of *Hypnea* was attributed to the polysaccharides.

Austin *et al.* (1992) reported that the supernatants and extracts derived from spray-dried preparation of *Tetraselmis Surcica* (Chlorophyceae) were observed to inhibit *Aeromonas hydrophila*, *A.*

salmonicida, *Lactobacillus* sp., *Serratia liquefaciens*, *Staphylococcus epidermidis*, *Vibrio anguillarum*, *V. salmonicida* and *Yersinia ruckeri* type *in vitro* methods .

Kulik (1995) found that a number of Cyanobacteria and eukaryotic algae, particularly macroalgae, produce various biologically active compounds as antibiotics and antifungal.

Mtolera and Semesi (1996) found that, the extracts of six marine green algae from Tanzania had antimicrobial activity against three bacterial species (*Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli*) and yeast (*Candida albicans*).

Namikoshi and Rinehart (1996) reported that, Cyanobacteria produce a large number of compounds with varying bioactivities.

Direkbusarakom et al. (1997) indicated that extra cellular products of *Chlorella* contained antibiotic activity which inhibited three strains of *Vibrio* isolated from diseased Shrimp (*Vibrio harveyi*, *V. paraphaemolyticus* and *V. penaeicida*).

Vlachos et al. (1997) found that, extracts from 56 southern African Seaweeds, from division's Chlorophyta, Phaeophyta and Rodophyta have antimicrobial activity against 12 bacteria, two yeasts and two moulds.

Reshef et al. (1997) found that, there are five novel diacylated sulfoglycolipids which were isolated from Cyanobacterium *Scytonema* sp. and four novel acylated diglycolipids which were isolated from Cyanobacterium *Oscillatoria raoi* inhibited HIV.1 and reverse transcriptase enzymatic activity. **Gustafson et al. (1997)** isolated a novel anti -HIV protein from a aqueous cellular extract of the cultured

Cyanobacterium (blue green algae) *Nostoc ellipsosporum*. **Blinkova et al. (2001)** proved that *Spirulina platensis* sulfolioids were effective against HIV; preparations were obtained from *Spirulina* biomass showed also activity against herpes virus, cytomegalo virus and influenza virus. **Huleihel et al. (2001)** reported that the cell wall sulphated polysaccharide of the red microalga *Prophyridium* sp. showed impressive antiviral activity against *Herpes simplex* viruses types 1 and 2 (HSV1, 2) and *Varicella zoster* Viruse (VZV).

Jaki et al. (1999) isolated a novel extracellular diterpenoid with antibacterial activity against *Bacillus cereus*, *Staphylococcus epidermidis*, and *Escherichia coli* from the Cyanobacterium *Nostoc commune*.

Jaki et al. (2000) reported that there are five novel extra cellular metabolites with an unprecedented diterpenoid skeleton isolated from the culture medium of terrestrial Cynobacterium *Nostoc commune* by means of bioguided isolation .The Molecules were designated as comnostins A-E. All comnostins showed antibacterial activities. Additionally, cytotoxic mollusscidal activities were found for comnostin B.

Klochenko et al. (2001) detected that the antifungal activity of different fresh water Cyanobacteria, using different species of *Fusarium* and other fungi as indicator organisms, and the strongest inhibitory activity of the Cyanobacteria tested was found with *Calothrix braunii*, *Tolypothrix tnuis*, *Spirulina platensis*, *Oscillatoria* sp. and *Lyngbya limnetica* .

Jaki et al. (2001) isolated two novel cyclic peptides with antifungal activity against the yeast (*Candida albicans*) from the Cyanobacterium *Tolypothrix byssoidea* (EAWAG195).

Lima- Filho et al. (2002) recorded that, extracts of *Amansia multifida* had antibacterial activity against Gram-negative strains such as *Enterobacter aerogenes*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *S. choleraesuis*, *Vibrio cholerae* and the Gram positive bacteria *Bacillus subtilis* and *Staphylococcus aureus*.

Tendencia and dela Peña (2003) reported that *Chlorella* sp. inhibits the growth of *Luminous* bacteria after 48h.

Tendenica et al. (2004) reported that stocking of tilapia at a biomass not lower than 300 g/m³ efficiently inhibited the growth of *Luminous* bacteria in Shrimp (biomass = 80 g/m³) rearing water.

Tendencia et al. (2005) reported that the presence of *Chlorella* sp. (10⁵ cells / ml) alone was not effective in the control of *Luminous* bacteria in Shrimp (biomass=80g/m³) rearing water. The presence of *Tilapia hornorum* alone (biomass = 500g/m³) was more efficient in controlling the growth of *Luminous* bacteria than the co-existence of tilapia and *Chlorella* sp.

Bacterial studies:-

Ross and Toth (1974) reported the first mortality for fish associated with *Lactobacillus* spp. was in rainbow trout within a California hatchery where caused pseudokidney disease, which caused distended abdomen against the fish.

Larsen and Jensen (1977) secured that the motile *Aeromonas* group, especially *Aeromonas hydrophila* is the cause of hemorrhagic septicemia. It is a disease affecting wide variety of fresh water fish species and occasionally marine fish. **Kuo and Kuo (1978)** secured that out breaks of red spot disease caused mass mortalities among pond, cultured eel in both Lu-Kong and Ping-Tung area. The causative organism is a Gram-negative, rod with a single polar flagellum. From the biochemical characteristics, it was identified as *Pseudomonas anguilliseptica*.

Csaba et al. (1981) and Badran (1993) recorded that *Oreochromis aureus* was the most fish affected with *Pseudomonas septicamia* where the examination of naturally infected tilapia by *Pseudomonas septicamia* were haemorrhages all over the body especially on the ventral surface, erythema at the base of fins, mouth, opercula and genital opening, slight ascitis. The postmortem examination revealed preasence of few amount of yellowish sanguineous fluid in the abdominal cavity. **Cone (1982)** recorded that *Lactobacillus* species were fish pathogens, which formed extensive damage in liver, kidney and spleen and petechial hemorrhages in muscles and hyperemic gas bladder, while gills appeared normal.

Nakai and Muroga (1982) reported that the cause of red spot disease occurred in European eel (*Anguilla anguilla*) was *Pseudomonas anguilliseptica*.

Newman (1983) and Sarieyyüpoğlu (1984) pointed that the motile *Aeromonad* species are often ubiquitous members of the aquatic

ecosystem, but all can be components of the microbial flora of aquatic animals and may be pathogens of poikilotherms, and humans.

Stewart *et al.* (1983) secured that the adults and elvers of European eel die within three weeks of transportation to fresh water. Developed small petechial hemorrhages over most the ventral surface. They isolated *Pseudomonas anguilliseptica* as a causative agent of red spot disease in eel.

Couch and Fournie (1993) reported that *Lactobacillus* have been identified as part of normal flora of both marine and fresh water fish and change to pathogens causing pseudokidney disease. **Badran (1993)** isolated *Pseudomonas fluorescens* from Nile tilapia and common carp where, it was gram negative, rod shape, motile, oxidative and produce catalase.

Austin and Austin (1993) and Abd El-Rhman (2003) pointed that *Lactobacillus* species are Gram positive, cocci, coccobacilli and bacilli, the naturally infected *Oreochromis aureus* by *Lactobacillus* species suffered from dark coloration, ulcers, eye cataract, congestion and enlargement in kidneys.

Loennstroem *et al.* (1994) indicated that strains of *Pseudomonas anguilliseptica* were isolated from *Baltic herring*, *Clupea harengus* membranes showing hemorrhages in the eyes.

Wiklund and Loennstroem (1994) reported that in 1986 the *Pseudomonas anguilliseptica* was isolated for the first time from diseased salmonids in Finland and *Pseudomonas anguilliseptica* was recovered mainly from diseased farmed rainbow trout, farmed Salmon, sea trout and white fish.

Lavilla-Pitogo et al. (1998 a, b) reported that in Philippine grow-out ponds, the onset of shrimp mortalities was always preceded by the increase in the number of *Luminescent* bacteria in the rearing water.

Taiwn et al. (2000) recorded that *Bacillus firmus* isolated from disease sea urchin caused Black mouth disease were Gram- positive, rod shaped. Catalase was positive and oxidase negative, they grow in 1% - 7% NaCl, reduced nitrate to nitrite, fermented glucose, xylose, arabinose and mannite. They can not grow in anaerobic medium.

Řehulka (2002) found that *Aeromonas* causes mass death of rainbow trout, *Oncrohyncus mykiss* (Walbaum) which formed skin lesions started as depigmented spots surrounded by a hyperaemic zone with the formation of ulcers.

Düğncl and Candan (2003) found that the *Aeromonas* strains which isolated from Salmon were Gram negative, rod-shape, oxidase-positive, fermentative, motile, glucose fermentation, ornithine decarboxilase positive.

Fungal studies:-

Olufemi et al. (1983) reported the details of Aspergillomycosis among intensive fish farm of tilapia in Kenya during the spring and early summer monthes. Morphological and postmortem changes were described. *Aspergillus flavus* and *Aspergillus niger* were isolated from heart, spleen and liver of the died fish.

Prasad et al. (1987) examined 40 fish samples collected from local market, 14 *Aspergillus flavus* strains of various morphological appearance were isolated.

Bhattacharya (1988) isolated *Aspergillus flavus* from infected fish (*Channa punctatus*) collected from shallow polluted pond water.

Bhattacharya et al. (1988) isolated *Aspergillus terreus* from hemorrhagic ulceritic patches on *Channa gachua*. The fungal growth was observed in injured fish and fish died within 5-11 days.

Udaya and Bhattacharya (1988) reported that pathes and some haemorrhic ulcers on the body and upper surface of the gills of infected fish collected from shallow pond water were of mycotic origin. Microscopic examination of gills and skin scraping confirmed the presence of the *Aspergillus niger*. **Marchenko (1988)** isolated *Mucor* spp., *Rhizopus* spp., *Penicillium* spp., *Aspergillus* spp. and *Alternaria* spp. from *Siberiam* and Hump backed salmon. **Isaeva and Kostik (1989)** isolated 21 species of fungi related to 11 genera from the surface of *salmogairdneri*. The isolated genera were *Aspergillus flavus*, *A. niger*, *Alternaria*, *Cladosporium cladosporium*, *Fusarium sporotrichioides* and *Penicillium*.

El-Hissy et al. (1989) isolated 46 species belonging to 22 terrestrial fungal genera from water after washing the external and internal organs of seven species of Nile fish (*Tilapia nilotica*, *Schillbe mystus*, *Bagrus bayed*, *Mormyrus kannum*, *Clarias lazera*, *Labeo niloticus* and *Synodontis schall*). The fungal isolated were *Aspergillus niger*, *A. flavus*, *Pencillium* spp., *Alternaria* spp., *Fusarium* spp., *Cladosporium* spp. and *Mucor* spp. were the most common.

Marzouk et al. (1990) described the different species of moulds caused tail and fins rot diseases and skin lesions in *Tilapia* species and Nile cat fish from different localities in Egypt. The isolated moulds

were *Saprolegnia* species, *A. Flavus*, *A. Niger*, *Penicillium* spp., *Fusarium* spp., *Alternaria alternaria*, *Mucor* spp., *Chladosporium* spp. and *Rhizopus* spp.

Ahmed et al. (1990) reported that 20 of Angle fish examined for the presence of saprophytic and pathogenic fungi in a trial to know the cause of the high mortalities among aquarium fish. Fungi were isolated from the internal organs (liver-spleen and kidneys) and from the surface of the gills and skin. Identification of fungal isolates revealed *Saprolegnia diclina*, *Achlya* spp. and *Aspergillius flavus*.

Hughes (1994) reported that all fish in fresh water can be infected with *Saprolegnia* spp. Salmonids are known to be particularly vulnerable and *Saprolegnia*-infected fish are easily recognized by the cotton-like, white to greyish patches on the skin and gills, visible to the naked eye.

Nagib (1994) described the different species of moulds caused skin lesions in *Tilapia nilotica* from different localities in Egypt. The isolated moulds were *Saprolegnia* spp., *A. flavus*, *A. niger*, *Fusarium* spp. and *Chladosporium* spp.

Plumb (1997) recorded that fungal infections usually increase on tilapia; the most common species was *Saprolegnia parasitica*.

Ahmed (1998) isolated 9 fungal genera from the apparently healthy fish, the samples were collected from fish farms at different localities in Egypt, from Abbasa Central Laboratory Aquaculture, Abbasa Fish Hatchery and Nawa Farm. These genera were *Aspergillus*, *Mucor*, *Fusarium*, *Penicillium*, *Alternaria*, *Chladosporium*, *Candida*, *Scopulariopsis* and *Rhodotorula*. The members of genus *Aspergillus*

were the most predominant with occurrence percentage of 21% in apparently healthy fish while in diseased fish its percentage was 20%.

Rach *et al.* (2004) secured that fish and fish eggs are commonly parasitized by numerous species of aquatic fungi belonging to family Saprolegniaceae. *Saprolegnia parasitica*, *Achlya hoferi* and *Dictyuchus* sp. Saprolegniasis is presumptively identified by the presence of fluffy, cotton-like, white to gray or gray to gray-brown growth on the skin, fins, gills or eyes of fish or on fish eggs.