Summary

A total number of 240 cultured and wild *O. niloticus* ((75± 5 gms) and 200 wild *C. gariepinus* (100 ± 10gms) were collected alive from Abo-Saleh fish hatchery (cultured) and khor Abo-Sleem (wild) and fish markets of Beni-suef governorate during spring and summer seasons for determination of edwardsiellosis prevalence. Edwardsiellosis prevalence in spring season was 13.33, 3.33 and 3% among cultured and wild *O. niloticus* and wild *C. gariepinus* respectively. On the other hand, in summer season, edwardsiellosis prevalence was 6.7, 0 and 0% among cultured and wild *O. niloticus* and wild *C. gariepinus* respectively. At the same time of *O. niloticus* collections, water samples were collected and examined for studying the effect of water quality on edwardsiellosis prevalence among cultured and wild *O. niloticus*. In spring season, there was fluctuation in water temperatures (20-30 ºC) and they were some what stable (28.3-29 ºC) in summer season in cultured habitat. DO. levels were high either in spring (8.2-9.4 mg/l) or in summer (6.56-7.4 mg/l). In addition, there was excessive unionized ammonia (NH3) either in spring season (0.3-0.4 mg/l) or in summer (0.5-0.7 mg/l). Highest prevalence of edwardsiellosis (30%) was detected in mid spring, while in the beginning and at the end of spring season and at the beginning and mid of summer season edwardsielosis prevalence was 10%. Similarly, in spring season, there was fluctuation in water temperatures (21-29 ºC) and they were some what stable in summer season (29-30.3 ºC) in wild habitat. DO. levels were low (3.13-4.5 mg/l) in spring season than that of summer season (5.0-6.04 mg/l). In addition, there was excessive unionized ammonia (NH3) either in spring season (0.2-0.3 mg/l) or in summer season (0.3-0.4 mg/l). At the beginning of the spring season edwardsielosis prevalence was 10%. Oppositely, there was no edwardsiellosis among wild *O. niloticus* at the mid and end of the spring season and all over the period of summer season.

Edwardsiellosis naturally infected *O. niloticus* showed variety of clinical signs including scale-loss, skin darkening, congestion and haemorrhages allover the body. Also, fin rot,

exophthalmia, opacity or hemorrhages of the eyes and protruded congested vent were seen. Moreover, abdominal distention, pale or congested liver, distended gall bladder and congested and enlarged spleen were detected.

On the other hand, edwardsiellosis naturally infected *C. gariepinus* showed ulceration of the head, peduncle region and dorsal musculature. In addition, swellings in the dorsal musculature when punctured emit a foul odor, disintegration of dorsal fin and fin rot were seen. The post-mortem lesions were congestion and enlargement of kidney and pale liver and the both organs were soft.

Isolation of edwardsiellosis causative agent *(E. tarda)*, from diseased *O. niloticus* and *C. gariepinus* was done using SS agar medium. Identification of the pure isolates of *E. tarda* was carried out on the basis of conventional biochemical tests and confirmed by API 20E biochemical system and PCR technique.

The morphological characters of *E. tarda* colonies on SS agar their variation in size and characterization by black centers to predominantly black colonies. In addition, the results of manual biochemical tests were analogues to that of API 20E biochemical system. The isolates of *E. tarda* in this study exhibited variation only in citrate utilization test, where, 2 only out of 17 isolates were failed in citrate utilization. By the PCR technique, type 1 fimbrial gene was detected in citrate negative and citrate positive *E. tarda* isolates which is specific for identification and pathogenicity of *E. tarda* isolates.

The pathogenicity test was carried out on citrate negative & citrate positive *E. tarda* isolates in *O. niloticus* by I/P injection of 0.3ml of 3×10^8 CFU/ml (McFarland 1 standard turbidity) and 1.5×10^8 CFU/ml (McFarland 0.5 standard turbidity). The results showed that citrate negative isolate was more pathogenic than citrate positive isolate especially at lower concentration (1.5×10^8 CFU/ml) as mortality rates were 80 % matching with 20% mortality in the citrate positive isolate. The experimentally infected *O. niloticus* manifested similar clinical signs of the naturally edwardsiellosis infected fish. The LD_{50} of citrate negative *E. tarda* isolate for *O. niloticus* was 1.5×10^6 CFU/ml.

In this study, trials for protection of *O. niloticus* against edwardsiellosis were conducted by using probiotic, *E. faecium* in feed. The mortalities were 40% and RLP% was 43% in fish fed *E. faecium* supplemented diet for one week. Oppositely, no mortalities and 100% RLP% were detected in fish fed *E. faecium* supplemented diet for two weeks, fish fed *E. faecium* supplemented diet for two weeks and challenged one week after feeding stop and fish fed *E. faecium* supplemented diet for two weeks and challenged two weeks after feeding stop. On the other hand, the control group showed 70% mortalities and zero% RLP%.

Differential leucocytic counts were characterized by predominance of lymphocytes & monocytes in the blood of *O. niloticus* fed *E. faecium* supplemented diet for two weeks, fish fed *E. faecium* supplemented diet for two weeks and challenged one week after feeding stop and fish fed *E. faecium* supplemented diet for two weeks and challenged two weeks after feeding stop. Contrary, number of lymphocytes & monocytes decreased in fish fed *E. faecium* supplemented diet for one week. Granulated leucocytic neutrophiles significantly decreased in the blood of fish fed *E. faecium* supplemented diet for two weeks and challenged one week after feeding stop and fish fed *E. faecium* supplemented diet for two weeks and challenged two weeks after feeding stop. Eosinophiles were found only in the blood of fish fed *E. faecium* supplemented diet for two weeks & fish fed *E. faecium* supplemented diet for two weeks and challenged two weeks after feeding stop. On the other hand, the basophiles did not found in all groups including the control. Regarding, total protein, albumin, globulin and A/G ratio, there was a significant increase in the total protein and globulin in the fish fed *E. faecium* supplemented diet for two weeks, fish fed *E. faecium* supplemented diet for two weeks and challenged one week after feeding stop and fish fed *E. faecium* supplemented diet for two weeks and challenged two weeks after feeding stop. On the other hand, there was a significant increase in albumin in the fish fed *E. faecium* supplemented diet for one week, fish fed *E. faecium*

supplemented diet for two weeks, fish fed *E. faecium* supplemented diet for two weeks and challenged one week after feeding stop and fish fed *E. faecium* supplemented diet for two weeks and challenged two weeks after feeding stop. A/G ratio in all fish groups did not show significant difference in comparison with control group.

Concerning effect of probiotic *E. faecium* on the level of lysozyme and complement 3, it was found that the level of lysozymes of fish fed *E. faecium* supplemented diet for one week and fish fed *E. faecium* supplemented diet for two weeks and challenged two weeks after feeding stop were increased insignificant compared to control group. Lysozymes levels in fish fed *E. faecium* supplemented diet for two weeks & fish fed *E. faecium* supplemented diet for two weeks and challenged one week after feeding stop showed significant increase than that of control group. On the other hand, the complement 3 of all experimental groups was insignificantly differed than that of control.

The total viable intestinal bacterial counts of *O. niloticus* fed *E. faecium* supplemented diet for one week, fish fed *E. faecium* supplemented diet for two weeks, fish fed *E. faecium* supplemented diet for two weeks and challenged one week after feeding stop and fish fed *E. faecium* supplemented diet for two weeks and challenged two weeks after feeding stop were 7×10^8, 2.2×10^8, 3×10^8 & 4×10^8 CFU g^{-1} respectively in comparison with $6.1 \pm 0.1\times10^9$ CFU g^{-1} in the control group.

Another trial for prevention and control of edwardsiellosis in *O. niloticus* by using plant extracts carvacrol & its biological precursor cymene was done. The synergistic effect of the plant extracts carvacrol and its biological precursor cymene at concentration of 100 ppm and 200 ppm could be able to prevent and control edwardsiellosis in *O. niloticus*, as there was no mortality in compared to 50% mortality of control group. In addition, fish fed plant extracts supplemented diet appeared silvery bright, active, alert and had good appetite. On the contrary, fish fed non supplemented diet exhibited typical edwardsiellosis clinical signs.