Discussion
In the present study, among the Indian major carps *Catla catla* and among the peninsular carps, *Labeo fimbriatus* were found most affected/susceptible species to *Lernaea cyprinacea* infection, which is in accordance with the observations made by Tamuli and Shanbhogue (1996) and Nandeeshia et al., (1984) that *C. catla* and *L. fimbriatus* are the most susceptible species to *L. bhadraensis* infection. Similarly, in an experiment to find out the comparative susceptibility of fingerlings of seven species of carps viz: *L. fimbriatus*, *L. rohita*, *L. calbasu*, *C. catla*, *Ctenopharyngodon idella*, *Cyprinius carpio* and *Hypophthamichthys molitrix* to *L. cyprinacea*, *L. fimbriatus* and *C. catla* along with *C. idella* and *H. molitrix* were found to be the most susceptible species (Hemaprasanth et al., 2011). In the present study, the infected fishes had abscesses in and around the site of infection and appeared pale and emaciated. In addition, *Lernaea* infection also had an adverse effect on the growth of the fish host. According to Putz and Bowen (1964), *Lernaea* attach to the exposed body surfaces of host fish, where they cause acute hemorrhage and ulcers at the site of penetration. The present observation is in conformity with Faisal (1988) who reported that *Lernaea* induces a severe and degenerative process in the skin and muscle tissue of infected fish and causes retarded growth of the fish. Apart from the physical and biochemical damage, the infection also affects the reproductive performance of the host. Similar observations were made by Baur (1962) and Yamaguti (1963) in case of mosquito fish. They observed the copepod destroying the host’s fins which is especially disastrous to the host when infection of the male’s sexual fin causes paralysis and thus sterility.
Culturing the parasite under laboratory conditions at 28°C resulted in successful hatching of the parasite egg and its further development. These finding are in agreement with Shields and Tidd (1968) who reported that optimal growth and development of *L. cyprinacea* occurred at 28°C to 36°C and Baur (1962) who stated that *L. cyprinacea* is an exceptionally thermophilic organism and develops successfully only at high temperatures. The hatching of eggs into naupliar stages and further into copepodid stages and the morphological changes during parasite development observed in the present study is in conformity with earlier reports of Grabda (1963); Bauer *et al.*, (1969); Fratello *et al.*, (1985) and Saleh and Ramadan (2002).

Attempts to arrest further development of the parasite and its prolonged maintenance under different conditions (at 4°C and 28°C) by supplementing the culture media with fish sera or liver extract (viz; Hank’s Balanced Salt Solution; Ringer Locke Solution; HBSS supplemented with 2% fish serum; HBSS supplemented with 2% liver extract; RL supplemented with 2% fish serum and RL supplemented with 2% liver extract) were not successful as indicated by the results. This clearly indicated that nutrients made available through the serum and liver extracts were not sufficient to create congenial conditions for increasing the time period for which *Lernaea* developmental stages can be maintained without losing its viability. These are ectoparasites and are reported to feed on fish mucus. The main constituent reported from the mucus of fish is a simple protein component with very little or no carbohydrate. Besides this protein component, the mucus also contains variable amounts of one or more glycoproteins (Erland and Ivar, 1957). The possible requirement of the above mentioned substances found in fish mucus
for the parasite's development can't be ruled out. Only further studies on survival of the developmental stages in culture media supplemented with fish mucus can validate this hypothesis.

This is the first report on electrophoretic separation of proteins of *L. cyprinacea*. The electrophoretic separation of soluble proteins of *Lernaea* eggs, nauplii, copepodid and adult parasite reveals that *Lernaea* egg has 12 (202,169,122,108,106,97,17,14,13,11,10 and 6 kDa) and nauplii has 6 (307,286,259,68,8 and 6 kDa) protein bands. Whereas copepodid has 8 (293,286,208,137,90,68,8 and 6 kDa) and adult parasite has 13 (202,169,111,106,97,56,29,12,11,10,9,7 and 6 kDa) protein bands of different molecular weights. The observed difference in the molecular weights of proteins from eggs to adult on electrophoretic separation may be due to protein modification during metamorphosis and development of the parasite. Proteins of 286 and 68 kDa are common for both nauplii and copepodid-I stages. However, none of the proteins from these two stages were shared by the adult parasite indicating a complete change in the protein profile upon metamorphosing into the adult which remain deeply embedded in the host tissue (as compared to the mobile developmental stages). According to Bauer *et al.*, (1969) egg yolk and other protein acts as a food reserve and are utilized by nauplii during its development within the eggs. This could be the possible reason for higher number of peptide units observed in eggs, when compared to nauplii and copepodid stages.

The development of *Lernaea* from egg to copepodid-I takes place without any hindrance in the aqueous culture, but further development of copepodid-I requires fish host. In the present study to establish optimal dose
of infective stages required to develop infection in carps, an infective dose of 70 copepodid-I/fish was found to be most suitable as evidenced by the relatively high percentage of infection, minimal pathology and associated mortality of the host and longer periods of parasite survival when compared to other infective doses. This is in agreement with Hemaprasanth et al., (2008) who established *Lernaea* infection under laboratory conditions in *L. fimbriatus* fingerlings by exposing the fishes to copepodid-I infective stage at the rate of 70/fish. Lower infective doses of 20, 40 and 60 copepodid per fish, eventhough resulted in nil or lower mortality of the host, the percentage of fishes getting infected and the quantum of parasites established per fish was much lower than those exposed to 70 copepodids per fish. This indicated that the lower doses were not sufficient for establishing the quantum of infection required for parasite maintenance and further experimental studies. Upon infecting the *L. fimbriatus* fingerlings with copepodid-I, fishes developed hemorrhage at the base of dorsal and caudal fins by day 5 PI. Several researchers reported significant parasite attachment at dorsal and caudal areas of the hosts (Bulow, 1979; Charles *et al.*, 1991). In the present study, it was observed that subsequent upon the parasites penetration of the host, tissue around the anchor turned into a granuloma or necrotic lesion which later transformed into a fibrotic encapsulation. This damage caused by the parasite upon attachment to the host is in agreement with Kabata (1970; 1985). Reduced feed intake and loss of equilibrium of infected fishes observed in the current study is in accordance with findings of Martins and Souza (1995). Secretion of more mucoidal substances by infected fishes observed in this study is an indicative of disease resistant activity (Shepard,
1994). This has also been reported as a part of fish defense mechanism to prevent colonization by parasites (Ebran et al., 1999). Mortality observed in the present study due to *Lernaea* infection is in agreement with the earlier reports of Kabata (1985) and Molnar (1987) according to which only a few parasites are enough to kill fish.

There are reports on copepodids grazing on the gill tissue of channel catfish (*Ictalurus punctatus*) and their feeding activity being associated with gill damage including epithelial hyperplasia, telangiectasis and hemorrhage (Goodwin, 1999). However, in the present study, the tissue damage caused by the early copepodid stages was not severe as evidenced by the relatively negligible or no differences in the protein patterns of tissue samples from the fish infected with copepodids (day 5 PI) when compared with control. This indicated that initial damage caused by the parasite at tissue level during the early phase (day 5 PI) of infection is minimal when compared to the later stages (18 PI) of infection. The absence of high molecular weight proteins (156, 116, 109, 103, 93, 85, 81 and 75 kDa) in tissue samples from infected fishes on day 18 PI may be due to the lytic action of the substances released by the parasite into the host body resulting in breaking down of these high molecular weight proteins.

Though, vast literature is available on serum protein fractions of various species of fish (Chandrashekar, 1959; Jane and Rao, 1988; Gerwick et al., 2002; Mark et al., 2006 and Shahida et al., 2011), information on electrophoretic pattern of serum proteins of *C.catta* and *L.fimbriatus* infected with *L.cyprinacea* is scanty. This is the first record of the serum protein profiles of *Lernaea* infected carps. In the present study, comparison was
made between protein profiles of serum from carps infected with *L. cyprinacea* with that of control serum from uninfected carps. The reduced intensity of the protein bands in infected fish serum of *C. calla* and *L. fimbriatus* when compared to that of control serum may be due to loss of serum proteins, which is further supported by the results of the blood biochemical changes in the infected carps where serum hypoproteinemia was observed. Nomenclature of the serum fractions as prealbumin, albumin and globulin was done based on the conventional increasing order of relative electrophoretic mobility. Similar nomenclature and relative positioning of the serum proteins were done by Chandrashekar (1959); Harris (1974); Jane and Rao (1988); Riaz Ahmad *et al.*, (2007) and Shahida *et al.*, (2011).

The blood biochemical variations in naturally and experimentally infected fishes were similar. Infected fishes showed decreased levels of total serum protein and alkaline phosphatase, which is in agreement with the findings of Kurovskaya (1984) that infection and associated reduced feed intake by infected fishes decreased the level of total protein and alkaline phosphatase indicating the parasite’s effect on the nutritional status of the host. Further, this is in accordance with the report of Finn (1970) that the interruption of host epidermis by parasite results in osmotic imbalance, loss of plasma ions and proteins. The significance of elevated cholesterol levels in infected fishes is not clear. However, similar findings were reported by Van Den Broek (1978) in case of *Merlangius merlangus* infected with *L. branchialis* and suggested that no deficiency of essential fatty acid (EFA) but increased cholesterol levels may be indicative of stress. According to Bell (1968), certain enzymes may be of diagnostic value with some fish diseases. The
pathological damage caused by the parasite in the host system is reflected in the levels of serum amino transferases studied. The activities of these enzymes in the serum of infected fishes increased to multiples of the physiological level. Parasitic load, immunological damage and immunological response against the parasites within the host system are likely to influence and result in elevated levels of amino transferases. Increased levels of both amino transferases also indicate liver cell insufficiency and liver cell damage which is further confirmed by the results of histopathological studies on liver tissue from infected fish.

This is the first report on histopathological studies on liver of Lernaea infected L. fimbriatus. When compared with the control tissue, the infected fish liver showed significant vacuolation along with mild to moderate degenerative changes- congestion, infiltration of connective tissue and presence of nuclear debris in the cytoplasm. A narrow zone of partly destroyed liver cells and granulation tissue is evident in infected fish liver. Several authors such as Bullock and Snieszko (1969); Hoffman (1976); Cusack and Cone (1986) and Dempster et al., (1988) reported that attachment of the parasite may facilitate the entry of bacteria causing secondary infections. This is in further agreement with Charles et al., (1991) who observed bacterial species of genus Aeromonas in histological sections of parasite attachment area as well as in the kidney. These secondary infections may also be responsible for the pathological changes observed in the infected fish. However, in the present study quantification of the effect of secondary infection on the host pathology was not carried out. Histopathological studies on tissue of fish from the site of parasite penetration revealed the presence of anchor of the parasite, mild to
moderate inflammatory changes and connective tissue infiltration. Areas of hemorrhages and congestion followed by moderated degree of vacuolation was also observed. The parasite reached the skeletal musculature of the host after having penetrated the epidermis and dermis and resulted in the formation of parasitic nodule. These observations are completely in agreement with the findings of Charles et al., (1991); Bauer et al., (1969); Khalifa and Post (1976) and Bastos (1996).

Adult *Lernaea* is visible to naked eye and the infected fishes can be easily segregated and quarantined based on visual observation. However, the developmental stages of this parasite are microscopic and remain loosely attached to the fishes and accordingly are a major route for spread of the disease. Killing or dislodging the developmental stages, especially copepodids attached to the host body by chemical dip bath treatment is one of the possible ways of preventing the spread of infection during the translocation of fish from affected ponds. In the present study, ammonium hydrogen difluoride at 0.1% and 0.05% concentrations showed high efficacy against developmental stages. However, fishes could not tolerate these concentrations of ammonium hydrogen difluoride and all the treated fish died subsequent on exposure. Oozing of blood from the gills of fish exposed to this chemical indicated its toxicity to fishes. Apart from this, fluorine compounds readily accumulate in fish with a particular affinity of fluoride for their bone tissue leading to contamination of the entire fish (Piotr et al., 2003). Pillai and Mane (1984) found delayed hatching and decreased protein concentrations in fertilized eggs of *C. catla* when exposed to fluoride media. In view of the present finding and earlier reports on fluoride being toxic to
fishes, this chemical even though effective in killing the developmental stages of the parasite, was not taken up for further studies. The present observation that ammonium chloride is effective against copepodid stages of *L. cyprinacea* is in agreement with earlier findings by Hemaprasanth et al., (2006). Fishes could tolerate 14 min exposure to 0.025% ammonium chloride as evidenced by minimal stress and nil mortality in 0.025% treatment when compared to treatments with 0.1% and 0.05% ammonium chloride. Decrease in pH and DO levels in 0.1% and 0.05% solutions of ammonium chloride could be one of the possible reasons for stress and lower tolerance of fishes to the above cited concentrations. These findings are in agreement with Shaffi (1980) who recorded inverse relationship between varying concentration of ammonium chloride and fall in pH and DO of water. The fact that fishes from Group I (infected and 0.025% ammonium chloride dip treated) did not develop *Lernaea* infection until the end of experiment (60 days) confirms the efficacy of ammonium chloride against the developmental stages of parasite. It is possible that when ammonium chloride gets dissolved, it dissociates into ammonium and chloride ions and it may be the ammonium ions which is toxic to the developmental stages. This was confirmed by the experiment on efficacy of sodium chloride against the developmental stages, where it showed no larvicidal properties and hence supporting our earlier hypothesis that ammonium ions are toxic to the developmental stages of *Lernaea*.

Blood analysis is an important tool to check the possible adverse effects of drugs, chemicals and pesticides. *Lernaea* infected fishes given dip bath treatment in 0.025% ammonium chloride solution showed elevated levels of plasma ammonia. Absorption of ammonia through gills may be the possible
reason for increase in the plasma ammonia concentration (p<0.001) after four hours of dip treatment in Group I (Infected and 0.025% ammonium chloride dip treated) and Group III (chemical control) fishes. However, their levels returned to near values reported for control group fishes within 24 hrs of exposure indicating reversible effect of ammonium chloride dip bath treatment on the Lernaea infected fishes. A less significant (P ≤ 0.10) decrease in the chloride level of infected control group 24 hrs of PI can be attributed to the interruption of host epidermis by parasite resulting in osmotic imbalance and loss of plasma ions (Finn, 1970). Insignificant decrease in serum protein level of group I (infected and 0.025% ammonium chloride dip treated) and III (chemical control) fishes indicates reduction in the rate of proteolysis and the rate of amino acid catabolism, which results in a decreased ammonia production, which may be another strategy adopted by the fishes to reduce ammonia toxicity (Randall and Tsui, 2002). Insignificant elevation of plasma Na⁺ and K⁺ ion concentrations in Group I and III (infected and 0.025% ammonium chloride dip treated and chemical control respectively) fishes may be attributed to accelerated NH₄⁺ excretion leading to rise in plasma Na⁺ (James, 1979) and K⁺ concentration (Randall and Tsui, 2002). The present study proved that dip treatment of fishes infected with Lernaea copepodids in 0.025% ammonium chloride solution can be effectively used to control this parasitic infection in carps.

In case of chemotherapeutic treatments, although doramectin and ivermectin has been effectively used to control various ectoparasites affecting different species of terrestrial animals (Franco and Mamann, 2004; George and Davy, 2004; Kasai, 2005; Paramar et al., 2005; Rambags et al., 2005;
Anju and Rath, 2006 and Campbell and Benz, 1984), only limited information is available on its use in aquatic animals. These studies have been undertaken mainly on the efficacy of ivermectin in the treatment and control of ectoparasitic infections of aquatic organisms. These includes use of ivermectin against *Lepeophtheirus salmonis* infestation in salmon (Johnson and Margolis 1993; Smith et al., 1993), *L. cyprinacea* infection in axolotl, *Ambystoma mexicanum* (Melidone et al., 2004), *Salmonicola californesis* infection in rainbow trout (Roberts et al., 2004) and in Chinook salmon (Johnson and Heindel, 2001), *Lernathropus kroyeri* in cultured sea bass (Athanasspoulou et al., 2001), *L. salmonis* and *Caligus elongus* in Atlantic salmon (O'Walloran and Coombs, 1993; Davies and Rodger, 2000) and *Caligus* sp in rainbow trout (Sievers et al., 1996). Compared to ivermectin, there are no reports available on the use of doramectin against parasites of aquatic animals.

In the present study on the efficacy of orally administered doramectin and ivermectin, *L. fimbriatus* fingerlings were not able to tolerate ivermectin at a dose of 1 and 1.5mg/k.b.wt, administered through feed as evidenced by the 60% and 100% mortality in respective groups on day 1. Further, the ruptured digestive tract with extensive hemorrhage observed in fishes that died subsequent upon ivermectin administration also indicates its toxicity to the host. Athanassopoulou et al., (2003) reported toxicity and histopathological changes in the intestinal tissue of sea bass *Dicentrarchus labrax* administrated ivermectin at various doses. There are reports of toxicity and adverse reactions of ivermectin in some group of aquatic animals. The reported LD$_{50}$ of a single oral dose of ivermectin for Atlantic salmon was
0.5 µg/kg b. wt, but in brown trout after intraperitoneal injection LD50 was 0.3 µg/kg b. wt. (Kilmartin et al., 1997). Johnson and Margolis (1993) found that steel head trout and Coho, Chinook and Atlantic salmon differed in their ability to tolerate ivermectin administered orally at a targeted dose of 0.05 mg/kg b. wt. Ivermectin at a dose of 0.4 mg kg\(^{-1}\) though effective against the copepods has been reported to cause up to 24% mortality of Atlantic salmon (Palmer et al., 1997). An accidental overdose of 0.75 mg kg\(^{-1}\) of orally administered ivermectin for controlling sea lice infestations killed 26% of treated Atlantic salmon (Smith et al., 1993). The toxicity to higher doses of ivermectin shown by \textit{L. fimbriatus} is further supported by the histopathological observations on the liver of fish fed ivermectin at the rate of 1 and 1.5 mg/kg b.wt. The complete evacuation of cytoplasmic contents and moderate to severe vacuolations along with loss of hepatic fabrication or architecture, nuclear debris and congestion indicate the acute toxic effects of the drug. However, an oral dose of ivermectin at the rate of 500 µg/kg b.wt, did not cause any toxic effects or histopathological changes in \textit{L. fimbriatus}. The mean time of clearance of parasites was found to be 28 days PT at a dose of 500 µg/kg b.wt, as compared to the normal course of 41 days in case of infected control which did not receive any drug. This finding is in agreement with the studies conducted in rainbow trout (\textit{Oncorhynchus mykiss}), wherein it was observed that fishes heavily infested with the lernaeapodid copepod \textit{Salmincola californiensis}, became free of parasite on day 31 post treatment (Roberts et al., 2004). Even though the feed and feeding habits of terrestrial and aquatic organisms are different, the lower bioavailability of endectocides following oral administration to aquatic animals due to its binding with gut
contents is quite possible. The reports of Alvinerie et al., (1999) and Hennessy et al., (2000) also confirms relatively lower bioavailability of endectocides because of binding with the gut content in terrestrial livestock. The present observation where oral administration of endectocides in multiple doses was required as compared to a single dose of IM administration to cure carps from *Lernaea* infection indicates the lower bioavailability and efficiency of orally administered endectocides.

*C. catla* and *L. fimbriatus* were able to tolerate oral administration of doramectin at 1 and 1.5mg/kg b.wt, without causing adverse effect on the host. The results of histopathological studies on liver of fish fed doramectin at the rate of 1 and 1.5mg/kg b.wt, also confirms the same. This is in accordance with the report of Pfizer (1987b) that organs in Long Evan rats treated with doramectin at varying concentration (0, 2.5, 5 or 10 mg/kg b.wt/day) for a period of 14 days has revealed no over signs of toxicity and no effects on body weight, hematology, serum chemistry, urine composition, liver or kidney weight and histopathology. The present study established the efficacy of orally administered doramectin at 1mg/kg b. wt, incorporated in the feed for control of *L. cyprinacea* affecting carps. No reports are available in the literature on the efficacy of this drug administered via the oral route to control ectoparasitic infections of livestock including aquatic animals and the present study appears to be the first report on efficacy of orally administered doramectin against ectoparasites of aquatic animals.

The observations that a whitish mass of encapsulation developed around the parasite in doramectin treated fish by day 2PT followed by the death and disintegration of the parasite within 19 days PT directly correlate
with the earlier reported pharmacokinetics of orally administered doramectin in donkeys (Gokbulut et al., 2005). They observed that following oral administration, the maximum plasma concentration ($C_{\text{max}}$) was reached at 24 hrs and this coincides with the initial reaction of the drug on the parasites leading to formation of a white mass around it within 48 hrs of drug administration and finally its death. The drug induced death or immobilization of the parasite is likely to result in release of increased levels of toxins and excretory/secretary products by the parasite and the formation of the white mass around the parasite may be part of the host response to this release of metabolites. The mean residence time of doramectin in plasma following oral administration is 9.1 days (Gokbulut et al., 2005). In the present study, all parasites were killed and fell off or disintegrated within an average of 9 days after the last dose of drug administration, indicating the activity of the drug during the reported plasma residence time.

In terrestrial animals, this drug was administered parenterally (either SC or IM routes) or in a few cases as a pour on application. In young fish, especially fingerlings, parenteral administration of the drug may lead to extreme handling stress and it is difficult from the operational viewpoint owing to their size, aquatic habitat and sheer numbers. Parenteral administration of the drug can be a viable proposition only in case of adult fish where few numbers are involved such as brood stock. In young fish, the preferred mode of drug administration is either orally via feed or as dip treatment. However, pour on application of doramectin has been found to be less effective than parenteral administration of the drug in terrestrial animals (George and Davy, 2004).
The comparative plasma dispositions of ivermectin and doramectin following subcutaneous and oral administration in dogs revealed that ivermectin produced a significantly higher maximum plasma concentration with slower absorption and larger area under the concentration versus time curve as compared with doramectin following oral administration of both drugs. No significant differences were observed on the pharmacokinetic parameters between ivermectin and doramectin after subcutaneous administrations. In addition, subcutaneously given ivermectin and doramectin presented a significantly lower maximum plasma concentration with slower absorption and larger area under the concentration versus time curve as compared with the oral administration of ivermectin and doramectin respectively. Considering the pharmacokinetics parameters, it was inferred that both doramectin and ivermectin could be used by either oral or SC routes for the control of parasitic infections (Gokbulut et al., 2006). Even though the SC route is the most preferred mode of doramectin application in terrestrial livestock, doramectin administered via the IM route to infected advanced fingerlings is found to be effective. Since the carps have scales all over their body, the SC administration of the drug can be difficult and time consuming proposition and among parenteral route, IM application is the best-suited one. IM route of administration of doramectin is further supported by the pharmacokinetics studies conducted in the cattle wherein it was shown that the doramectin is absorbed with equal bioavailability in terms and extent and rate of absorption with IM and SC routes (Nowakowski et al., 1995).

A single IM application of doramectin and ivermectin at 200μg/kg b. wt, was more efficacious than multiple administrations in divided dose in
controlling *Lernaea* infection in *L. fimbriatus*. The $C_{\text{max}}$ and the resultant higher bioavailability of doramectin is reported to occur within 12-24 hrs of drug administration and this drug has a mean plasma residence time of 9.1 days (Wicks *et al.*, 1993) and the corresponding value for ivermectin is reported to be 19.2 hrs and 6.5 days respectively (Gokbulut *et al.*, 2005). Loss of appetite, lethargy and darkening of the site of injection during the first week post injection is in accordance with findings of Katharios *et al.*, (2001). In comparison, multiple divided doses of the drug is likely to result in its relatively lower plasma concentration at a given point of time leading to lower bioavailability, although the period of its availability may be longer. The longer period of bioavailability resulting from multiple administrations in divided doses may not be required for the treatment of adult *Lernaea* infection in fish. Further, the maximum plasma concentration ($C_{\text{max}}$) of these two drugs is responsible for the initial reaction of the drug on the parasites leading to formation of a white mass around it within 2 days of drug administration and finally its death. This explains the better efficacy of a single IM application of ivermectin and doramectin at 200 μg/kg b. wt, as compared to its multiple applications in divided doses against this parasite. In view of the handling associated stress and the other operational difficulties involved, single IM administration of ivermectin and doramectin at 200 μg/kg b. wt, is recommended for treatment of heavily infected brood stock of carps under culture conditions.

Wound healing process in infected fish was also augmented by doramectin as evidenced by the early healing of the abscesses caused by the parasite in treated fish than the wounds in untreated fish. This is in conformity
with the report of Oliveira et al., (1993) that the wound of doramectin treated infected cattle healed more rapidly than the infected wounds of the untreated calves and also that of Moya borja et al., (1993) that doramectin administered at 200μg/kg b. wt., prevented damage caused by Dermatobia hominis in cattle. Subsequent to doramectin administration to infected fish, the parasites egg production was affected leading to decreased egg output, followed by the disintegration of the egg sacs and death of the parasites. It was also observed that early administration of doramectin was more effective as evidenced by the lower hatching and survival of Lernaea eggs from infected C. catla treated as early as 16 days PI. Better hatching and survival of parasite eggs were recorded from infected fishes administered doramectin from day 22 PI. This relatively lower efficacy of doramectin on the eggs of the parasite can be explained by the fact that by day 22 PI, most of the parasites have developed into adults with gravid and well-developed egg sacs and possibly the drug is not able to penetrate through the fully developed egg shell. Based on these observations it can be presumed that doramectin has a direct impact on the reproductive potential of the parasite. The ability of doramectin to affect the reproductive capacity and development of other ectoparasites was reported earlier. The oviposition and hatchability of the few Boophilus microplus that complete their lifecycle on the doramectin treated cattle were severely reduced (Gonzales et al., 1993). Similarly, Macedo et al., (2005) studied the post embryonic development of Stomoxys calcitrans fed in the feces of bovines treated with different avermectins and found that doramectin reduced the viability of Stomoxys calcitrans to develop from larvae to adult by 92%. This ability of doramectin, which is detrimental to the development and
reproductive potential of the parasite, can possibly be utilized to develop prophylactic strategies where animals can be treated in a manner that limits the contamination of aquatic environment.

There are good numbers of reports on the effect of doramectin on blood biochemistry of higher animals (Bal et al., 2003; Seri et al., 2006). Seri et al., (2006) evaluated the effect of intramuscularly injected doramectin on the blood constituents of donkeys and found that all the studied blood parameters except urea are not affected. However, no information is available on its effect on blood biochemical parameters of *Lernaea* infected fishes. Even though the effect of *Lernaea* on host serum protein, intestinal enzymes (Kurovskaya, 1984) and hematological parameters (Silva et al., 2000) were evaluated earlier, the present study appears to be first report on the effect of doramectin on serum biochemical parameters of *L.fimbriatus* infected with *Lernaea*. Oral administration of doramectin at the rate of 1 mg/kg b. wt, did not result in adverse effect on the host. Variations in values of serum biochemical parameters in Group I (infected and doramectin treated at the rate of 1mg/kg b.wt) fishes came to near by values reported for the control group fishes on day 23 PT, indicating the role of doramectin in recovery of the host from *Lernaea* infection, resulting in the physiological parameters returning back to their normal values. The present observation on the efficacy of doramectin in restoring the serum biochemical parameters of *Lernaea* infected fish to near normal values is in accordance with Bal et al., (2003) who reported improvement in the serum biochemical levels in sheep naturally infected with gastrointestinal parasites after treatment with doramectin. Possible explanation for the sluggish movement and lowered
response to external stimulations shown by drug treated fishes lies in the report of EMEA (1997) which states that doramectin at the rate of 50-100mg/kg b.wt, fed orally to rats induced decreased activity, respiration, tremor and ataxia. Similarly, dogs fed at the rate of 2>mg/kg b. wt, of aqueous form of doramectin also showed tremor and ataxia. This is further in agreement with Pfizer (1989a) that Long-Evans rats administered doramectin at the rate of 0, 2, 5 or 10 mg/kg b.wt/day for 38 days showed hunched appearance, ataxia and tremors. However, in the present study after discontinuation of the treatment, the fishes recovered and resumed their normal behavioral activity.

On comparison, it appeared that doramectin at higher concentration is safer than ivermectin for use in aquatic organisms, as its administration through intramuscular and oral route did not result in any noticeable adverse reaction or toxicity in the fish host. This finding is in agreement with studies conducted in veterinary livestock, wherein it was proved that doramectin is more potent and safer drug for use against ectoparasites (Hendrick et al., 1993; Traeder, 1994; Kassai, 2005; Parmar et al., 2005). Meanwhile the present finding that orally administered doramectin is equally effective as the parenterally given drugs to control of L.cyprinacea infection in fish correlates favorably with the result of the plasma disposition and pharmacokinetics studies of doramectin in dogs (Gokbulut et al., 2006). In case of ivermectin the parenteral administration of the drug leads to an overall higher bioavailability in plasma compared to oral that accounts for the lesser time required for the clearance of parasite infestations. However, the pharmacokinetic behavior of these drugs vary extensively and their plasma concentration, bioavailability
and resident time are influenced by a number of factors which include the species under study, body weight, physiological, nutritional and health status of the animals, route of administration and vehicle used in commercial formulations. Hence, it is difficult to extrapolate data from one species to another and the prolonged prophylactic effect against Lernaea infestation in avermectin treated carps observed in the present study may be influenced by a combination of factors mentioned above.

In the present study, higher concentrations (12.5μg/l and 6.25μg/l) of deltamethrin proved to be toxic as evidenced by the 100% mortality of the host within five minutes of exposure time. The possible reason could be the entry of the chemical into the fish body via gills (Elliot, 1976) causing gill damage and thereby causing oxidative stress (Costin et al., 2007) to the host. This is in further agreement with Iqbal sayeed (2003), who reported oxidative stress in Channa punctatus exposed to deltamethrin at 0.75 μg/l for a period of 48 hrs. The concentrations (12.5μg/l and 6.25μg/l) used in the present study are higher than the LC50 values reported for other fishes by several authors viz; 24 h LC50 value for catfish, Clarias gariepinus 0.01 μg/l (Datta and Kaviraj, 2003), LC50 value for guppy, Poecilia reticulata 0.016 μg/l (Mittal et al., (1994), 48 hrs LC50 and LC99 value for P. reticulata 5.13 and 33.09 μg/l, respectively (Viran et al., 2003), 96 hrs LC50 value for C. carpio 0.058 μg/l (Svobodova et al., (2003) and LC50 value for Oncorhynchus mykiss, 0.39 μg/l; C. carpio, 1.84 μg/l; and Sarotherodon mossambica, 3.50 μg/l (Mestres and Mestres, 1992). However, deltamethrin at a dose of 3.125 μg/l or lower concentration was found safe for treatment of Lernaea adult parasites affecting fish. Stress or adverse effects were not observed in fishes
exposed to deltamethrin at 3.125 μg l\(^{-1}\) or lower concentrations and taking into account its ability at this dose levels to remove *Leranaea* infection from carps, a deltamethrin dose of 3.125 μg l\(^{-1}\) is most suited for control of adult *Leranaea* infection in carps.

The study which evaluated the natural products against *Leranaea*, found neem seed extracts to be effective against the developmental stages of the parasite. Winkaler *et al.*, (2007) reported the use of aqueous extract of neem in fish farms as an alternative for the control of fish parasites. The effectiveness of neem seed extracts against *Leranaea* probably lies in azadirachtin, which is found in high concentrations in seed. Zahedi *et al.*, (2010) and Rembold (1989) reported that azadirachtin is much more concentrated (85%) in the neem seed. In the present study neem seed powder treated with solvent and further solubilized with hot water (90°C) was more effective in preventing the hatching of eggs and further development of the parasite, than any other types of extracts. This may be because the active components of neem seed are likely to yield more when treated with solvent. Even though the high temperature could inactivate volatile compounds; it could also increase the release of active compounds and free radicals. Similar observations were made by El-Mahmood (2010); Majumdar *et al.*, (1998) and Schmutterer and Singh (Cited by Ogbuewu *et al.*, 2011) according whom the active components of neem are slightly hydrophilic, but freely lipophilic and highly soluble in organic solvents. The initial stress shown by the fishes upon exposure to all types of neem seed extracts is in agreement with Winkaler *et al.*, (2007) who reported typical stress response of *Prochilodus lineatus* upon exposure to neem leaf extracts. In the present
study up on exposure to neem extracted with water at 28°C in both the types (solvent treated and untreated), fishes survived up to an average of 3 hrs and 50 minutes. The possible reason for this observation could be that the neem seed along with major component azadirachtin contain 45% oil (Schmutterer, cited by Zahedi et al., 2010) and the presence of volatile compounds could have reduced the survival rate of the fishes. However, the concentration used for the present study (i.e 100μg/ml) proved to be non-toxic to the fishes. This is in agreement with Elijah Oyoo-Okoth (2011) who reported that extracts of neem are less toxic at low concentrations and concentrations exceeding 3,200 mg/l influence physiological and biochemical disturbances. This is in further agreement with Mordue and Blackwell, (1993) that neem components are non-mutagenic, biodegradable and non-toxic to mammals.

The present study established the high degree of tolerance shown by nauplii stages of Lernaea to neem seed extracts. The possible reason could be the impermeability of outer membrane or shell of nauplii. However the copepodid stages were susceptible to long term exposure to neem seed extracts leading to their mortality. The fact that nauplii stages are resistant to the herbal or chemical treatment was further supported by the report of Lahav et al., (1964) who found nauplii stages to be resistant to chemical treatments until they moult to the first copepodid stage. The ability of neem seed extract to kill the copepodid stages of Lernaea can be exploited and used as a prophylactic and control measure to disrupt the developmental cycle of the parasite.