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Prevalence of parasites in tilapia farms and their management practices in Tamil Nadu, India

Subramani Sathish, Pushparaj Chidambaram, Arumugam Uma and Pandiyan Yuvarajan

Abstract
A six month field study was conducted to investigate the incidence of parasitic infection and its interactive factors in the Tilapia farms at Tamil Nadu. About twenty tilapia farms were selected from the Thiruvarur, Krishnagiri, Nagapattinam and Thanjavur districts. Culture water and fish samples were collected from the each selected farms and transported to the laboratory for parasite identification. The protozoan parasites (*Tetrahymena* sp., *Trichodina* sp., *Epistylis* sp., and *Chilodonella* sp), monogenean trematode (*Dactylogyrus* sp.) and crustacean parasite (*Argulus* sp.) were identified from twenty farm samples. Among that, protozoan parasitic infection was found to be maximum (69%) followed by monogeneans (26%) and crustaceans parasites (5%). High stocking, poor water quality and poor feeding practices influenced the parasitic incidence. Farmers has been using the commercial antiparasitic agents to control the parasitic infection in the 18 farms. The Clinar (Cypermethrin) antiparasitic agent was mostly utilized by farmers followed by Doom (Dichlorovus-76% E.C), Nuvan (Dichlorvustech.83.0%, 0%) and Butox Vet (Deltamethrin 1.25%). The present study found that, the tilapia farms of Tamil Nadu has mostly infected with protozoan parasites due to the poor biosecurity. Hence, farmers should improve the biosecurity to minimize the disease and avoid the synthetic chemicals for environment safety.

Keywords: tilapia farms, parasite prevalence, disease incidence, antiparasitic agents, control measures

Introduction
World scenario of Tilapia aquaculture production in 2017 was 6.65million metric tonnes and grown to 6.97million metric tonnes in 2018. World Bank predicted that Tilapia production in 2030 would be 7.3million metric tonnes. Tilapia has emerged as the second most cultured aquatic species after the carp [1]. It is a hardy species which can tolerate poor water quality and the ability to convert organic and domestic waste into high quality protein [2]. Nile Tilapia (*Oreochromis niloticus*) are being used in commercial farms in more than 100 countries of the world from extensive to super-intensive and one of the important food fish in the aquaculture industry in this century. With the increase in demand for fish, the practice of aquaculture has been intensified. Consequently, aquaculture has been facing various problems, particularly, disease occurrence due to virus, bacteria, fungi and parasites [3]. Majority of fish serves as an intermediate host for many parasites which reduces the food value of fish and cause mass mortality [4, 5]. The majority of freshwater fishes carry heavy infestation of parasites. They are mainly grouped as protozoans, helminths and crustacean parasites. Parasitic infective disease are hindering the productivity of Tilapia culture. Improper water aeration would lead to faecal deposits at the bottom of the pond and become a suitable medium for parasite growth [6]. Fish parasites can inflict a variety of damages such as irritation, wound, injury or atrophy of tissues and occlusion of the alimentary canal and blood vessels [7]. Higher stocking density, maintenance in poor water quality, mixing of stock fishes from different regions in single ponds, unhygienic feeding procedure paved way for the occurrence of parasites. In recent years, several authors recorded parasites like *Tricodina* sp., *Epistylis* sp., *Tetrahymena* sp., *Chilodonella* sp., *Dactylogyrus* sp., *Gyrodactylus* sp., *Argulus* sp., *Ergasilus* sp., and *Lernaea* sp in tilapia. [8, 9, 10, 11]. A study on the prevalence of parasites will be of much importance to assess the economic loss to the fish farmers. Under natural conditions, 50 to 90 percent of freshwater fishes harbor at least one species of parasites. The parasitic infection gives indication of water quality since they were commonly increase in polluted water. Parasites and their host usually live in equilibrium. However, in crowd conditions of the host parasitic diseases can spread very rapidly and cause heavy mortality.

Therefore, proper health management procedures should be followed with appropriate control measures to boost up aquaculture production. The paper deals with the systematic survey on incidence of fish parasites and to record pathological changes in Tilapia. This study also records the different management measures and chemicals used by the farmers for control of parasites in farms of Tamil Nadu, India.

Materials and Methods

Study area and Sample collection

Tilapia fish samples in apparently healthy and moribund conditions were randomly collected from twenty farms located in Thiruvarur, Krishnagiri, Nagapattinam and Thanjavur districts of Tamil Nadu, India (Figure 1). The study was conducted for six months from December 2018 to May 2019. Fish and water samples were taken for investigation on the prevalence of parasites and interactive factors causing the disease incidence. GPS locations were recorded from the sampling points of the study area (Table 1). Information on remedial measure adopted by farmers for treatment of fish parasite in farms was collected through a pretested proforma.

Skin biopsy and wet mounts preparation

For identification of external parasites, a skin biopsy was carried out by following two methods viz., (1) Skin scrapping and (2) Fin clipping. Skin scrapping was performed by taking a spatula or a scalpel and gently scraping along the side of the body and fins. The scrapings were then placed on a glass slide with a drop of distilled water and put the cover slip immediately and were examined under the light microscope with in-built digital camera as per the description of Waltman [12]. The wet mount was examined immediately for parasites. Identified parasites were carefully preserved in 10% formalin acetic acid alcohol solution for long time storage. The identification of protozoan under the light microscope with high magnification (400X) and with oil immersion (1000X) was made as per standard protocol [13-17]. Water quality parameters were measured as per standard protocol [18]

Statistical analysis of data

Statistical analysis of the data on the occurrence of parasites in the fishes was carried out using the following formulae; Prevalence (%) = Number of infected hosts/ Number of hosts examined × 100 [19].

Results and Discussion

During the present study, Tilapia fish and water samples were collected from twenty tilapia fish farms and used for screening for the prevalence of parasitic infections. Farm codes were allotted with prefix of the districts (Thi-Thiruvarur, Kri-Krishnagiri, Nag-Nagapattinam, Tha-Thanjavur) and coordinates were recorded (Table 1). Protozoan parasites, Monogenean trematodes and Crustacean parasite infections were observed (Table 2). Among the 20 farms, 13 farms had protozoan parasitic infection with the prevalence of 69%. Monogenean trematodes were recorded in three farms (26%) and crustacean parasite *Argulus* infestation was recorded in one farm (5%) (Figure 3). The protozoan parasites recorded were *Tricodina* sp, *Epistylis* sp, *Tetrahymena* sp and *Cholodinella* sp. Among these protozoans, *Tetrahymena* sp was found to be dominant followed by *Tricodina* sp, *Cholodinella* sp and *Epistylis* sp (Table 3). *Tetrahymena* sp was confirmed by the presence of circular, toothed disc and characteristic hook like structure within the body (Plate 1). *Tetrahymena* sp infected fish showed spiralling football type swimming. Microscopic aggregates of parasitic protozoan forms are noted in the kidney, brain, gill lamellae, muscle, and sub dermal tissue. Trichodinids infected fish looked weak and exhibited flashing. The ectoparasitic ciliates are observed on scrapings of skin mucus. Microscopic examination of the scrapping of the mucus in skin showed the presence of peritrichal ciliated protozoans and the parasite appeared as ornate disc with a characteristic ring of interlocking denticles that form a circle in the middle of the organism. The epidermis of the infected fishes is found to exhibit necrosis (Plate 2). In the present study, *Trichodina* sp was noted in farms where the water level is less than 3 feet. *Epistylis* sp (Plate 3) also was also recorded and the body shape is conical with oral cavity with peristomic disc. The Monogenean trematode observed was *Dactylogyrus* sp (Plate 4). The clinical signs observed were lethargy, swimming near the surface, loss of scales, swollen gills, increased respiration rate, piping and gulping at the surface of the water. *Dactylogyrus* (Gill fluke) were observed on the gill of tilapia. It was identified by its orange brown vitelline glands and eye spots in its body. They were flattened and leaf like, with four anterior eyespots and with attachment organ opisthohaptor as anchors. Crustacean parasite *Argulus* infestation was recorded in one farm (Plate 5). Most of the parasites were found over the body. The clinical symptoms observed were frequent rubbing and flashing and ulcers over the infected area of the fish. Heavily infected fishes showed symptoms also like restless, lethargic and secrete excessive mucus. The water quality parameters like temperature, dissolved oxygen, ammonia and total organic carbon were assessed in the samples collected from 20 farms. The relation between the water quality parameters and the parasitic incidence was correlated. The statistical analysis proved that poor water quality parameters viz., high levels of ammonia, nitrite, nitrate and total organic carbon have a significant correlation with parasite abundance (P<0.05). The results of the observations made are presented in Figure 2. In the survey 18 farms were known to use antiparasitic agents and no antiparasitic agent was used in two farms. The drugs and chemicals commonly used to control parasitic infestations in fish culture are Nuvan, Butox Vet, Cliner, Ectodel (2.8%), Emamectin Benzoate (EB), Hitek Powder and Paracure-IV. Among these Butox Vet and Cliner has comparatively higher use than other products. The survey revealed that most farmers used the common antiparasitic agents like Cypermethrin, Dichlorvas, Ivermectin and deltamethrin at minimal standard dosages as parasitic control agent (Figure 4).

Parasite infestation is the greatest threat in the fish culture system. Many fish species are affected by various types of parasites and as a result, fish production decreases significantly and it causes economic loss to the farming community. Constant monitoring of the parasites, good water quality management, hygienic feeding procedures and quarantine procedures by the farmers will help to prevent the fish from parasitic diseases and the loss due to death of fishes. Analysis on the prevalence of parasitic infections in twenty farms was carried out during December 2018 to May 2019. Parasitic infections like Protozoan parasites followed by Monogenean trematodes and Crustacean parasite were observed and their percent of infection was (69%), (26%), and (5%) respectively (Figure 3). Similarly, Patrick TK Woo [20] reported that parasitic infestations are a major cause of

concern and ectoparasites like protozoan, multicellular trematodes and arthropods are major parasites infecting Tilapia. Parasitic infection was noted in 17 farms; similarly Tiya Amdisa Areda [21] recorded the prevalence of parasites in small scale fish farms in Ethopia and reported that out of the fish samples, 77.60% were found to be infected with parasites. He reported a higher prevalence of external parasites 32.29% than internal parasites (20.31%) which corroborated with our findings. He also reported higher prevalence in January to February. In the present study, external parasites like *Tricodina* sp, *Epistylis* sp, *Tetrahymena* sp and *Cholodinella* sp, *Dactylogyrus* sp, and *Argulus* sp were identified in Tilapia farms. *Trichodina* parasite was found to be more prevalent than other parasites and *Trichodina* sp. was extensively isolated from gills of tilapia which corroborated with the findings [22, 23]. Kabata [13] reported that shallow ponds and stagnant water favours the multiplicity of ciliate, *Trichodina*. Similarly in the present study, *Trichodina* sp was noted in farms where the water level is maintained less than 3feet. *Epistylis* sp also was recorded in the pond. The body shape is conical with the oral cavity and peristomic disc. Similarly El-Tantawy [24] described peritrichid cilated protozoans with a conical body shape with contractile and nutritive vacuoles. Monogenean trematodes, *Dactylogyrus* were identified in three farms and it was found attached in the gills of fish. Similarly, Donald [25] also reported *Dactylogyrus* infection in gills of tilapia and also *Dactylogyrus* presence is associated with stress to fish due to negative water quality. The present study revealed a low prevalence of crustacean parasite (5%). The results were similar to that of Noor El deen *et al* [26]. Similarly lower ectoparasitic infestation in Nila Tilapia (*Oreochromis niloticus*) in cage culture at MPAKA dam, Ghana was reported by Alhassan *et al* [27]. He reported that a low level of crustacean infestation may be due to high resistance of Tilapia for parasitic infection. The farms infested with parasitic incidence are with increased level of ammonia, over-stocking, water depth, improper feeding, lack of farm hygiene, temperature fluctuations leading to stressful conditions for fish and they become susceptible to parasitic infection. The present observation corroborated with the similar results of Ray [28] who reported attack by *Argulus* sp in *Catla catla*. Significant correlation was found between the dissolved oxygen level and the incidences of parasites. This observation was similar to that made by Ojwala *et al* [29] who reported a higher incidence of *Gyrodactylus* in cage farming of tilapia in Subukia, Kenya where low dissolved oxygen was recorded; low oxygen weakens immune system of fish, resulting in heavy infection of fish host. Paredes-Trujillo *et al* [30] also noted that the mean abundance of *Gyrodactylus* sp increases in farms when the dissolved oxygen value was 3-8ppm. In the present study, the water temperature ranged from 19 °C to 32 °C with mean of 27.6±0.15. The incidence of parasites varied with temperature, similarly temperature as an important abiotic water characteristic that influences the dynamics of parasitic assemblages, prevalence and abundances in fish species was explained by Heera and Harish [31]. In this study, monogenean parasites are observed in the summer period of higher temperature similar observation was made by Ojwala *et al* [29] who reported higher incidence of monogenean in ponds with temperature 26. The variation of parasite level with higher temperature may be due to favourable temperature for reproduction and higher availability of intermediate host [32, 33].

The higher use of antiparasitic agents (90%) observed in the present study is due to higher incidence of parasitic infestations (65%) than bacterial infections (30%). The drugs and chemicals commonly used to control parasitic infestations in fish culture recorded are Nuvan, Butox Vet, Cliner, Ectodel (2.8%), Emamectin Benzoate (EB), Hitek Powder and Paracure-IV. Among these Butox Vet and Cliner has comparatively higher use than other products. Similarly, application of a wide range of aqua medicines, drugs and chemicals in aquaculture to control production loss [34, 35]. Mishra *et al* [36] also reported higher usage of Clinar and Butax Vet in fresh water fish farms. Apart from antibiotics, some common chemicals adopted by farmers to control parasites included Sodium Chloride, Formalin, Malachite green, Methylene blue, Potassium permanganate, Hydrogen peroxide and Glutaraldehyde [37]. Large numbers of aquaculture consultants and representatives of pharmaceuticals, feed companies and chemical sellers are involved in the marketing chain for the delivery of products to end users [38]. However, there has been no official recommendation for use of such products in Aquaculture, although many such products have been permitted for use in animal medicine and agriculture as insecticides. Hence, we conclude that, common antiparasitic agents such as Cypermethrin, Dichlorvas, Ivermectin and deltamethrin were used at minimal standard dosages as parasitic control (Figure 4). The survey revealed that most farmers did not have proper knowledge about the chemicals and they use such aqua drugs as per the advice of fish-consultants or feed/chemical suppliers in the region.

Table 1: Farm codes, Location and Corresponding coordinates of Selected Tilapia Farms

Sl. No	Farm code	Location	GPS Location
1	Thi-1	Thiruvarur	80°12'748.44" E-13°7'476.25"N
2	Thi-2	Thiruvarur	79°21'1279.91" E-10°43'2618.11"N
3	Thi-3	Thiruvarur	79°21'1280.10" E-10°44'2650.89"N
4	Thi-4	Thiruvarur	79°34'9372.00" E-10°67'6516.89"N
5	Thi-5	Thiruvarur	79°41'2332.20" E-10°76'7701.00"N
6	Kri-6	Krishnagiri	78°10'42.65" E-12°29'37.10"N
7	Kri-7	Krishnagiri	78°31'1398.15" E-12°24'1493.19"N
8	Kri-8	Krishnagiri	78°22' 1335.26" E-12°13'815.26"N
9	Kri-9	Krishnagiri	78°19' 1150.85" E-12°17'1036.67"N
10	Kri-10	Krishnagiri	78°18' 1103.96" E-12°17'1062"N
11	Kri-11	Krishnagiri	78°18'1094.13" E-12°18'1119.13"N
12	Nag-12	Nagapattinam	79°41'2473.93" E-11°17'1039.43"N
13	Nag-13	Nagapattinam	79°45'17" E-11°13'28"N
14	Nag-14	Nagapattinam	79°42'4073.33" E-11°17'1635.15"N
15	Nag-15	Nagapattinam	79°41'2471.46" E-11°18'1085.97"N

16	Tha-16	Thanjavur	79°16'985.80" E-10°36'2161.96"N
17	Tha-17	Thanjavur	79°16'973.11" E-10°36'2165.27"N
18	Tha-18	Thanjavur	79°30'571.1" E-10°70'7014.02"N
19	Tha-19	Thanjavur	79°14'1864.02" E-10°52'3127.90"N
20	Tha-20	Thanjavur	79°34'8828" E-10°48'9696"N

Table 2: Parasitic disease incidence in selected Tilapia Farms

Sl. No	Farm No.	Disease Incidence		
		Protozoan Parasites	Monogenean Trematodes	Crustacean Parasites
1	Thi-1	※	-	-
2	Thi-2	※	-	-
3	Thi-3	-	-	-
4	Thi-4	※	-	-
5	Thi-5	※	※	-
6	Kri-6	-	-	-
7	Kri-7	※	-	-
8	Kri-8	※	※	-
9	Kri-9	-	-	-
10	Kri-10	※	※	-
11	Kri-11	※	-	※
12	Nag-12	-	-	-
13	Nag-13	※	-	-
14	Nag-14	※	-	-
15	Nag-15	-	-	-
16	Tha-16	-	-	-
17	Tha-17	※	-	-
18	Tha-18	※	-	-
19	Tha-19	-	-	-
20	Tha-20	※	-	-

*__Note:__ The symbol ※ indicates the presence of parasitic infestation in fish

Table 3: Prevalence of Parasitic disease incidence in Tilapia Farms

Sl. No	Farm No.	Protozoan Parasites				Monogenean Trematodes		Crustacean Parasites	
		Trichodina	*Epistylis*	*Tetrahymena*	*Chilodonella*	*Dactylogyrus*	*Gyrodactylus*	*Argulus*	*Lernaea*
1	Thi-1	※	-	※	-	-	-	-	-
2	Thi-2	※	-	※	-	-	-	-	-
3	Thi-4	※	-	※	-	-	-	-	-
4	Thi-5	-	-	-	-	※	-	-	-
5	Kri-7	※	-	※	※	-	-	-	-
6	Kri-8	※	-	※	-	※	-	-	-
7	Kri-10	※	※	※	-	※	-	-	-
8	Kri-11	※	-	※	※	-	-	※	-
9	Nag-13	-	-	※	-	-	-	-	-
10	Nag-14	※	※	※	※	-	-	-	-
11	Tha-17	※	-	※	-	-	-	-	-
12	Tha-18	※	-	※	-	-	-	-	-
13	Tha-20	※	※	※	※	-	-	-	-

*__Note:__ The symbol ※ indicates the presence of parasitic infestation in fish

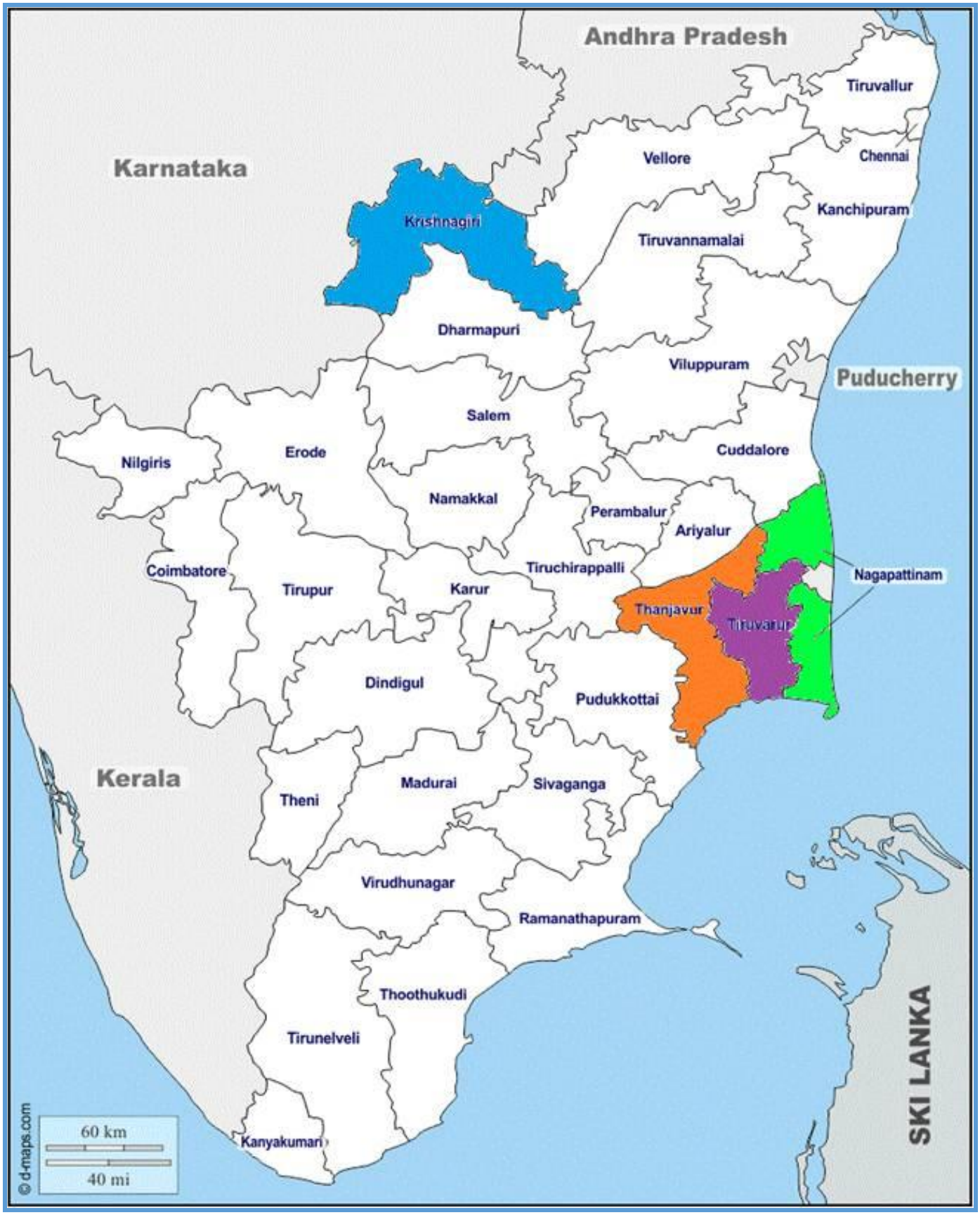
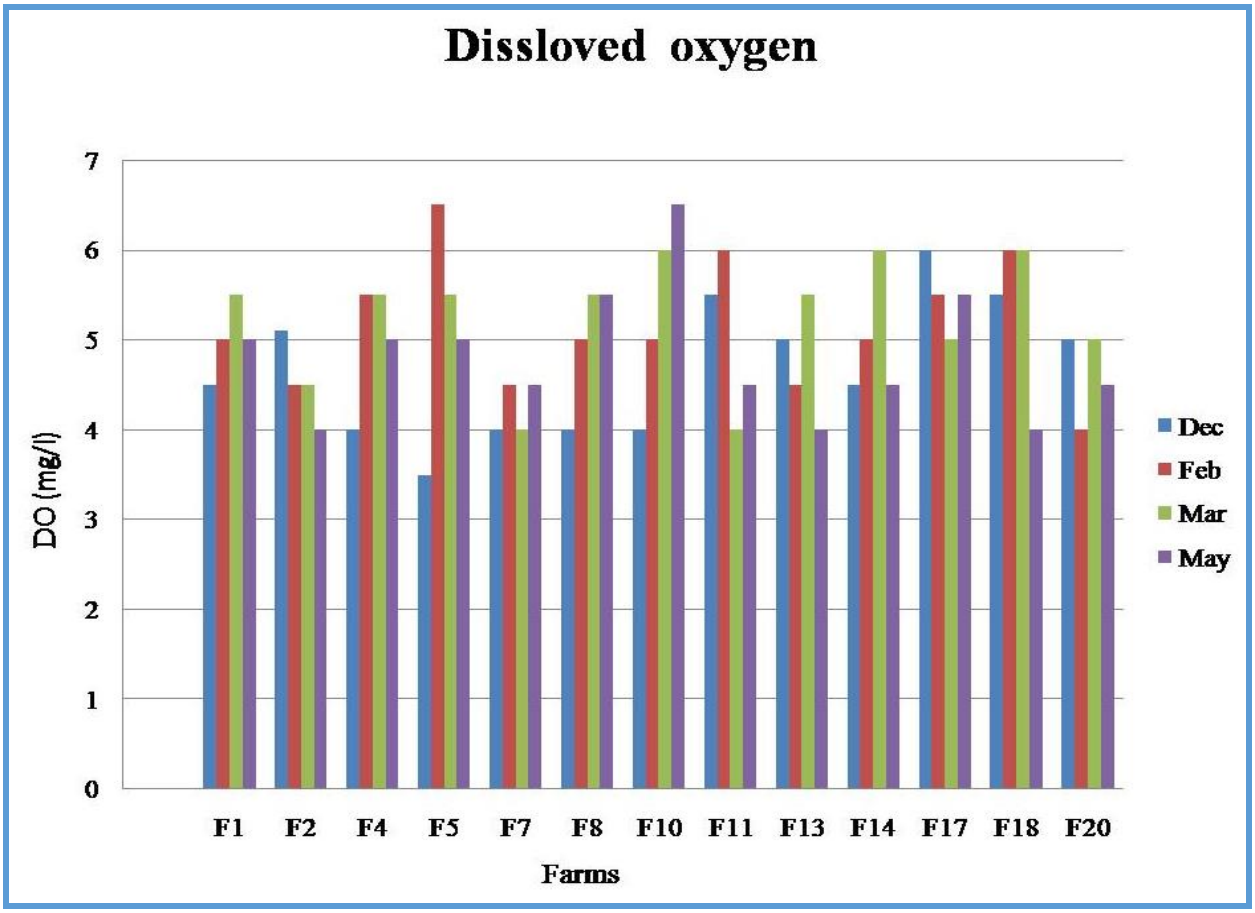
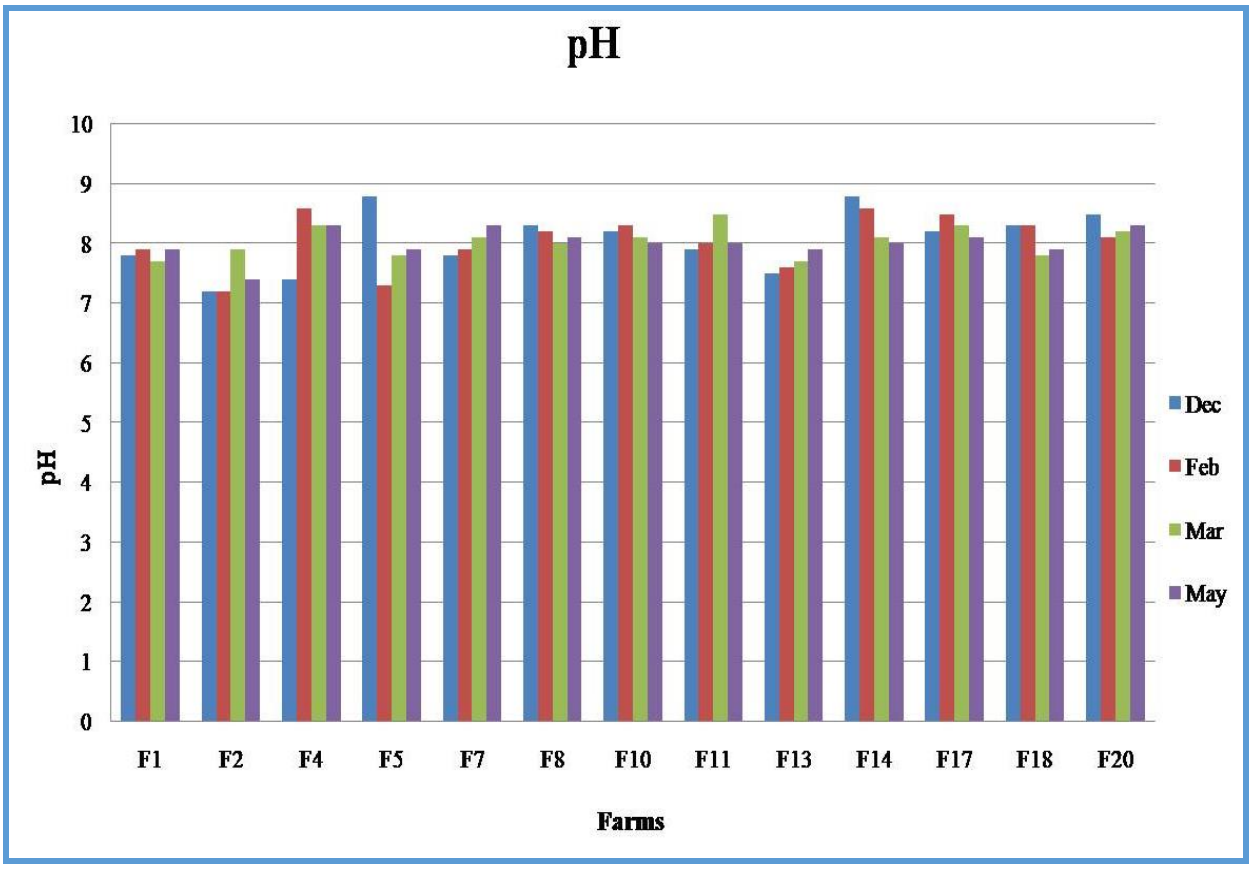
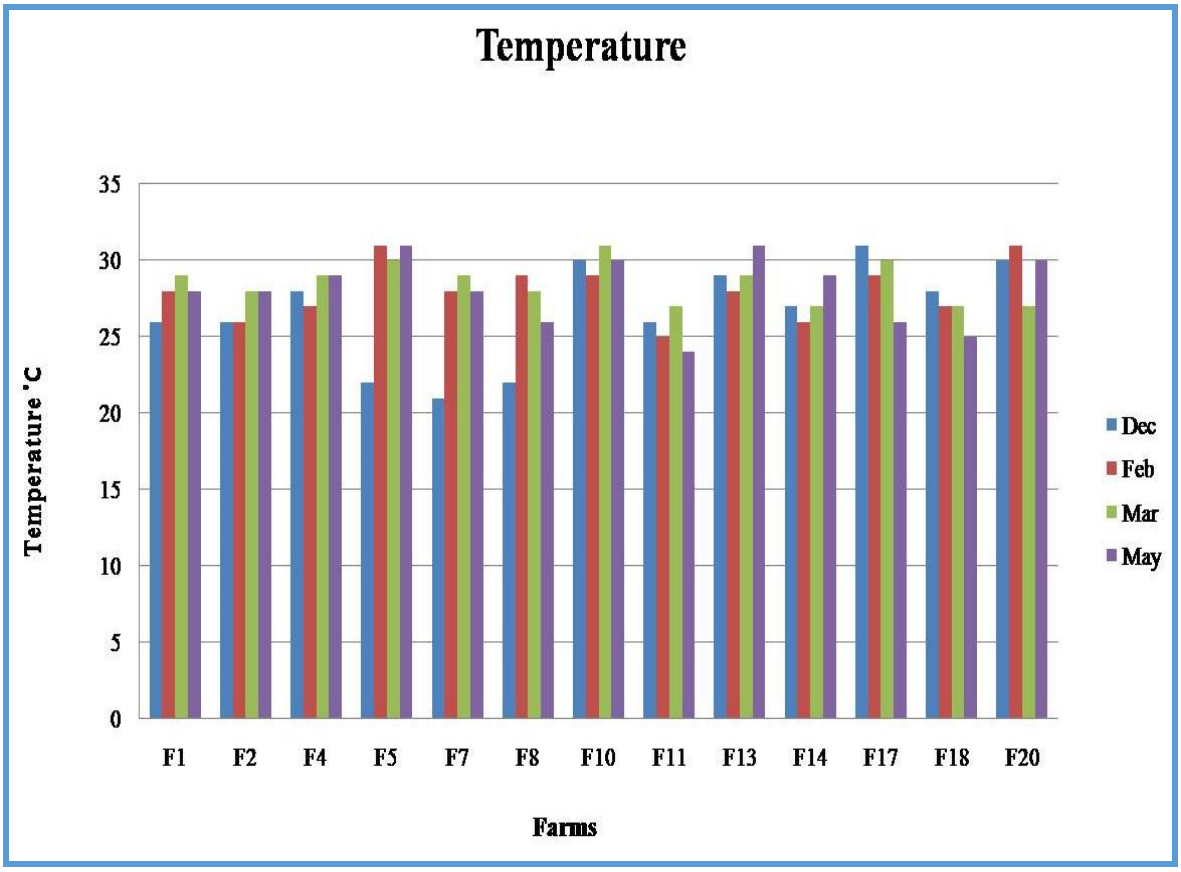
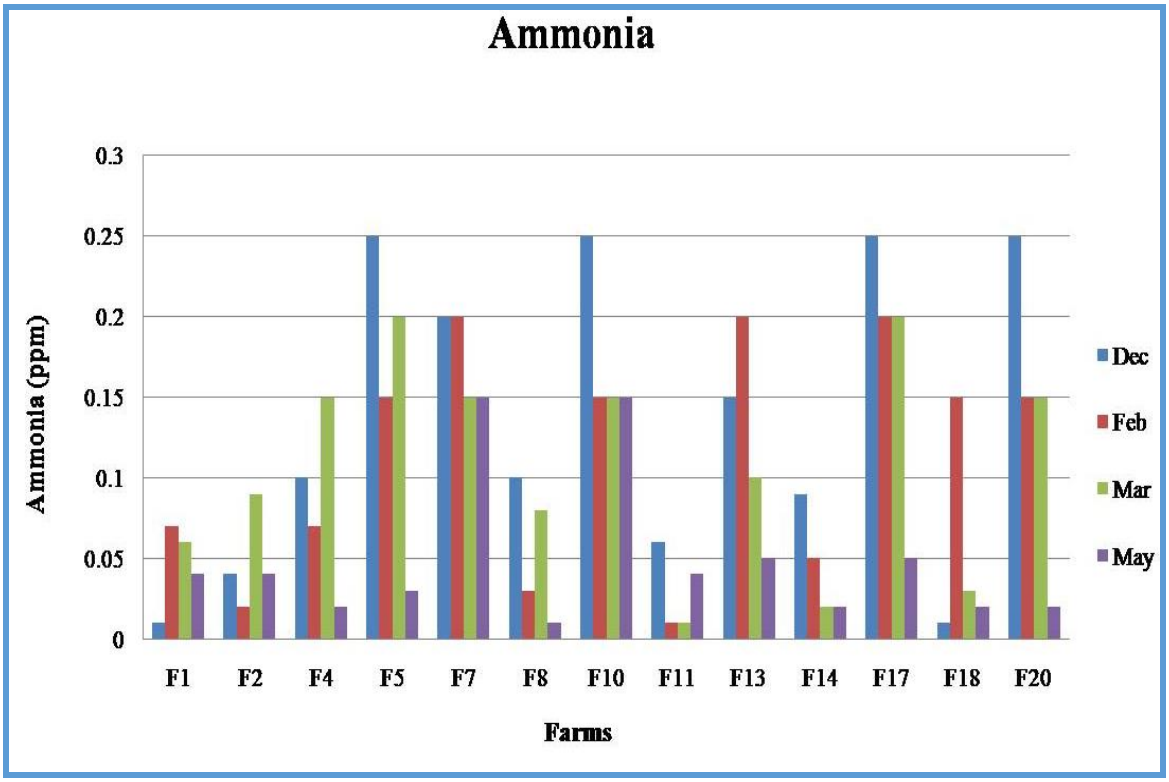


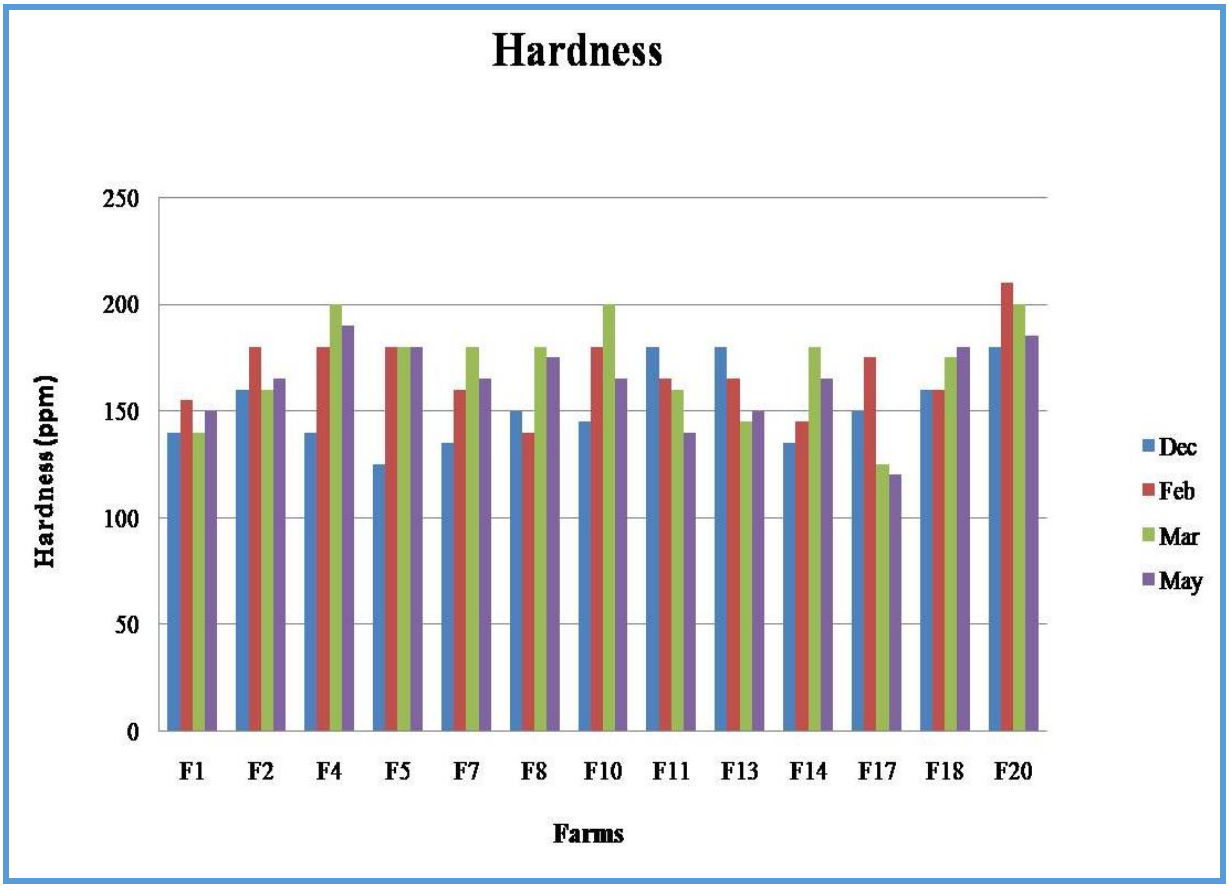
Fig 1: Tamil Nadu Map showing districts selected for study (Highlighted)

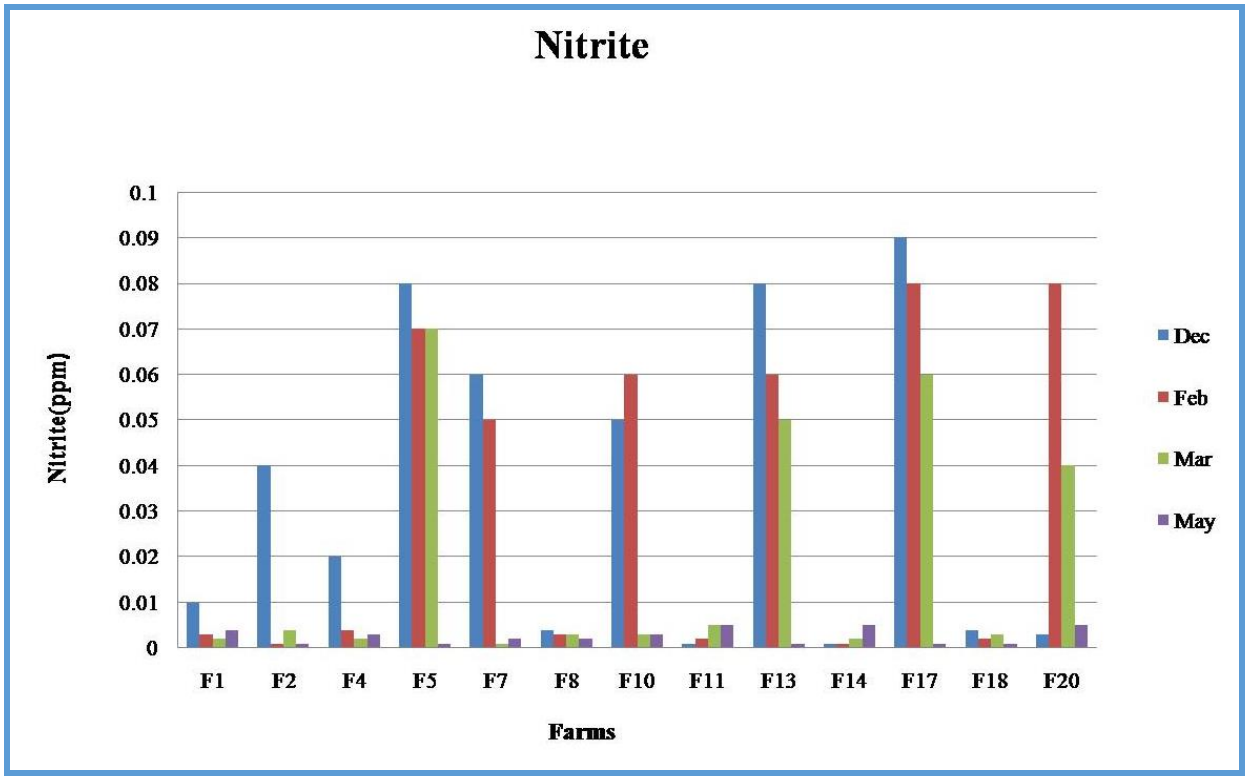

Dissloved oxygen
DO (mg/l)
0
1
2
3
4
5
6
7
F1
F2
F4
F5
F7
F8
F10
F11
F13
F14
F17
F18
F20
Farms
Dec
Feb
Mar
May


pH
pH
0
1
2
3
4
5
6
7
8
9
10
F1
F2
F4
F5
F7
F8
F10
F11
F13
F14
F17
F18
F20
Farms
Dec
Feb
Mar
May


Temperature
Temperature °C
35
30
25
20
15
10
5
0
F1
F2
F4
F5
F7
F8
F10
F11
F13
F14
F17
F18
F20
Farms
Dec
Feb
Mar
May


Ammonia
Ammonia (ppm)
0.3
0.25
0.2
0.15
0.1
0.05
0
F1
F2
F4
F5
F7
F8
F10
F11
F13
F14
F17
F18
F20
Farms
Dec
Feb
Mar
May


Hardness
Hardness (ppm)
250
200
150
100
50
0
F1
F2
F4
F5
F7
F8
F10
F11
F13
F14
F17
F18
F20
Farms
Dec
Feb
Mar
May


Nitrite
Nitrite(ppm)
0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
F1
F2
F4
F5
F7
F8
F10
F11
F13
F14
F17
F18
F20
Farms
Dec
Feb
Mar
May

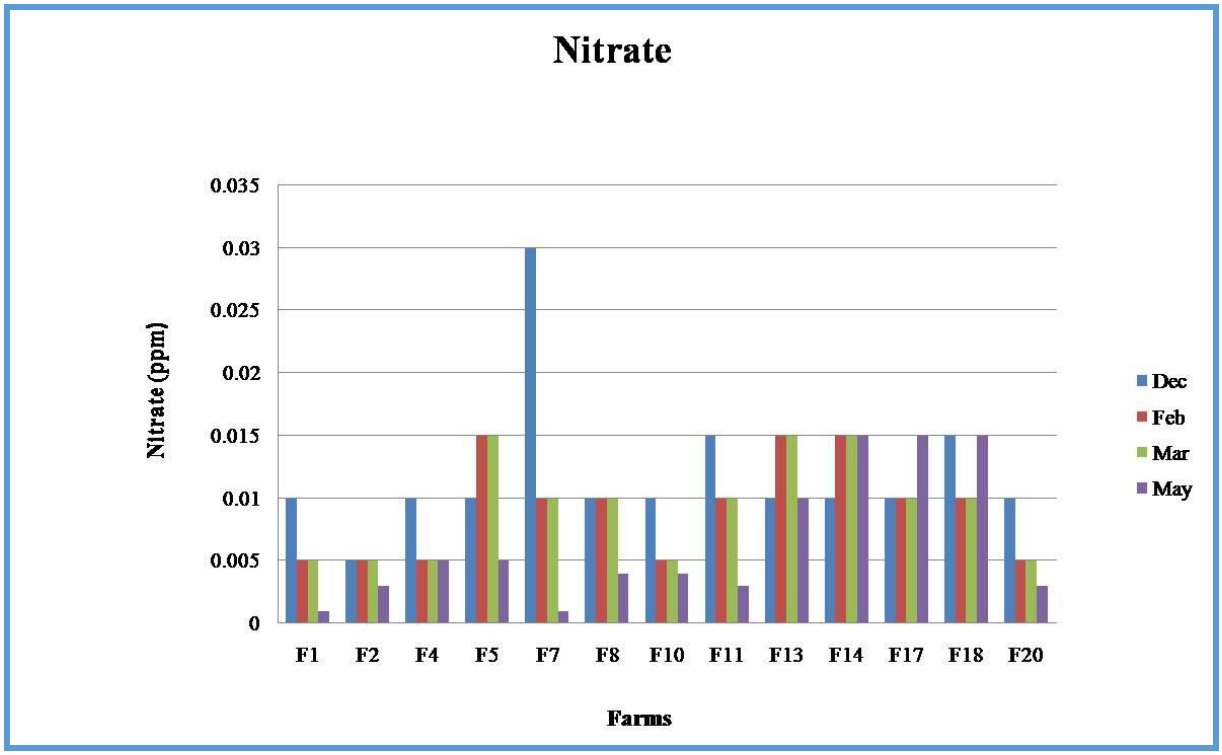


Fig 2: Water quality parameters in Disease incidence farms over different sampling periods

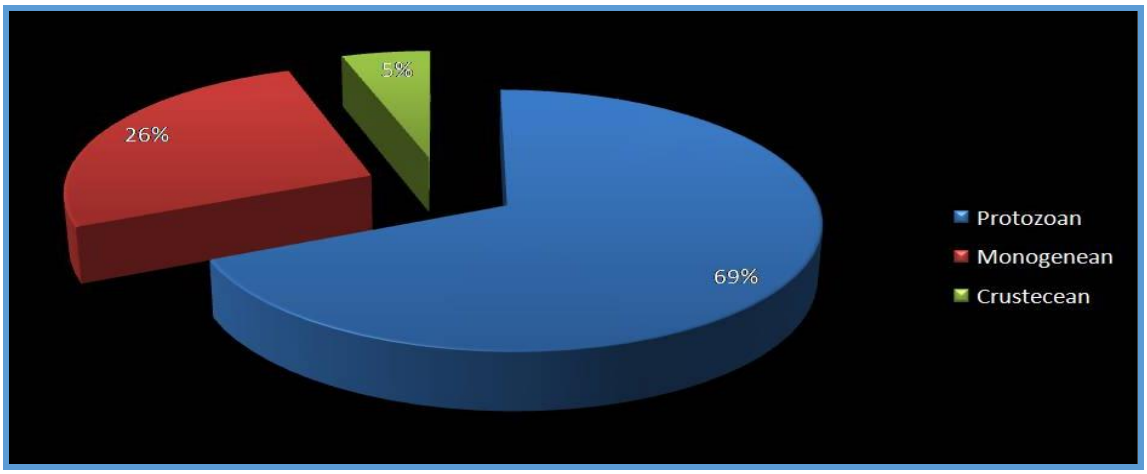


Fig 3: Prevalence of parasitic diseases in Tilapia farms (%)

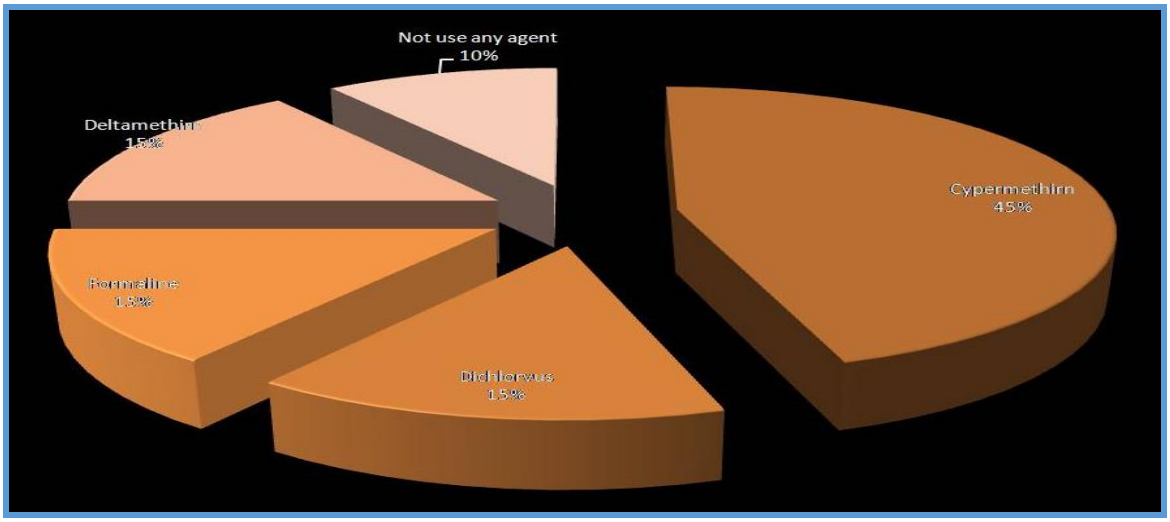
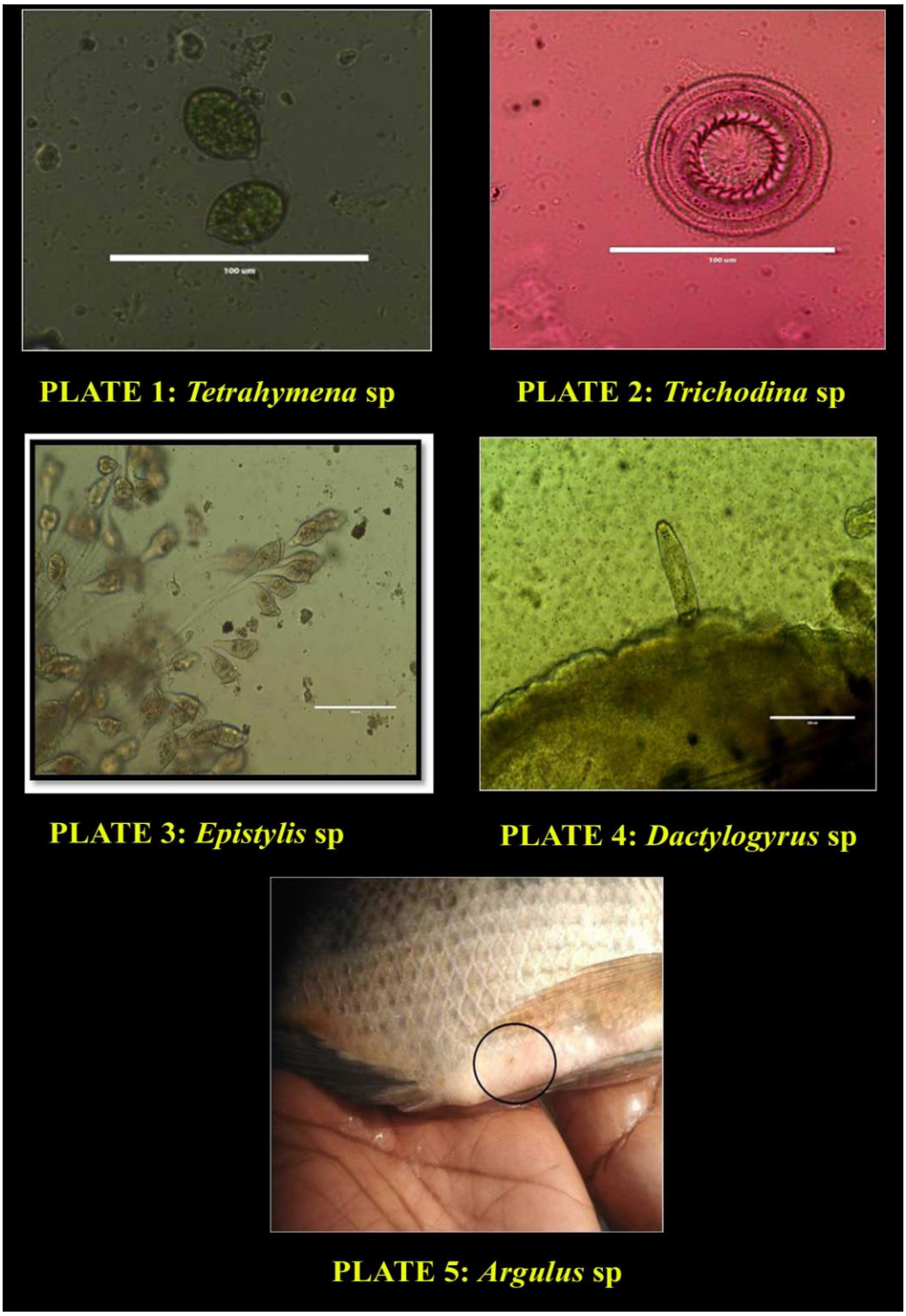


Fig 4: Antiparasitic agents used in Tilapia farms (%)



Conclusion

This study concluded that maintenance of good water quality management is necessary for protecting the fish from parasitic infestation. The presence of intermediates also enhances the availability of the parasite. Hence, snails and other intermediate hosts to be removed for reducing the parasite incidence in farms. The water level in the pond and optimal fertilization are necessary for keeping the ammonia and total organic load in pond at an optimal level and prevent stress in fish making it healthy against infection with parasites. Survey revealed that most farmers did not have proper knowledge about the application of chemicals and aqua drugs in farms and hence farmers should be educated on the effective use of chemicals without causing damage to the environment and fish.

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