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Efficacy of Algal Nutrition on Crustacean Infestation of Some Cultured Marine Fish, Ismailia Governorate

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THE aim of this study was to assess the impact of major parasitic crustacean invasions on farmed marine fish in Ismailia Province, Suez Canal Region, Egypt. A total of 540 premature, 180 *Dicentrarchus labrax* (225 ± 25 g), 180 *Sparus aurata* (150 ± 25 g) and 180 *Mugil cephalus* (125 ± 25 g) were collected from assimilatory ponds in Ismailia farms. They were investigated for crustacean infestation between May 2021 and August 2022. Infected fish were characterized by respiratory symptoms, reduced food intake and growth rate, abrasion, erosion, and ulcers of varying sizes. The overall prevalence in untreated premature animals studied was 53.44%, *D. labrax* 63.87%, *S. aurata* 53.60%, and *M. cephalus* 36.92%, respectively. While, the prevailing of infestations after treating the same fish with 5% of microalgae (*Amphora spp* and *Nanochloropsis spp*) that added to feed were 21.48 % (33.88, 23.61, and 16.94% for *D. labrax*, *S. aurata,* and *M. cephalus*, respectively). The parasite species detected were the crustaceans *Lernanthropus* spp. and *Caligus* spp. from *D. labrax*, *M. cephalus,* and *S. aurata*. Histopathological alterations were recorded in the gills and musculature. Microalgae played an important role in the spread of fish diseases in the studied fish.

Keywords: *Dicentrarchus labrax, Sparus aurata, Mugil cephalus*, *Lernanthropus spp, Caligus spp,* and microalgae.

Introduction

A little over 20% of the world's marine aquaculture production is produced in saltwater and most infectious diseases that are known to harm fish are caused by parasitic infestations [1]. The economic burden of parasitic infections might rise due to their direct (mortality rate) or indirect (morbidity rate) effects on wild and cultivated animals [2,3]. More than 80 fish species and one cetacean have both been documented to harbour *Caligus* sp., which has very low host specificity [4]. The clinical signs of most examined fish revealed no pathognomonic abnormalities on some of the infested fish except for congestion, excessive mucous secretion, sticky or pale gills, and grayish coloration with hemorrhage all over the body surface, especially at the base of the fins and the abdomen [5]. Infested fish displayed secondary gill lamella hyperplasia, as well as oedema and inflammatory cell infiltration. Severe vacuolar degeneration and a limited amount of leucocytic infiltrations were reported by El-Lamie [6].Algal biomass contributes to the improvement of edible fishes' development abilities, disease resistance, and skin colour, notably in the case of coloured fishes [7]. Dietary algal meal improved the physiological health of fish, and muscle protein levels increased in response to the algal level. Chemicals derived from marine algae, according to several experts,

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serve a variety of biological functions [8]. Green and brown algae, as well as invertebrates, contain sulfated polysaccharides (SPS) that may have antiprotozoal and antiparasitic properties [9]. SPS can compete with bacteria, viruses, and parasites for glycoprotein receptors on the surface of the host cell by binding to a variety of receptors there [10]**.**

The biological activities of seaweed have been the subject of numerous studies in recent years, and they have been identified as potential sources of natural antioxidants [11]. As a result, it is now important to thoroughly examine the use of microalgal biomass as a cost-effective feed supplement for various aquaculture animals [12]. The study of the *Amphora spp and Nannochloropsis* spp marine microalgae species, which are utilized in aquaculture for some marine fishes' food, could be employed as a green water strategy to maximize growth and survival rates [13].

The current study's focus was on the function of various marine algae in bio-form as a preventative measure against several common parasite diseases of gilthead seabream (*Sparus aurata*), sea bass (*Dicentrarchus labrax*), and mullet (*Mugil cephalus*).

Material and Methods

Fish sampling

A total of 540 premature fish were gathered from heterotrophic ponds for studies: 180 *D. labrax* (225±25 g), 180 *S. aurata* (150±25 g), and 180 *M. cephalus* (125±25 g). Initially, prematurity fish were randomly chosen from each pond and tested for parasitic infestation. Fish were caught from cement ponds at the Faculty of Agriculture, Suez Canal University, in Ismailia Province. Fish were randomly distributed in triplicate into three treatment groups for the examined fish infestation (540 fish) in the Fish Nutrition Laboratory, Research Center, Ismailia. Treatments were performed in 12 cement ponds at a rate of 45 fish per pond. The collected marine fish for experimentation were acclimated to the culture system for 2 weeks.

Diet

First, we determined the total weight of fish collected from each tank and recorded the average initial weight per species, then examined for parasite infestation. Water temperature, dissolved oxygen, salinity, and pH were adjusted to approximately 23±١°C, 6 mg/L, 35 mg/L, and 7.5, respectively. A basal diet was formulated to contain (32.5% crude protein, 3.50 crude fibers, 3.91% ash and 4420 Kcal kg-1 total energy). *Amphora spp.* and *Nanochloropsis spp.* were collected in the laboratory. *Amphora spp.* and *Nanochloropsis spp.* were collected in Lab. of algae in National Research Center. Subsequently, several marine fish (*D. labrax*, *S. aurata*, and *M. cephalus*) were added to the primary aquaculture marine fish diet at a concentration of (2 g, 3 g and 5 g) microalgae/kg per pellet diet. According to some references[14,15], microalgal feed additives contain microalgae. Basal diet represents levels of 2, 3, and 5 g/kg-1 diet. Premature fish were fed each diet at 5% of their body weight twice daily at 8:00 a.m. and 1:00 p.m. for 8 weeks. Collected samples were carefully examined infestation and weight gain recorded for signs of disease, with particular attention to crustacean infestations

Clinical picture

All of the collected fish were clinically evaluated and parasitological examined in accordance with Robert and his colleagues [16], the crustaceans were immediately fixed in 70% ethanol, stored in clearly labeled tubes, and transported to the lab for identification. Using a dissecting microscope, the morphological characteristics of the isolates were investigated.

Histopathological Examination

The afflicted skin, gills, and skin of premature *D. labrax*, *S. aurata*, and *M. cephalus* were preserved in 10% phosphate buffered formalin, then dehydrated in ascending grades of alcohol and cleaned in xylol, then embedded in paraffin wax and cut into thin sections (5 um) and floated on warm water (just below the melting point of the paraffin), the sections were removed from the water bath on slides, coated with a minimal quantity of Myers' albumin then allowed to dry thoroughly and then stained with (H & E) stain according to Noga [17].

Result and Discussion

Concerning the Clinical signs, were nearly similar in all the infested fish spp. regardless of their species. These fishes suffered from sluggish movement, emaciation and rubbing the body against any hard object.

In the present study, two crustacean parasites (*Lernanthropus spp.*, *Caligus spp.*) isolated from three fish species (*D. Labrax, S aurata* and *M. cephelus*). Fish were infested by one or two *Lernanthropus sp.* which seen unilaterally on gills, occupying gill arch. These results came in accordance with the previous finding [18]. Caligidae were observed on the skin and buccal cavity. The parasites were severely attached leaving a hemorrhagic lesions or necrotic ulcers on body surface and buccal cavity. These species belong to the family: Caligidae. These results came in accordance with the finding of Toksen et al. [19].

Parasitological examination

As shown in plate (1), the parasites under examination were collected from gills of *S. aurata,* based on the morphological and parasitological examinations, the isolated Crustacea was belonged to *Lernanthropus spp*. (Copepoda, Siphonostomatoida: Lernanthropidae). The parasites under examination were collected from the mouth of *D. labrax*. Based on the morphological features, the isolated Crustacea was belonged to family Caligidae, genus Caligus, species. The parasites under examination were collected from the skin of *S. aurata* and *M. cephalus*. Based on the morphological and parasitological examinations, the isolated Crustacea was belonged to family Caligidae, genus *Caligus.*

Histopathological examination of the infested fish

The presence of congestion and mild to moderate hyperplasia of gill lamellae was detected by histopathological examination of the gills of a naturally wild *D. labrax* infested with dangerous crabs (*Lernanthropus spp.*). In addition, high infestation demonstrated presence of severe hyperplasia of gill lamellae and leucocytic infiltration predominantly lymphocytes and few macrophages. Furthermore, it demonstrated significant vacuolar degeneration with little leucocytic infiltration (Plate 2). There is extensive gill destruction, severe haemorrhage, and exsanguination as a result of the parasite›s adhesion and feeding [6]. Histopathological alteration of the musculature of infested fish were characterized by scale detachment , excessive

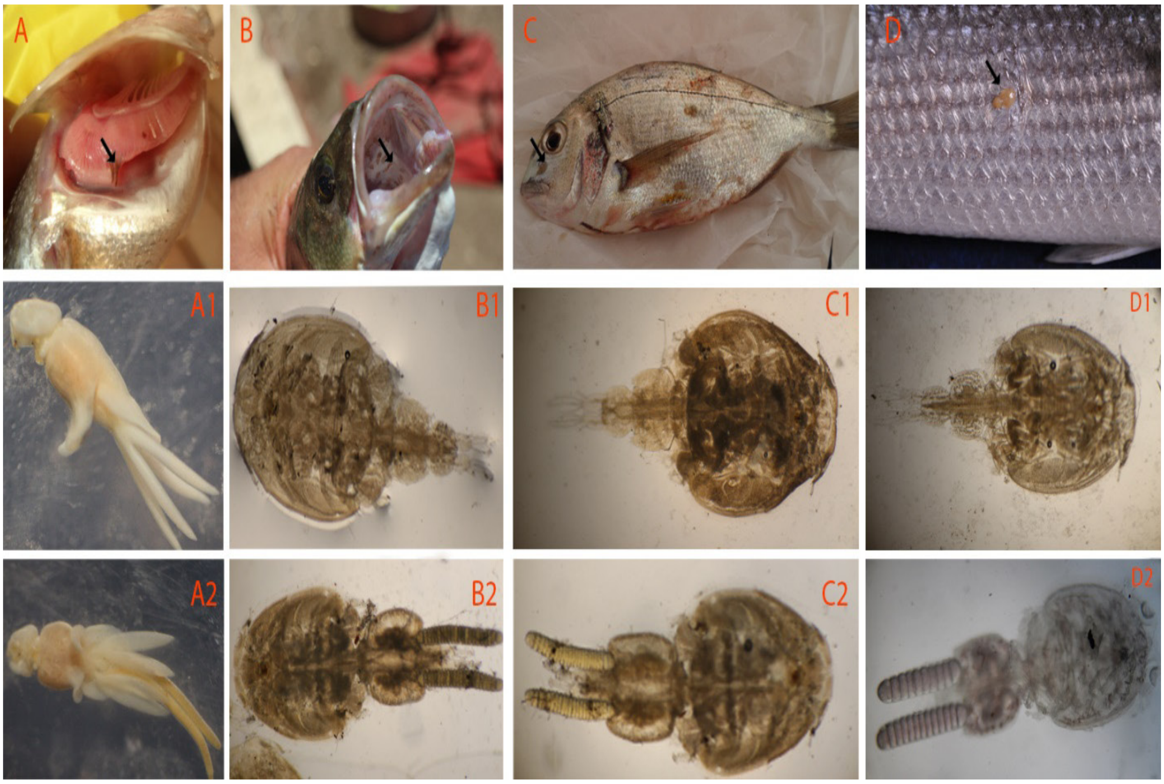


Plate 1. Showing a Crustacea Lernanthropus sp. embedded in gill arch of *D. labrax* (arrows) (A), light photomicrograph of (A1) male and (A2) female *Lernanthropus spp.* and, isolated from *D. labrax,* copepod *Caligus sp.* in buccal cavity of *D. labrax* (arrows) (B) (B1) female and (B2) male *Caligus spp.* isolated from *D. labrax*. While *Caligus spp.* (whole copepod), on *S. aurata* (arrows) (C). (C1) female and (C2) male *Caligus spp.* isolated from *S. aurata*, Also, *Caligus spp.* (whole copepod), (D) and male *Caligus spp* (D1) female and (D2) male (copepod), isolated from *M. cephalus*. (X40).

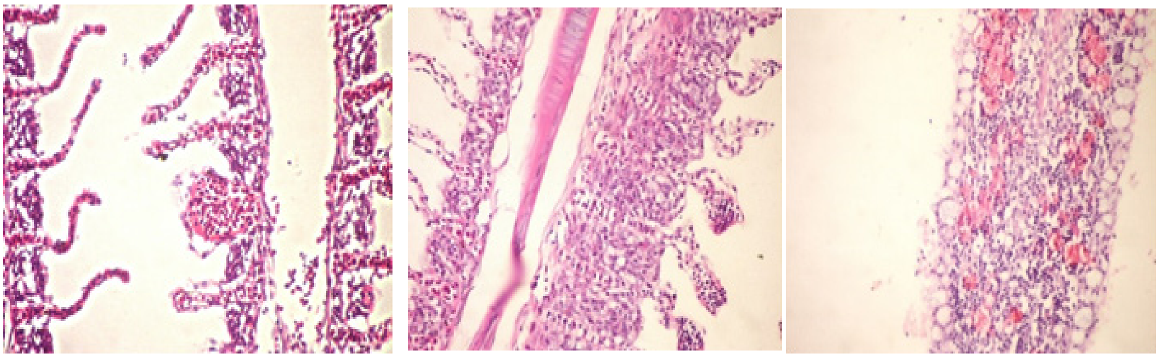


Plate 2. Gills of infected fish showing (A) congestion and mild to moderate, hyperplasia of gill lamellae, (B) severe hyperplasia of gill lamellae and leucocytic infiltration mainly lymphocytes and few macrophages,(C) severe vacuolar degeneration along with little leucocytic infiltration.

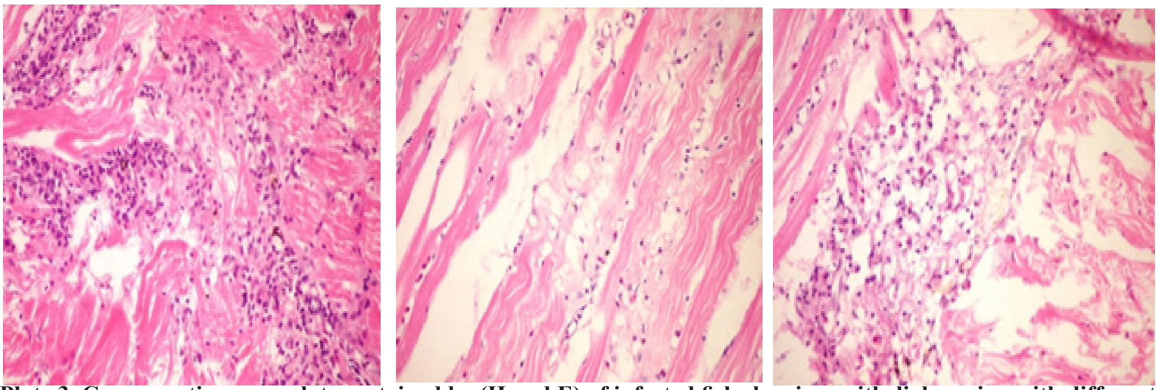


Plate 3. Crosse section musculature stained by (H and E) of infected fish showing epithelial erosion with different degrees, increase of goblet cell number and melanomacrophage cell .

mucus secretion, erosion of various degrees at the external epithelium of skin with ulcer formation (plate 3) which consider the main cause of the secondary bacterial infection [20].

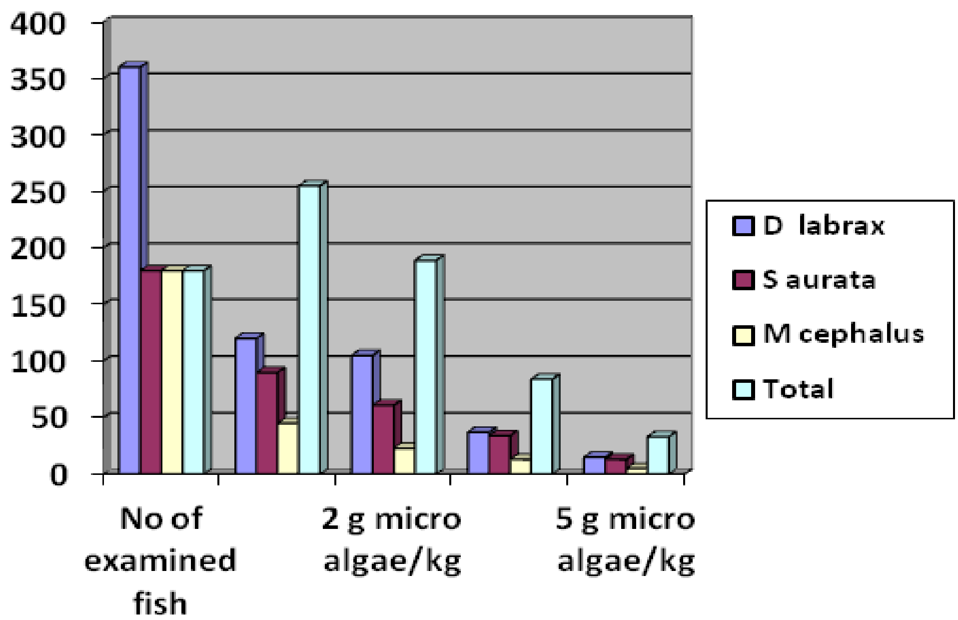
Prevalence of parasite infestation in premature marine fishes evaluated before and after therapy.

The total prevalence of parasite infestation in no-treated premature fishes was 53.44%, as indicated in the table (1). *D. labrax* had the highest proportion (63.87%), followed by *S. aurata* (53.60%), and *M. cephalus* had the lowest percentage (34.92%). In contrast, the overall prevalence of parasite infection in treated preterm fish was 21.48%. *D. labrax* had the highest proportion (33.88%), followed by *S. aurata* (23.61%), and *M. cephalus* had the lowest percentage (16.94%).

In the last few years, parasites developed resistance to common traditional method of treatment and drugs which leads loss of its effect on the parasite. In addition most of these drugs has several adverse effects on the fish and its toxicity, therefore several researches searching for effective safe alternative therapy for parasitic infestation prevention [8]. Microalgae are harmless and have antiparasitic properties [21]. Amphora (microalgae) is high in total lipids which composed of glycerol, sugars or bases, esterified to fatty acids having carbon numbers in the range of C12-C22, which may be either saturated or unsaturated. Furthermore, fatty acids with a high concentration of polyunsaturated fatty acids (PUFAs), particularly sulfated polysaccharide (SPS) and a high concentration of necessary amino acids that works as growth promoters [22]. The amino acids contained in all algae consider as functional proteins, with good quality [23]. *Tetraselmis suecica*, *T. chuii*, and *Pavlova lutheri* have high arginine content and are considered to be highly nutritious products [24]. Furthermore, uncommon molecules like as betaine lipids, chloro-sulfolipids, and various other sulfolipids may be important components of certain species or orders [25]. In the present study addition of microalgae with 5 g micro /kg algae to the feed led to decease the total parasitic infestation from 53.44 to 21.48 and (63.87 to 33.88, 53.60 to 23.61, 36.92 to 16.94) in *D. labrax*, *S. aurata*, *M. cephalus,* respectively. This result explains that addition of microalgae to the feed of fish led to decrease the total parasitic infestation from non-treated fish with species different [8,21]. As shown in the Table (1), several concentrations of microalgae were added to fish diet (2 g micro algae/kg, 3 g micro algae/kg, 5 g micro algae/kg), with the best results obtained with 5 g micro algae/kg. The amino acids pattern of practically all algae corresponds fairly with that of other food proteins,

TABLE 1. Showing total parasites infestation in non-treated premature examined fish.

Fish species	No. of examined fish	No. of infested fish not feed with algae	No. of infested fish different algae feed concentrations						
			2 g micro algae/kg		3 g micro algae/kg		5 g micro algae/kg		
		No	%	No	%	No	%	No	%
D. labrax	180	115	63.87	90	50	75	41.5	59	33.88
S. aurata	180	96	53.60	75	41	60	27	47	23.61
M. cephalus	180	86	36.92	65	29	55	30.5	41	16.94
Total	540	297	53.44	220	40.7	190	35	147	21.48



with modest shortages in sulfur containing amino acid methionine and cysteine [23]. High arginine concentration in *Tetraselmis suecica*, *T.chuii* and *P. lutheri*, considering them as better in nutritional quality [24]. SPS can compete with bacteria, viruses, and parasites for glycoprotein receptors on the surface of the host cell by binding to a variety of receptors there [9,10] and these explain why the algae should be used in the fish feed for decreasing the parasitic infestation.

Conclusion

Microalgae are regarded as a good supplement to fish meal in marine fish feed due to their economic impact on the growth rate and survivorship of treated fish, as well as the premature growth performance of studied fishes (*D. labrax*, *S. aurata* and *M. cephalus*), Concentration of 5% of microalgae in ration considered the best concentration that produced positive results for decrease of parasitic infestations. In addition, microalgae had an essential impact in the control of fish illnesses in the studied species. When obtained at 5 g/kg diet, it is promising for protection against microbial diseases and increases resistance to invading pathogens. The current study revealed that replacing fish meal in marine fish feed decreased parasite infestation when compared to those fed on ration free algae. Additional research.

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Conflicts of Interest

No conflict

Funding statements

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دراسات عن أهم الامراض الطفيلية السائدة التى تؤثر على بعض الأسماك البحرية المستزرعة فى محافظة الاسماعلية مصر مع التركيز على أستخدام بعض أنواع الطحالب

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يمثل الإستزراع السمكي البحرى أحد أهم مصادر الأنتاج السمكي في العالم حيث أن أنتاجه يصل الي أكثر من ٢٠٪ من الأنتاج السمكي العالمي. وتعتبر الامراض الطفيلية من أهم المسببات المرضية التى تصيب هذه الاسماك بطريقة مباشرة او غير مباشرة بالرغم من أن الغالبية العظمي من هذه الطفيليات قد لاتظهر اعراضها المرضية بوضوح الا اذا كانت مصحوبة بعدوي بكتيرية ثانوية ممرضة. ويهدف هذا البحث لعمل دراسة لمعرفة أهم الأمرض الطفيلية السائدة وعزل مسبباتها من الأسماك المصابة فى بعض أسماك المزارع السمكية البحرية فى منطقة الاسماعلية بمصر. ومعرفة مدى انتشارها وتأثيرها على أسماك الدنيس والقاروص والبورى. ولقد تمت الدراسة علي ٥٤٠ من الأسماك المستزرعة في بعض المزارع المصابة ببعض الطفيليات القشرية الممرضة في عدد ١٨٠ سمكة من اسماك القاروص والتي تتراوح اوزانها مابين ٢٢٥ ± ٢٥جم وكذلك ١٨٠ سمكة من أسماك الدنيس والتي تتراوح اوزانها مابين ١٥٠ ± ٢٥ جم . بالأضافة الي ١٨٠ سمكة من اسماك العائلة البورية والتي تتراوح اوزانها مابين ١٢٥±٢٥ جم في منطقة الأسماعلية. وقد لوحظ من الفحص الظاهري للأسماك محل الدراسة وجود أعراض تنفسية مع نقص فى معدل التغذية ومعدل النمو مع أحمرار وتقرحات جلدية في بعض الأسماك المصابة وقد تم عزل الطفيليات القشرية الموجودة عليها وكانت من سلالة *Lernanthropus spp*. و *Caligus* . ولقد كانت نسب الأصابة كالتالي قبل العلاج الإصابة الكلية 53.44% (53.44%, (*D. labrax* 63.87%, *S. aurata* 53.60%, and *M. cephalus* 36.92%).بينما كانت نسب الأصابة بعد أضافة الطحالب من سلألة الأمفورا والنانوكلوربسس علي العلف بنسب ٢ و٣ و ٥ جرام /كيلو علف وبنسبة علف ٥٪ من الوزن الكلي للأسماك ولمدة ٨ اسابيع وقد تلاحظ أن أقل نسبة أصابة كانت مع ٥ جرام أضافة طحالب وكانت كالتالي :-الأصابة الكلية 21.48% (21.48 % (33.88, 23.61, and 16.94% for *D. labrax*, *S. aurata,* and *M. cephalus*). وقد سجلت التغيرات الباثولوجية علي الخياشيم وفي عضلات الأسماك في أماكن أصابتها الطفيلية.

الكلمات الدالة: أسماك الدنيس والقاروص والبورى ، الاصابات الطفيلية , الطحالب , محافظة الاسماعلية.