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Harnessing Garlic Extract and AI for Sustainable Disease Mitigation in Aquaculture

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Abstract

This comprehensive study delves into alternative and sustainable disease management in aquaculture, particularly in cultured rabbitfish (Siganus rivulatus) susceptible to Pseudomonas aeruginosa infections. Acknowledging the vulnerabilities in conventional veterinary medications and the need for rapid diagnosis, the research investigates the use of raw garlic extract as a dietary supplement alongside machine learning-based diagnostic methodologies. Using histo-biochemical analyses, the study finds that fish treated with garlic extract showed significant resistance to infection without visible signs of lethality. Furthermore, machine learning classifiers achieved an accuracy rate of 97.2% in distinguishing healthy and infected fish. The study thus provides evidence for garlic's potential role as a sustainable antimicrobial agent, and machine learning's efficacy for rapid, accurate diagnosis.

Keywords: *Siganus rivulatus, Pseudomonas aeruginosa, Aquaculture, Garlic, Artificial Intelligence*

1. Introduction

Aquaculture serves as an invaluable cornerstone in human nutrition, providing not only essential proteins but also a variety of vital nutrients [1]. According to data from the Food and Agriculture Organization (FAO), global fish production consists of a staggering 93.2 million tons harvested from natural marine and freshwater ecosystems, augmented by an additional 48.1 million tons derived from aquaculture operations. Despite their high productivity, these aquaculture systems remain alarmingly vulnerable to catastrophic disease outbreaks, which substantially impede production capabilities [2].

Various environmental and operational stressors - ranging from overcrowding and inconsistent handling to variable water conditions - can provoke physiological modifications in fish. These changes frequently result in alterations to oxidative stress pathways, rendering the fish increasingly susceptible to bacterial infections [3]. Notably, Pseudomonas species have been identified as key culprits in disease outbreaks among cultured rabbitfish (*Siganus rivulatus*) maintained in Recirculating Aquaculture Systems (RAS) in the Red Sea.

Timely and precise disease diagnosis stands as a critical element for optimizing yields in aquaculture. However, conventional diagnostic methods often prove inadequate in swiftly identifying early indicators of infections [4]. Emerging advancements in image processing technologies are paving the way for unprecedented improvements in diagnostic accuracy. This study seeks to explore the potential of raw garlic as a dietary supplement for *S. rivulatus* in combating infections caused by *Pseudomonas aeruginosa*, utilizing an integrated approach of histo-biochemical analyses and machine learning methodologies for diagnostic evaluation.

Traditionally, the aquaculture industry has leaned heavily on veterinary medications for disease management [5]. These drugs are delivered through a variety of means, including incorporation into fish feed, therapeutic water baths, or even targeted vaccinations [6]. However, rising apprehensions regarding the ecological impact and potential human health risks linked to the use of these pharmaceuticals have emerged [7, 8]. Most alarmingly, the indiscriminate use of antibiotics can catalyze the development of antibiotic-resistant bacterial strains, thereby exacerbating treatment complexities [9].

In light of these considerations, there is a burgeoning demand for more sustainable and ecologically benign disease management solutions. The influence of fish stress on increased disease susceptibility has been extensively documented [6]. Consequently, contemporary approaches are gravitating toward innovative treatments aimed at enhancing the fish's innate immune system [1, 10].

Within this framework, the utilization of natural remedies such as plant-derived substances in aquaculture is gaining momentum [11, 12]. Our study is specifically tailored to investigate the physiological ramifications of using raw garlic as a dietary supplement for *S. rivulatus*. Garlic is rich in bioactive compounds that are known for their antimicrobial efficacy and have demonstrated potential in fortifying animal immune systems. Through a synergistic blend of histo-biochemical analyses and state-of-the-art image processing technologies, this research aspires to evaluate the effectiveness of garlic-enriched diets in mitigating disease outbreaks and fortifying fish health.

2. Materials And Methods

Rabbitfish (*S. rivulatus*) of approximately uniform age, boasting an average weight of about 15 grams, were obtained from the specialized Aquaculture Unit at the Marine Science Station (MSS) in Aqaba. The garlic used for the experimental trials was meticulously sourced from local markets in Aqaba, Jordan.

The isolation and identification of *P. aeruginosa* followed rigorous protocols as outlined in the existing literature [13]. The isolated bacterial strains were then subjected to a freeze-drying process for preservation, as meticulously documented in [14].

The rabbitfish were systematically segregated into seven distinct cohorts, each comprising 10 individual specimens. The negative control group (C) received a diet devoid of garlic extract and was shielded from the bacterial challenge. Conversely, the positive control group (PC) was intentionally exposed to *P. aeruginosa* but did not receive any form of garlic supplementation. This latter group was immersed directly in the bacterial solution.

The experimental cohorts, designated as G1 through G5, were all exposed to the bacterial challenge. These groups received varying daily dosages of garlic extract, specifically 25, 50, 75, 100, and 125 mg per kilogram of body weight, over a span of 14 days.

Upon the experiment's conclusion, targeted tissue samples—specifically from the hepatopancreas and muscles—were expeditiously harvested post-scarification from all designated groups. These samples were then cleansed using a normal saline solution enriched with heparin, weighed, and subsequently homogenized in a phosphate buffer saline, maintaining a pH level of 7.2. The prepared tissue samples were stored at a temperature of -20ºC, pending subsequent biochemical analyses.

The Lipid Peroxidation (LPO) stress biomarker assay was administered across all the experimental groups. Concentrations of Malondialdehyde (MDA) within the hepatocytes, indicative of LPO levels, were quantified in accordance with methodologies delineated in [15, 16, 17]. Additionally, the cytotoxicity biomarker, Lactate Dehydrogenase (LDH) activity, was assessed utilizing protocols detailed in [18, 19].

AI-Assisted Analyses

For the specialized task of image analysis, Artificial Intelligence (AI) was employed to discern potential morphological tissue damage subsequent to pathogen exposure, as visually represented in Figure 1. The Support Vector Machine (SVM) algorithm was adeptly leveraged to construct a model proficient in analyzing fish tissue images. This model effectively categorized the images into two primary classifications: healthy and infected.

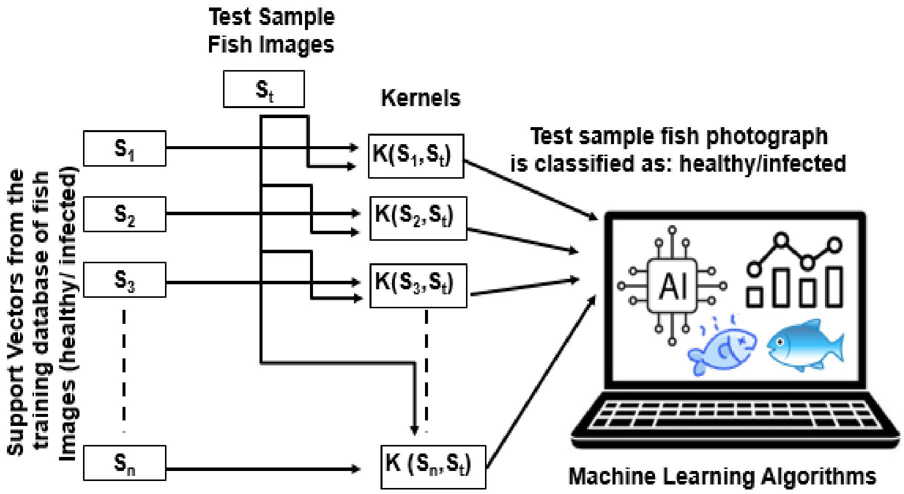

***Fig. 1**: Classification using support vectors for classification of healthy and infected fish*

3. Results and Discussion

In the course of a rigorously executed experiment, it was discerned that fish subjected to a regimen of garlic extract exhibited formidable resistance to mortality. Remarkably, this specific group displayed no visible indicators of lethality. Conversely, the fish exposed to the infectious agent -along with those administered raw garlic - manifested conspicuous signs of compromised vitality. These symptoms encompassed a reduced enthusiasm for swimming, evident in their decreased energy levels, and a significant diminution in food consumption, especially when contrasted with the baseline metrics of the control group.

In addition, among the fish that were subjected to infection but had not been treated with raw garlic, a conspicuous and acute alteration in the coloration of their hepatopancreas was evident. This organ assumed a more somber red shade, a striking divergence from its typical hue. Intriguingly, when these infected cohorts were administered garlic extract, a laudable reversion to the organ's normal appearance was achieved, underscoring the potential therapeutic merits of garlic extract in counteracting the deleterious effects of the infection.

Further scrutiny revealed that the fish in the infected group exhibited an elevated ratio of hepatopancreas weight to overall body mass. This intriguing phenomenon could conceivably be attributed to acute intrahepatic hemorrhage, compounded by the localized accumulation of blood within the hepatopancreas itself. This finding accentuates the harmful repercussions of the infection on the general well-being of the fish.

Turning our focus to the MDA concentrations within tissue homogenates, it is essential to recognize that these serve as unequivocal and reliable biomarkers for bacterial toxicity. The control group, which had remained unexposed to infection, manifested lipid peroxidation (LPO) levels that fell comfortably within the expected parameters, quantified at 0.172 µM/g in hepatocytes and 0.104 µM/g in muscle homogenates. In sharp contrast, the infected cohorts experienced a statistically significant ($P<0.05$) elevation in their MDA levels. This was demonstrative of a staggering 11-fold and 10-fold escalation in the hepatopancreas and muscle homogenates, respectively, relative to the control group. This compelling data corroborates the existence of a dose-dependent relationship. It is noteworthy that when the infected fish were administered varying dosages of raw garlic, a subsequent reduction in MDA concentrations ensued. The most notable restoration was observed when the fish were given a daily dose of 75 mg of garlic extract per kilogram of body weight, consistently administered over a 14-day period. For a graphical illustration of these findings, one may refer to Figure 2.

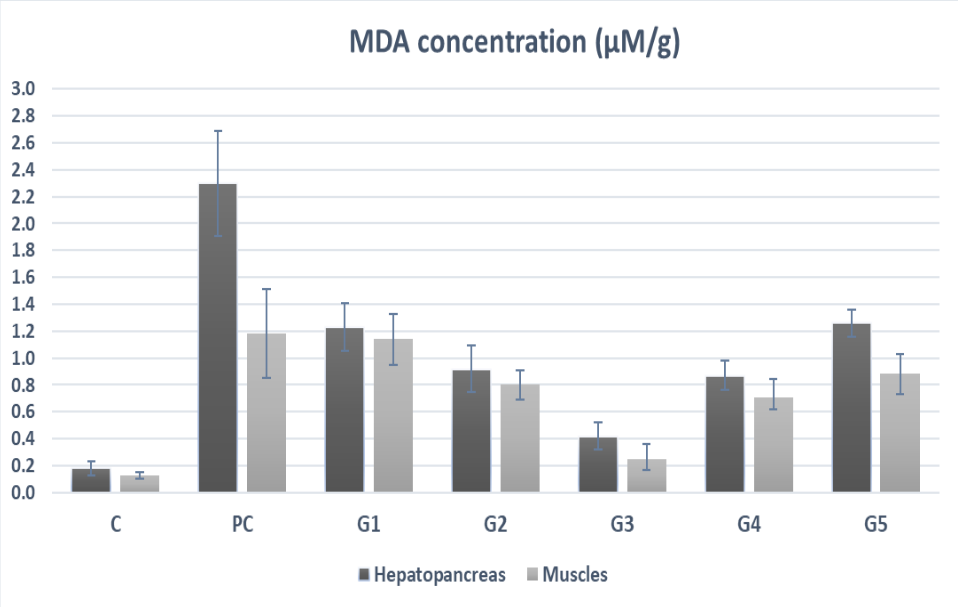

Fig. 2: *MDA concentration (µM/g) ±S.D in the two examined tissues of the experimental fish.*

A highly refined spectrophotometric assay was scrupulously calibrated to assess the cytotoxic ramifications exerted by the bacterium *P. aeruginosa* on two target tissues: the hepatopancreas and skeletal muscle. This assessment was facilitated by measuring the release of lactate dehydrogenase (LDH), a key marker for cellular damage arising from cell lysis. Upon evaluating the fish groups infected with the bacterium, we observed a conspicuous escalation in LDH concentrations. More precisely, LDH levels surged by an estimated 2.7-fold in the hepatopancreas and approximately 2.3-fold in the muscle tissues, as depicted in Figure 3. Intriguingly, when the infected specimens were administered a diet enriched with raw garlic, a marked reduction in LDH concentrations was observed, in comparison to the control group. This protective effect was most pronounced in group G3, which received daily doses of 75 mg of garlic extract per kilogram of fish weight, administered over a 14-day period.

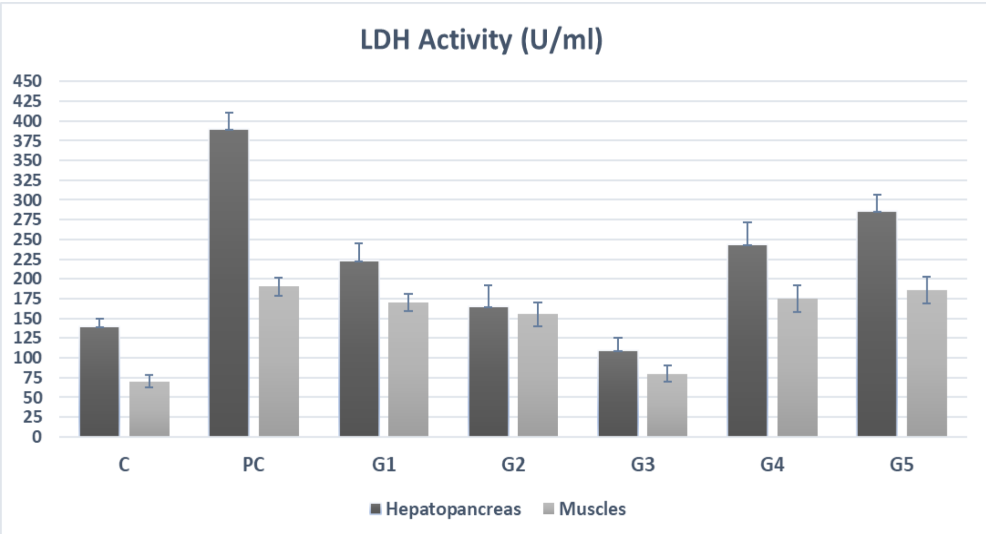

Fig. 3: *Lactate Dehydrogenase (LDH) activity (U/ml) ±S.D in the two examined tissues of the experimental fish.*

To construct the model dataset, a meticulously curated collection of no fewer than 60 photographs was assembled. These visual data points were subsequently digitized and uniquely categorized into two distinct classifications to streamline the analytical process.

Artificial Intelligence (AI) plays an indispensable role in the field of image analysis. It facilitates the utilization of sophisticated image filters, capable of isolating a wide array of image attributes. These range from fundamental visual components like corners and edges to more complex characteristics such as color schemes and histograms. Adding another layer of depth to this capability are machine learning classifiers. These tools enhance the feature extraction process by accurately identifying textural attributes, encompassing but not limited to co-occurrence matrices and local binary patterns (LBP). Of equal significance are shape features, which serve as key elements in capturing the intricate geometries of objects within images.

When deployed in the context of marine studies, AI-powered fish image segmentation proves to be invaluable. It assists in the precise demarcation and differentiation of individual fish species. This technological advancement holds monumental importance for the advancement of marine research and conservation efforts, as illustrated in Figure 4.

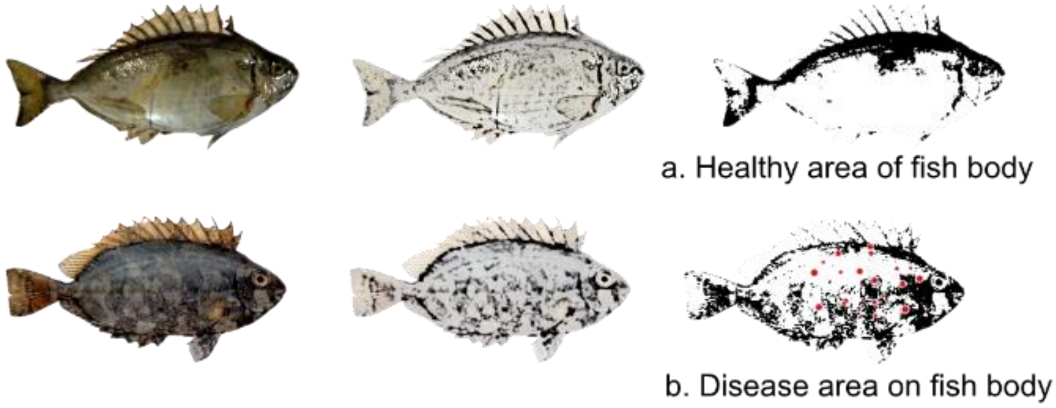

Fig. 4 : Fish image segmentation using AI techniques

Additionally, the integration of machine learning classifiers plays a crucial role in identifying particular attributes within images, such as the precise dimensions and spatial orientation of objects. Key statistical metrics, such as the mean and variance, provide valuable quantitative perspectives on the distribution and central tendencies of pixel intensities throughout these images. By synergizing the capabilities of artificial intelligence and machine learning, a more nuanced and holistic analysis of digital imagery becomes achievable (Refer to Fig. 1).

Table 1 presents the comprehensive accuracy metrics for a Support Vector Machine (SVM) classifier, which was deployed in a classification task aimed at differentiating between two distinct categories: "Healthy" and "Infected."

Table 1. *Comprehensive Accuracy Results of the SVM Classifier (97.2%)*

Class	*TP Rate*	*FP Rate*	*Precision*	*Recall*	*F-Measure*
Healthy	*0.971*	*0.027*	*0.973*	*0.971*	*0.972*
Infected	*0.973*	*0.029*	*0.970*	*0.973*	*0.972*
Weighted Avg.	*0.972*	*0.028*	*0.972*	*0.972*	*0.972*

As delineated in Table 1, the Support Vector Machine (SVM) classifier demonstrated exceptional performance, boasting an extraordinary accuracy rate of 97.2%. The True Positive Rate (TP Rate) serves as an illuminating metric that reveals the proportion of instances accurately identified. Notably, both classes exceeded the 97% threshold: The "Healthy" class attained a 97.1% TP Rate, whereas the "Infected" class slightly outperformed it with a rate of 97.3%.

Turning our attention to the False Positive Rate (FP Rate), this metric quantifies the proportion of inaccurately identified instances. It remained impressively low across both categories: a mere 2.7% for the "Healthy" classification and 2.9% for the "Infected" class.

Precision, which specifies the proportion of true positive instances among all instances labelled as positive, registered at 97.3% for the "Healthy" category and 97.0% for the "Infected" category. Similarly, Recall—often equated with the True Positive Rate or Sensitivity—captures the percentage of true positive instances among all actual positive cases. Both classes showcased extraordinary figures, with the "Healthy" category registering at 97.1% and the "Infected" category at 97.3%.

The F-Measure, a harmonic mean of precision and recall, provides an encompassing perspective on the classifier's overall efficacy. Both classes achieved consistent scores of 0.972, indicating a harmonious and robust performance in terms of both precision and recall.

Considering all this, the SVM classifier, armed with its commendable accuracy rate of 97.2%, exhibited exceptional proficiency in segregating instances into the "Healthy" and "Infected" classifications. This robust performance underscores the SVM's considerable potential as an invaluable tool for the specific classification task at hand.

The recent study delves into the promising therapeutic applications of raw garlic (*Allium sativum*) for improving the health of rabbitfish (*S. rivulatus*) in RAS that are challenged by the bacterial pathogen *P. aeruginosa*. Strikingly, the group treated with garlic exhibited a substantial reduction in mortality rates as well as fewer adverse health symptoms. Application of *A. sativum* notably mitigated discoloration in the hepatopancreas and led to decreased levels of malondialdehyde (MDA), a critical biomarker for bacterial toxicity, in tissue samples. These compelling findings endorse the efficacy of *A. sativum* as a viable alternative in aquaculture treatments, offering solutions to both environmental and health challenges posed by conventional veterinary pharmaceuticals.

The study further incorporates cutting-edge Artificial Intelligence (AI) and Machine Learning (ML) techniques to analyze images and categorize fish as either "Healthy" or "Infected." Remarkably, the Support Vector Machine (SVM) classifier achieved an astounding accuracy of 97.2%, underlining its formidable capability to distinguish between the two health states. The application of AI and ML technologies enhances our understanding of visual data and refines the assessment of fish health, heralding a new era in disease prevention within aquaculture.

Garlic, renowned for its potent antimicrobial properties, releases allicin, a powerful oxidant, when crushed. Numerous studies corroborate the antioxidant capabilities of both fresh and powdered forms of garlic [20]. The data indicates that *P. aeruginosa* can inflict significant tissue damage, presumably caused by an excess of free radicals leading to oxidative stress [21]. Such oxidative activities predominantly target organic molecules, such as polyunsaturated fatty acids in cellular membranes, resulting in lipid peroxidation (LPO) via hydroxyl radicals [22, 23]. By evaluating LPO levels in the fish's hepatopancreas and muscles, the study illuminates how the dietary inclusion of raw garlic mitigates MDA formation by neutralizing hydroxyl radicals [17, 24]. Most notably, the maximum protective effect was observed in group G3, administered a daily dosage of 75 mg of *A. sativum* extract per kilogram of fish body weight over a 14-day period. The observed protection appears to be dose-responsive, achieving optimal efficacy at this specific daily dosage.

Emerging strategies in aquaculture disease management focus on bolstering the fish's innate defenses through the proactive administration of antioxidants, presenting a sustainable alternative to traditional chemotherapy and vaccines. Antioxidants, lauded for their broad-spectrum activity, cost-effectiveness, and environmentally-friendly profile, play a crucial role in disease prevention [25]. They amplify both specific and generalized defense mechanisms, thus enhancing the overall health and resilience of aquatic organisms [26]. A plethora of bioactive compounds, including those derived from both plant and animal sources, have demonstrated efficacy as immune stimulants [25]. Garlic and its derivatives are rich in bioactive compounds such as phenolics, polyphenols, and alkaloids [27], many of which are gaining recognition as antibiotic substitutes [20]. Extensive studies have scrutinized garlic's antioxidant properties across a diverse range of species, including rodents, poultry, and human cell cultures [28]. Nevertheless, the utilization of medicinal plants as feed additives in aquaculture remains a relatively untapped avenue [29].

Antioxidants not only bolster immune function but also promote growth, exhibit antimicrobial activity, stimulate appetite, and offer anti-stress effects [30, 34]. Conventional approaches to microbial disease management in aquaculture often entail economic and environmental drawbacks. Standard treatments can discharge substantial amounts of chemotherapeutic residues into aquatic ecosystems, thereby posing risks to human health [31]. A shift towards eco-friendly alternatives, such as medicinal plants, is becoming increasingly prominent [32, 33]. Various studies have emphasized the antibiotic efficacy of selected plants against pathogens in diverse animal models [27]. Survival rates of fish, even under severe infection conditions, can be significantly improved using immune enhancers, vaccines, and probiotics. For instance, herbal treatments have exhibited promise against *Aeromonas hydrophila* [26]. Extracts from Ayurvedic herbs like *Solanum trilobatum*, *Andrographis paniculata*, and *Psoralea corylifolia* have demonstrated protective effects against a range of pathogens, including *P. aeruginosa* in Penaeus species. In another noteworthy example, a concoction of four traditional Chinese medicinal herbs emerged as an effective preventive strategy, replacing antibiotics in the treatment of enteritis in grass carp [34].

The introduction of natural flavonoids into Jordanian aquaculture represents an innovative undertaking. To the best of our knowledge, such an approach remains largely unexplored in the region.

4. Conclusion

It The study reveals that garlic extract holds promise as a sustainable alternative to traditional veterinary medications, showing considerable efficacy in preventing *P. aeruginosa* infections in rabbitfish. Additionally, machine learning classifiers, specifically Support Vector Machines (SVM), demonstrated high accuracy (97.2%) in differentiating between healthy and infected fish. The dual approach of using garlic for treatment and machine learning for diagnosis could revolutionize sustainable disease management in aquaculture.

Recommendations

Further investigations are warranted to ascertain the optimal dosages of garlic extract that could be beneficial across various fish species and environmental settings. Long-term ecological impact studies of garlic extract application in large-scale aquaculture are also recommended for a comprehensive understanding of its sustainability. Expanding the machine learning models to additional fish species can offer a broader diagnostic accuracy and applicability. Incorporating machine learning technologies as standard diagnostic tools in the aquaculture industry could provide rapid, precise evaluations and mitigate the impact of disease outbreaks. In light of these findings, policymakers should consider amending current health management protocols in aquaculture to include garlic extract as an approved treatment method.

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