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Use of crude extract of garlic (*Allium sativum*) as an alternative in the prevention of saprolegniosis in rainbow trout (*Oncorhynchus mykiss*)

Aprovechamiento del extracto crudo de ajo (*Allium sativum*) como alternativa en la prevención de saprolegniosis en trucha arcoíris (*Oncorhynchus mykiss*)

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Article Data

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Abstract

The objective of the study was to evaluate the use of garlic (*A. sativum*) in the prevention of saprolegniosis in rainbow trout (*Oncorhynchus mykiss*). Descriptive statistics were applied. We used juvenile fish distributed in 10/pond, in 15 ponds, 150 specimens (fish from 10 to 15 cm in length, with weights from 150 to 200 g), there were positive controls with symptoms of saprolegniosis and one negative individual in optimal health conditions. Once the crude garlic extract was elaborated with the determined samples, the results were: the presence of fungal structures in the form of cotton specks based on the appearance of this sign in T₁ 11, T₂ 13 and T₃ 15 trouts respectively affected and it was analyzed during the garlic treatment the reduction and complete absence of the sign we have for T₁ 22, T₂ 35 and T₃ 33 with frequency and representing 24 %, 39 % and 37 % respectively obtaining better results T₂ based on 500 mg of crude garlic extract. The depigmentation of the skin is another sign of Saprolegnia presence which was manifested in all fish and analyzing its reduction and absence throughout the treatment, results were obtained in T₁ and T₂ with frequencies of 59 each, representing 33.5 %, and T₃ with a frequency of 58 representing 32.9 %, the three treatments showed efficacy since there is no significant percentage difference. It is concluded that the analysis of Saprolegniosis by *Saprolegnia* spp. determined that T₂ (500 mg/L water/tank) was the one that controlled macroscopic lesions of *Saprolegnia* spp.

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Resumen

El objetivo del estudio fue, evaluar el aprovechamiento de ajo (*A. sativum*) en la prevención de saprolegniosis en trucha arcoíris (*Oncorhynchus mykiss*). Se aplicó estadística descriptiva. utilizamos peces juveniles distribuidos en 10/estanque, en 15 estanques, 150 especímenes (peces de 10 a 15 cm de longitud, con pesos de 150 a 200 g), existió testigos positivos con patologías de saprolegniosis y un individuo negativo en óptimas condiciones de salud. Una vez elaborado el extracto crudo de ajo con las muestras determinadas los resultados fueron: la presencia de estructuras fúngicas con forma de motas de algodón partiendo de la apariencia de este signo en el T₁ 11, T₂ 13 y T₃ 15 truchas respectivamente afectadas y se analizó durante el tratamiento a base del ajo la reducción y ausencia completa del signo tenemos para el T₁ 22, T₂ 35 y T₃ 33 con frecuencia y representando el 24 %, 39 % y 37 % respectivamente obteniendo mejores resultados T₂ a base de 500 mg de extracto crudo de ajo. La despigmentación de la piel es otro signo que presencia Saprolegnia la cual se manifestaba en todos los peces y analizando su reducción y ausencia a lo largo del tratamiento se obtuvo como resultados en la T₁ y T₂ con frecuencias de 59 cada una, representa 33.5 %, y la T₃ una frecuencia de 58 que representa el 32.9 %, los tres tratamientos mostraron eficacia ya que no existe una diferencia porcentual significativa. Se concluye que el análisis de Saprolegniosis por *Saprolegnia* spp., determinó que T₂ (500 mg/L de agua/estanque) fue el que controló de las lesiones macroscópicas de *Saprolegnia* spp.

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Introduction

The use of plants and substances such as plant extracts have been considered the most ancient in the world, referring to various investigations, medicinal plants have similar efficacy to conventional medications, as well as the use of garlic bulb (*Allium sativum*) used since time immemorial, dating back to the beginning of humanity, studies focused on its organosulfur compounds such as allicin and ajoene¹, they own beneficial effects on the cardiovascular system, immunological, as antifungal, antimicrobial and anticancer².

The *Allium* genus has more than 300 varieties, *A. sativum* in Latin, means "odorous" due to its characteristic odor when crushed, cut or crushed, a vegetable 30 to 40 cm tall, very narrow ensiform leaves, stem with white flowers, its main part, the bulb, is underground during its growth and maturation³. garlic is one bulbous plant composed of 6 to 12 bulbils, known as garlic cloves, attached to a covered base by a semi-transparent membrane that forms its head. Several studies have reported its antioxidant, antimicrobial, anticancer, antifungal among others⁴, originally from Asia, European traders facilitated its distribution, being introduced to the American continent at the end of the 19th century by the Spanish⁵.

It is cultivated in cold climates with temperatures between 13 and 24 °C, a maximum of 30 °C and a minimum of 7 °C, on soft ground little sandy, with good drainage, it is a demanding crop, it needs agricultural practices, from the selection and preparation of the soil, the seed, as well such as irrigation, pest control, weeds among others. It is harvested after 6 to 7 months, depending on the characteristics of the environment, for its harvest it can be taken into account certain characteristics such as color, texture a

of leaves⁶. There are more than 600 varieties of garlic classified into two subspecies, soft neck, hard neck, also classified according to their color, white, purple, pink, violet, red, brown, among other classifications, Chinese garlic, Japanese, elephant, male⁷.

Phytotherapy indicates multiple benefits for human and animal health, thanks to the use of plants and their extracts, it was possible to prevent and control numerous diseases, which is how garlic was subjected to different studies that refer to its therapeutic properties⁸. Various studies conducted on animals have reported the use of fresh garlic, it has antioxidant effects, due to its ability to inhibit the formation of free radicals, reinforcing the mechanism of capturing endogenous radicals, increasing cellular antioxidant enzymes, among other beneficial characteristics, the compounds responsible for the antioxidant property is S-allyl-cysteine and allicin⁹. Garlic contains large amounts of selenium, acts as a coenzyme, increases antioxidant activity in our body. Allicin has been considered the main inhibitor of platelet aggregation, it reduces calcium levels in smooth muscle cells causing vasodilation, allicin and its ajoenes also cause inhibition of cyclooxygenase and lipoxygenase⁹. According to observational studies in humans, it is mentioned that garlic induces apoptosis of leukemic cells by stimulating the production of peroxide and activating the nuclear factor kappa B¹⁰.

Saprolegniosis, an opportunistic disease, affects freshwater fish at all stages of development, causing multifocal cottony lesions due to the proliferation of hyphae in the skin and gills¹¹, however, severe infections can occur, invading internal organs such as the intestine, stomach¹². *Saprolegnia* affects the majority of salmonids that are under artificial breeding, better

known as aquaculture, in all their life stages, the most likely being the eggs, juvenile fish, have also been affected by this type of aquatic mould, tilapia and aquarium fish¹³. Saprolegnia, an opportunistic aquatic mold present in freshwater aquariums, of the class Oomycetes, family Saprolegniaceae¹⁴, of the kingdom Chromista, phylogenetically closer to algae, without.

However, *Saprolegnia* grows in culture media for fungi, produces coenocytic hyphae and mycelium due to its morphological characteristics, it was previously classified within the kingdom Fungi¹⁵. The genus *Saprolegnia* has around 12 species, *Saprolegnia parasitica*, the main pathogenic species of aquatic organisms, acts as a secondary pathogen¹⁶, develops in dead plant material, its spores affect fish, inhabits their gills, taking advantage of the stress caused by improper handling, colonizes host skin causing fungal infection, the factors that favor infection are changes in water temperature, salinity, pH, most infections occur at temperatures below 10 °C, however, *Saprolegnia* can live in environments between 3 at 33 °C, with salinity 1.75 % ClNa¹⁷.

In general, saprolegniosis manifests itself chronically, and can be complicated by the presence of bacteria, causing the death of the fish in an acute form¹⁸. The hyphae of *S. parasitica* focally invade the skin of the fish, progressively penetrating the superficial layers such as the epidermis, dermis, even the hypodermis, muscle, causing imbalance of organic fluids and peripheral circulatory failure, due to the impossibility of maintaining the volume of circulating blood¹⁹.

The trout is native to North America from the basins that drain into the Pacific, it was introduced for sport fishing, beginning its breeding in Spain in the 60²⁰. It

has a fusiform body, with fine scales, coloration varies according to the environment, age and stage of sexual maturation, the name rainbow trout is due to the presence of a red stripe with different shades on its edges in the shape of a rainbow on the lateral part of the body²¹. Anatomically, it has two even fins (one pectoral pair, one ventral or pelvic pair), and three unpaired fins (dorsal, anal and caudal), like all salmonids they have an adipose fin that does not have a function defined²². They can reach a weight of 4.5 kg in captivity and 7-10 kg in lakes, rivers and seas²³. The trout inhabits uncontaminated aquatic spaces with crystalline fresh water, with causes that present marked topographic unevenness that cause shock or shock of water, producing greater oxygenation²⁴. The optimal temperature for its aging ranges from 11 to 16 °C²⁵. It is a carnivorous animal in free life, it feeds on live prey such as: fly larvae, molluscs and aquatic insects, its requirements are high, in captivity all its nutritional needs must be strictly met²⁶.

Trout farming, as in all animal farming, there is the presence of etiological agents that take advantage of, in one way or another the susceptibility of the individual, they are capable of causing diseases by altering the natural development of the animal, so some diseases could be named of bacterial, viral, parasitic and fungal type in the rearing of this type of salmonids²⁵. Among the bacterial diseases, furunculosis caused by *Aeromonas salmonicida* can be named, characterized by blisters on salmonid skin, loss of appetite and hemorrhages in the liver. Among other bacterial diseases we have yersiniosis or red mouth disease, *Aeromonas* septicemia, vibriosis among others²⁷.

As an example of a viral disease we have Infectious Pancreatic Necrosis caused by a birnavirus, characterized by a septic condition with high mortality in

fingerlings²⁸. As parasitic agents that affect trout, we have ectoparasites such as *Ichthyophthirius multifiliis*, which causes white spot or Ich, protozoa such as *Myxobolus cerebralis*, diplostomiasis caused by a stryroid metacercaria known as *Diplostomum spathaceum*²⁹. The fungal disease that most affects freshwater fish is caused by the genus *Saprolegnia*, whose etiological agent is an opportunistic fungus that colonizes the skin and gills of the host³⁰. The objective was to evaluate through the use of crude extract of garlic (*A. sativum*) as an alternative in the prevention of saprolegniosis in rainbow trout (*Oncorhynchus mykiss*) of the “El Porvenir” fish farming complex located in the Píllaro canton, province of Tungurahua.

Materials and methods

It was carried out in the “EL PORVENIR” fish farm complex located in the San Andrés parish of the Píllaro canton, Tungurahua Province. Latitude -1.17 longitude -78.53 altitude 3300 masl, with an average minimum temperature of 8 °C and a maximum of 16 °C, Relative Humidity, 88.57%, Coordinates X = 9870622, Y = 772342 (-1.1694472, -78.552773), IN-AMHI M0127³¹.

The specimens were acquired from Mr. Wilfrido Salas, both the sick and healthy animals were used for the negative control, they were placed in 30 x 40 cm plastic drawers that allowed the entry and exit of water, it served to separate the treatments and repetitions. throughout the research project, its daily management, at the time of performing the immersion baths, then each labeled treatment was placed in a single clean tank, 1.5 x 3 m of brick covered with cement, which were approximately 25 days without lodging rainbow trout (*O. mykiss*), previously disin-

fected, the fish selected for the experiment had an approximate weight of 120 to 200 g and a length of 10 to 15 cm, at the time of passing the trout to their ponds it was done through manual nets, avoiding stress and mistreatment as little as possible. 24 h after they were located in their new habitat, they were marked by the use of earrings, for easy registration and to individualize the animals, after these process 10 days were taken as setting, the water that was provided to them was from a natural spring. uncontaminated, at a temperature with an interval of 10-12 °C. Once the setting time had passed, the corresponding dose of the fresh garlic macerate was administered for each treatment that was carried out for six consecutive days, the evaluations and records per animal were made at a fixed time of 6:30 am, before feeding the fish, each animal was registered, with an artisanal type ladle-shaped mesh, which allowed access to the trout, to avoid stress on the specimens under study.

To obtain the fresh garlic maceration, it was crushed in a mortar with the help of a pestle, and it was immediately filtered to prevent the external components of the peel from remaining in the preparation. Once the pertinent dose for each treatment was obtained, it was I mix with 20 L of water contained in tubs, and then immerse the trout in the immersion bath with the help of a wooden shovel, which will distribute the preparation evenly covering the entire surface of the animals, the immersion was for 20 min for each treatment approximately, this procedure was done daily, with the doses T₁ 250, T₂ 500 and finally T₃ 750 mg/L, with two witnesses, who did not include the fresh garlic macerate, the effect of the inclusion on saprognelliasis was analyzed at 24, 48, 72, 96, and 120 h.

Calculation of dosage of fresh garlic macerate

T₁ 250 mg/L X= 0.25 g/L= 20 L X= 5 g/20 L X=5 mL/20/L
 T₂ 500 mg/L X= 0.50 g/L= 20 L X= 10 g/20 L X=10 mL/20/L
 T₃ 750 mg/L X= 0.75 g/L= 20 L X= 15 g/20 L X=15 mL/20/L

Study Factors. Application of the fresh macerated garlic at the following times: 24, 48, 72, 96 and 120 h, in the following doses: T₀ negative: 0 mg/L of fresh macerated, T₀ positive: 0 mg/L, T₁ 250 mg/L, T₂ 500 mg/L, T₃ 750 mg/L.

To characterize the clinical symptoms and lesions of Saprognelliasis in juvenile rainbow trout (*O. mykiss*), during the time of exposure to fresh garlic maceration per treatment. We worked with juvenile fish distributed in 10 per 1 m² pond, which were built of brick with cement plaster protected with a zinc cover, with controlled water inlets and constant flow, adding a total of 15 ponds, with a total of 150 specimens (10 to 15 cm in length with an average weight of 150 to 200 g), a positive control with diagnosed saprognelliasis, a negative control made up of completely healthy animals, and the last three ponds, with doses of fresh garlic maceration for six days.

The response variables, the clinical symptomatology was determined for all the fish, it was monitored every day since the animals were exposed to the fresh garlic maceration, each symptomatology was characterized independently, it was observed and recorded

in individual records, all types of signs from lethargic problems. The lesions were established in all the fish, each external lesion, hemorrhages at the base of the fins, cottony structures in the fins and gills, as well as spots on the body of the animals, were examined, the fish were taken using manual nets and the improvement or not when exposed to raw garlic maceration was recorded, in these two variables the percentage of mortality was recorded, the last day of the experiment, a necropsy was performed on each of the specimens to observe possible internal injuries such as hemorrhages.

It was analyzed through a descriptive statistics of frequency, in which the time that the fresh maceration of garlic (*A. sativum*) needed was assessed, in addition, the most effective concentration was evaluated, and finally, the analysis of Saprognelliasis by *Saprolegnia* spp., the pathology was detailed for each pond of all the animals, two controls were used, one positive and one negative for the validation of the research.

Results

Table 1 Presence of cotton balls

Treatment alternatives/ efficacy (mg)	Trout N=90	Frequency/presence			Percentage %	
		P	R	A		
T ₁ 250	30	11	11	0	22	24
T ₂ 500	30	13	4	18	35	39
T ₃ 750	30	15	4	14	33	37
Total	90	39	19	32	90	100

Table 2 Skin depigmentation

Treatment alternatives/ efficacy (mg)	Trout N=90	Frequency/presence			Percentage %	
		P	R	A		
T ₁ 250	30	30	29	0	59	33.5
T ₂ 500	30	30	17	12	59	33.5
T ₃ 750	30	30	10	18	58	32.9
Total	90	90	56	30	176	100

Table 3 Skin erosion

Treatment alternatives/ efficacy (mg)	Trout N=90	Frequency/presence			Percentage %	
		P	R	A		
T ₁ 250	30	30	29	0	59	33.5
T ₂ 500	30	30	29	0	59	33.5
T ₃ 750	30	30	23	5	58	32.9
Total	90	90	81	5	176	100

Table 4 Congestion

Treatment alternatives/ efficacy (mg)	Trout N=90	Frequency/presence			Percentage %	
		P	R	A		
T ₁ 250	30	30	29	0	59	33.5
T ₂ 500	30	30	20	9	59	33.5
T ₃ 750	30	30	0	28	58	32.9
Total	90	90	49	37	176	100

Table 5 White-yellowish pigmentation

Treatment alternatives/ efficacy (mg)	Trout N=90	Frequency/presence			Percentage %	
		P	R	A		
T ₁ 250	30	17	16	0	33	32.3
T ₂ 500	30	18	17	0	35	34.3
T ₃ 750	30	18	16	0	34	33.3
Total	90	53	49	0	102	100

Table 6 Scale loss

Treatment alternatives/ efficacy (mg)	Trout N=90	Frequency/presence			Percentage %	
		P	R	A		
T ₁ 250	30	30	29	0	59	33.5
T ₂ 500	30	30	29	0	59	33.5
T ₃ 750	30	30	28	0	58	32.9
Total	90	90	86	0	176	100

Table 7 Ulcers

Treatment alternatives/ efficacy (mg)	Truchas N=90	Frequency/presence			Percentage %	
		P	R	A		
T ₁ 250	30	16	15	0	31	32.2
T ₂ 500	30	17	16	0	33	34.3
T ₃ 750	30	17	15	0	32	33.3
Total	90	50	46	0	96	100

Table 8 Necrosis

Treatment alternatives/ efficacy (mg)	Truchas N=90	Frequency/presence			Percentage %	
		P	R	A		
T ₁ 250	30	7	6	0	13	23
T ₂ 500	30	12	11	0	23	41
T ₃ 750	30	11	9	0	20	36
Total	90	30	26	0	56	100

Discussion

The research carried out by Ñahuincopa Vergara³² used the Probit LD₅₀ (Median Lethal Dose) method, obtaining as a result the concentration of garlic extract at 70.36 % at 24 h and 68.34 % at 48 h of inhibitory effect, based on the results obtained. mentions that *Saprolegnia* sp. is sensitive to the treatment applied with garlic extract³². On the other hand, Armuelles Bernal et al.³³ evaluated the effect of adding garlic powder at 2 and 4 % to the diet of Jack mackerel (*Seriola lalandi*) as a preventive treatment against infestations of *Zeuxapta seriolae*, a total of 180 organisms. Distributed in three experimental groups, one control and 2 treatments with 3 repetitions for each one, the diet supplemented with garlic powder was administered for 32 days, then the jack mackerel culture was infested with the parasite *Z. seriolae*, negative bacteria, making it an antibiotic with effective action when used in doses of 50 mg per day during treatment. three consecutive days.

Similarly, Prieto et al.³⁴, states that the antibacterial effect of *A. sativum* is similar to penicillin, acting specifically against Gram-negative bacteria, making it an antibiotic with effective action when used in doses of 50 mg per day during treatment. three consecutive days. Villamar Ochoa³⁵, points out that the use of garlic and lemon as a substitute for antibiotics and disinfectants in shrimp production has given good results in the treatment of diseases, pointing out that thanks to the action of these two natural products, the presence of microorganisms is significantly reduced. pathogens, the defenses of the shrimp are stimulated, reducing the appearance of diseases and therefore the mortality of the crustaceans.

Barriga González & Clavijo Rojas³⁶, when evaluating malachite green and methylene blue versus garlic and tobacco extract on the control and eradication of the ornamental tiger tigrato, obtained better results with malachite green and methylene blue as chemical treatment at a dose of 7 drops. per 40 L of water, eliminating the protozoan 5 days after treatment. The study carried out by Juárez-Segovia et al.³⁷ has shown that the garlic extract obtained by maceration in a phosphate-buffered saline solution has antifungal effectiveness against fungi of the *Aspergillus* genus, inhibiting their growth.

Silva Blanco³⁸, states that garlic eliminates 100 % of the *Trichodina* ssp. ectoparasite, applied as a bath with a concentration of 800 ppm to tilapias in two days, in terms of weight gain and feed conversion, no significant differences were found between treatments. However, it states that the ration including the garlic extract is well tolerated by the fish and also acts by strengthening the immune system, protecting them from the different pathogens and thus increasing the fish survival. Agurto Rodríguez & Rivera Intriago³⁹ in their study carried out on shrimp evaluated the antibacterial, antioxidant and immunostimulant properties of extracts of garlic, oregano, cranberry green tea, Astragalus and propolis, the antibacterial effect was evaluated by using 5 pathogenic bacteria of which 2 are of importance in aquaculture, 2 are enteric bacteria that affect consumers of contaminated aquatic animals and 1 opportunistic bacteria in humans. García Gómez & Sánchez-Muniz⁴⁰, conducted tests based on plant extracts used as antibacterials in fish, for which garlic in saline solution in-

hibited the growth of *Aeromonas hydrophila* at a concentration of 6.25 mg/mL⁻¹ and *Photobacterium damsela* at a concentration of 6.3 mg/mL⁻¹.

It was observed that the group of trout from the positive control previously sick with *Saprolegnia* sp., increased macroscopic lesions over time and some of the trout died, on the other hand the negative control that at the beginning of the experiment the specimens did not show associated lesions to the fungus showed lesions on the 4th day of observation, on the contrary, the trout subjected to the treatment based on fresh garlic maceration improved their appearance and from the 2nd day of application, a decrease in the lesions caused by the fungus was noted, that is why It is concluded that fresh garlic maceration serves as a natural antifungal and controls saprolegniosis in rainbow trout.

The macroscopic lesions that were identified in the rainbow trout used in the experiment were the following: (loss of scales, skin depigmentation, erosion, congestion, yellowish-white pigmentation, presence of fungal structures with the appearance of cotton specks, ulcers and necrosis). Other symptoms that were identified were: (lethargy, imbalance, exhaustion and loss of reflection, even causing the death of the trout).

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Conflicts of interest

The manuscript was prepared and reviewed with the participation of the author, who declares that there is

no conflict of interest that jeopardizes the validity of the results presented.

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Ethical considerations

The research complied with the ethical standards of the information process.

Authors' contribution to the article

Núñez-Torres Oscar Patricio, contributed with the structure and writing of the article. *Paredes-Sandoval Johana Cristina*, contributed with the field work. *Artieda -Rojas Jorge Rodrigo*, contributed with the tabulation of results and *Muñoz- Espinoza Manolo Sebastián*, contributed with data tabulation.

Research limitations

There was no limitation for the development of the research.

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