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Bee propolis (*Apis mellifera*) as a Growth Promoter in Tilapia (*Oreochromis niloticus*)

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ABSTRACT

A study was conducted to determine the effect of ethanolic extracts of propolis collected by bees from Tapachula, Chiapas on the development of 225 juvenile tilapia (Oreochromis niloticus), fed in commercial starter feed in one of five daily rations with different concentrations: T0 (0 g Kg^{-1}), T1 (10 g Kg^{-1}), T2 (15 g Kg^{-1}), distributed for 4 weeks in 9 containers of 60 l each. Tilapia growth was evaluated through morphometric data such as weight (g), total length (cm), and standard length (cm) for each week in order to identify changes. Physicochemical parameters were measured in the water, such as temperature (°C), salinity (ppm), electrical conductivity (uS/m), total dissolved solids (ppm), pH, and total ammonium (mg/L). Significant differences ($p<0.05$) were found between treatments in the different weeks evaluated, and it was T1 that showed the greatest positive effect on fish development. The physicochemical parameters that explain 72% of the behavior of the treatments were salinity, total dissolved solids, and electrical conductivity. It is concluded that ethanolic extracts of bee propolis (Apis mellifera) from the lower zone of Tapachula, administered in feed, can promote the development of juvenile tilapia (Oreochromis niloticus) under experimental conditions.

Keywords: *Plant resins, Phenolic compounds, Additives, Aquaculture production*

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INTRODUCTION

Aquaculture activity has positioned itself as one of the most important activities worldwide, since it represents approximately 50% of the fish destined for food and is considered an excellent source of proteins, fatty acids, vitamins, minerals, and essential micronutrients. Current aquaculture production (approximately 80%) is derived from animals lower in the food chain, such as omnivorous fish, herbivores, and mollusks (FAO, 2017).

The cultivation of tilapia "mojarra" (*Oreochromis niloticus*) is one of the most successful in different countries of the world, due to its adaptability to a wide range of environmental conditions and the ability to grow and reproduce in captive conditions, besides feeding on lower trophic levels (El-Sayed & El-Sayed, 2017; Thazha *et al*., 2023).

Tilapia is the second group of farmed fish with the greatest economic importance for developed and developing countries. Due to its food habits, tilapia tolerates foods with a higher percentage of source protein from vegetables such as soy (FAO, 2009; Makhoahle & Gaseitsiwe, 2022).

Propolis is a viscous waxy resin collected from plants by bees as a combination of plant exudates, wax, pollen, and enzymes present in bees' saliva; this is also a source of antiseptics, antimicrobials, antiparasitic, bacteriostatic, anti-inflammatory, and antioxidant properties. The use of propolis has been documented since 300 BC for its pharmacological properties in pathological entities of humans and animals. In animal production, this could be used as a dietary supplement by increasing the digestibility of structural and soluble carbohydrates, stimulating the immune system, and increasing growth rates and microbiota in the digestive system (De la Cruz *et al*., 2018; Dhanasekar *et al*., 2022).

Propolis composition varies depending on the vegetation, geographic and ecological region, time, and method of collection. Likewise, its botanical origin and the composition of its chemical structure are different for each physiographic region; therefore, the importance of evaluating and using them by this condition.

Flavonoids are the main components of propolis, and according to the chemical structure, flavones, flavonoids, chalcones, dihydrochalcones, isoflavones, isodihydroflavones, and neoflavonoids are identified, and could contribute to their nutritional properties (Huang *et al*., 2014; Graefen *et al*., 2023; Watanabe *et al*., 2024). Therefore, products such as propolis are added to rations in order to promote growth and development in several fish species (Buck *et al*., 2017; Patil, 2022; Wilhelmy *et al*., 2022).

The great demand for tilapia mojarra meat (*O. niloticus*) is being intensified, implying a greater efficiency and a lower cost. Nonetheless, the muscle mass that needs to be increased requires a greater amount of protein but also promoters that increase performance and reduce the adverse effects of intensive production on the animals, besides antibiotics, ionophores, probiotic microorganisms, and other growth promoters such as steroid hormones, with the consequent adverse effects of residuality. Recent studies showed that propolis administration in diets present a positive effect on the number of muscle fibers in the initial stage of development (juveniles) caused by the increase in the expression of genes for myostatin, although later this effect decreases and only muscle hypertrophy is observed (Buck *et al*., 2017; Kulkarni *et al*., 2023; Pavlova, 2024). Therefore, this study aimed to evaluate the effect of propolis ethanolic extracts added to the diet on tilapia mojarra (*O. niloticus*) growth.

MATERIALS AND METHODS

Study site

This study was carried out during a period of 28 days, inside Santa Rosa ranch, located in the second section of "tinajas" (14°48'22" N and 92°20´23" W), in Tapachula, Chiapas, Mexico. We used nine plastic containers (60 L) with an aeration system and a culture density of 1 fry per 2.4 liters of water, doubling the surface of agreement to Alcantar *et al.* (2014).

Each one of the ponds was marked randomly for each of the repetitions and treatments (T0, T1, and T2). The fish were kept for 1 week in the acclimatization phase and provided commercial food without propolis, before we started the experiment. 25 tilapia (*O. niloticus*) fingerlings weighing an average initial: 4.7g ± 0.7 per experimental unit and their corresponding repetitions, totalizing 225 hatchlings of mojarra tilapia.

Propolis and treatment preparation

The preparation of propolis ethanolic extracts was based on the technique described by NOM-003-SAG/GAN-2017 (NOM-003, 2017); 100 g of raw propolis was placed in a glass container and 300 ml of 100% pure undenatured cane alcohol in a proportion of 1:3. This solution was kept without light for twenty days.

Three treatments were evaluated (with three repetitions): T0 (0% of inclusion of propolis Kg^{-1}), T1 (10 g of inclusion of propolis Kg^{-1} of food), and T2 (15 g of propolis inclusion $Kg^{-1}$of food) according to Kaplan and Erdogan (2021) and Dongmo *et al*. (2023). The addition of propolis was carried out by using an ethanolic extract and subsequent mixing in the sprinkling diet; for this, a large aluminum tray was used to be able to extend the food and to ensure that the extract was fully incorporated. Subsequently, the food was left exposed in a closed place so that the alcohol (HE) could evaporate.

Tilapia feeding (*Oreochromis niloticus*).

Tilapia were fed five times a day (7, 10, 13, 16, and 19 h), in a ratio of 10% of the biomass of each tank (2% for each meal; approximately 15 g daily). The calculation of the biomass of each tank was carried out based on Csirke (1980) on a weekly basis to adjust the amount of ration placed on the fish.

Physical-chemical analysis of water

The physical-chemical parameters of the water were taken daily (7, 12, and 17 hours): temperature, total ammonium, pH, total dissolved solids, and electrical conductivity.

To measure the physical parameters, a Generic brand potentiometer, model EZ-9909, was used, and a colorimetric kit was used to measure total ammonium. The water used for fish establishment was changed every 5 days, removing feces and sedimented food by siphoning with the help of a plastic hose.

Morphometric parameters

Tilapia were weighed and measured throughout the experiment period, each week, to observe changes in development. The estimated parameters were: Weight (P): weight each week (g); Total length (TL): measurement of the anterior portion of the head at the beginning of the caudal fin (cm); Standard length (SL): measurement of the anterior portion of the head to the insertion of the caudal fin (cm).

Data analysis

The data obtained were subjected to a normality test and subsequent analysis of variance ($p<0.05$), a multivariate analysis, a Wilks' lambda discriminant analysis, and a Hotelling test with the Infostat MR statistical software. Likewise, a principal components analysis was used to identify the physicochemical variables as explanatory of the behavior of the response variables.

RESULTS AND DISCUSSION

It was found that there were significant differences between the weights due to the treatment effect and week ($p<0.05$), where T1 was the different one (Tukey <0.05). No evidence was found for the effect of the container or the other factors **(Figure 1)**.

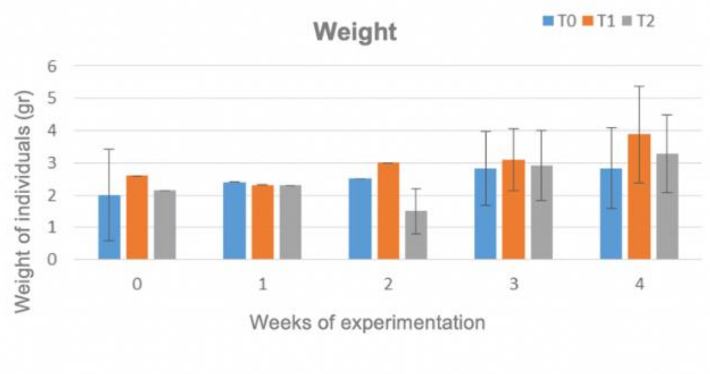

Figure 1. Total weight increment during the experiment weeks for 28 days. Mean ± standard deviation of the treatments evaluated with the total length variable.

In the fourth week of the experiment, T1 showed a significant increase in fish with regard to other weeks evaluated (p>0.05). The total length variable showed significant differences between the treatments evaluated (p<0.05) and due to the effect of the study week p<0.001), and for this variable also treatment 1 was the one with the greatest effect (Tukey < 0.05). In the following graph **(Figure 2)**, you can see the increase in length due to treatment 1.

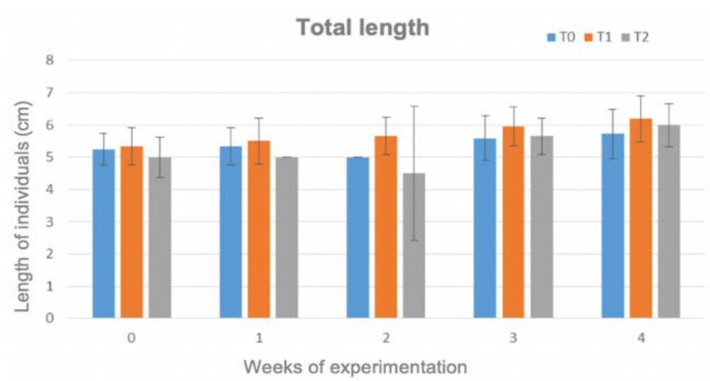

Figure 2. Total length increment during the experiment weeks for 28 days. Mean ± standard deviation of the treatments evaluated with the total length variable.

Standard length

Significant differences (p>0.05) were found between the treatments, where again T1 was the one with the greatest effect (Tukey <0.05). It was observed that individuals in treatment 1 had the greatest increase in standard length **(Figure 3)**.

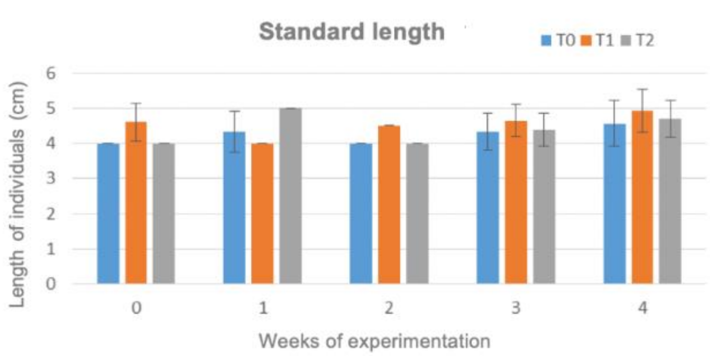

Figure 3. Standard length increment during the experiment weeks for 28 days. Mean ± standard deviation of the treatments evaluated.

The multivariate analysis allowed us to observe that the variables under study were influenced by the treatments, where weight is in first place, followed by the total length, and finally the standard length.

On the other hand, the discriminant analysis (Lambda of Wilks) indicates that the treatment ($p<0.05$) and week ($p<0.05$) are growth determinants. The Hotelling test alpha = 0.05 indicated that the most significant treatments showed were treatment two in the initial week (T2:0), treatment zero in week two (T0:2), treatment two in week one (T2:1), and treatment zero in the initial week (T0:0).

Physicochemical parameters

During the experiment on *O. niloticus* breeding, they tended to increase except for temperature, which showed changes that ranged between 25 to 27 ° C. The Hotelling test (=0.05) was carried out for the physicochemical parameters that at all moments (HE) were kept controlled, but that can be modified by external factors.

Using a principal components analysis to identify the main factors that explain the behavior of the treatments were the total of solids dissolved, salinity, and electric conductivity showed 74.2% of variation **(Figure 4)**.

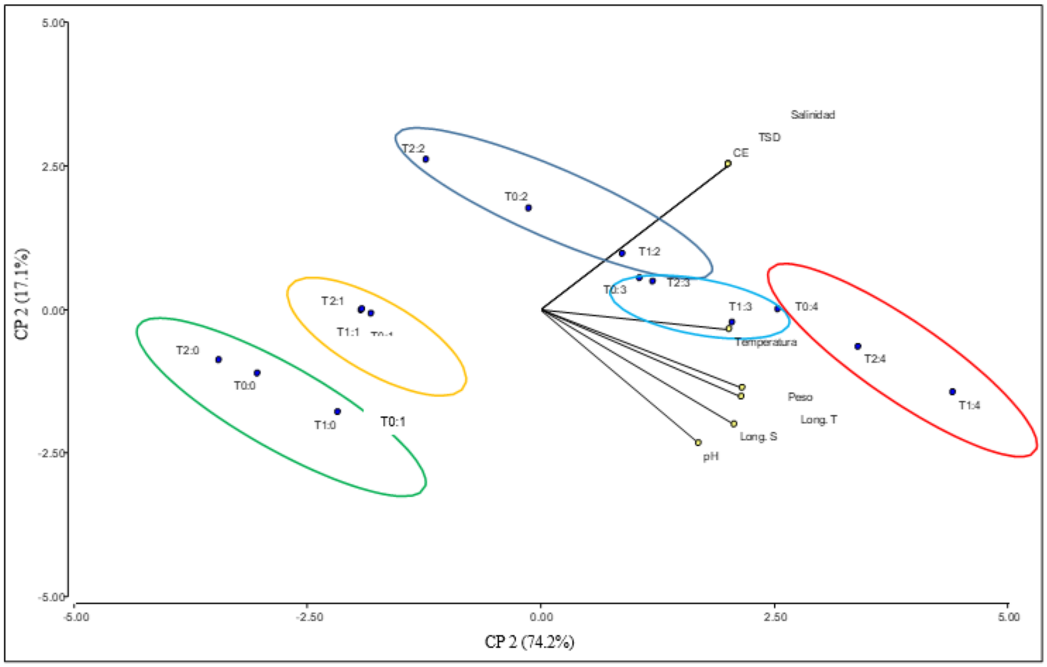

Figure 4. Physicochemical parameters as the main explanatory variables of the effect of the treatments on the variables of the answer.

In the present study, a significant difference could be observed between the three variables of growth of the specimens that were sampled in relation to the treatments and weeks evaluated. Treatment 1 (T1) showed an increase in the revenue of weight, length, total and the length standard in the fourth week; contrasting difference with regard to the treatments 0 (T0) and 2 (T2), where the effect of the propolis as growth promoter in other fish species have been evaluated. Kaplan and Erdoğan (2021) reported that the specific growth rate (SGR) and the revenue of weight (WG) varied quadratically with the concentration of propolis and this do not decrease growth rates except at higher levels, which could be the answer for T1 in our experiment, with the lowest concentration of propolis.

Our results are similar to Abbass *et al.* (2012), who found that propolis added to the diets significantly improved ($P<0.05$) the specific growth rate (SGR), the average daily gain (ADG), and the feed efficiency ratio (FER) on tilapia. Tukmechi *et al.* (2014), Fiodorova *et al.* (2022) and Ravoori *et al.* (2024) also tested the effect of different concentrations of bee propolis (*A. mellifera*) added to the diets supplied in a short-term period (0, 1, 2 and 5 g Kg^{-1}) and a long-term period (0.5, 1, 2 g Kg^{-1}) in rainbow trout (*O. mykiss*), obtaining a significant increase in the growth performance of the specimens. Although *O. mykiss* is a completely different species in taxonomic and physiological terms with respect to *O. niloticus,* there was a positive effect of propolis on the growth of rainbow specimens, which could be observed by the incorporation of propolis in the fish diet, regardless of the species.

In contrast, Uczay *et al.* (2011), Marian *et al.* (2024) and Fernandes *et al.* (2022) evaluated five concentration levels of propolis in the diets of common carp (*Cyprinus carpio*) for 45 days, and observed that there were no differences in any of the variables analyzed between the control treatment and the levels of propolis added to the diet. Therefore, it suggests that the addition of the propolis ethanolic extracts was not efficient as an additive for the growth of common carp. However, the concentrations of added propolis and the number of times the fish were fed could have influenced, since food was only supplied three times a day (9, 13, and 17 hours).

Therefore, De la Cruz *et al.* (2018), Dipalma *et al.* (2022) and Shaheen *et al.* (2023) suggested that the origin and composition of propolis an important factors that can also influence the growth effect of various species of fish or other farmed animals with commercial interest. Based on the botanical origin, we can find differences in propolis composition, such as flavonoids, essential oils, minerals, and other organic compounds such as ketones, lactones, quinones, steroids, and sugars.

The physicochemical parameters measured during the period of time that included the experiment were salinity, pH, temperature, total dissolved solids, electrical conductivity, and total ammonium. Through the analysis of principal components in relation to the growth variables, treatments, and weeks evaluated, the result was that the growth variables associated with the physicochemical parameters of temperature, electrical conductivity, total dissolved solids, and salinity had differences in the 3 treatments in the four weeks of evaluation. Kaneshima *et al*. (2022), Maneea *et al*. (2023) mention that temperature is an important factor in the development of tilapia (*O. niloticus*), since it slows down its metabolism, affecting its physiological processes, which means there is no optimal growth in the fish. The optimal range suggested by Bonilla *et al*. (2018) is 25 – 30 °C, which indicates a similarity with what was found in the present work since during the four weeks of evaluation the temperature varied between 26.47 and 26.81 °C, explained by the changes that occur during the day and night as a result of the presence and absence of the sun and which in commercial production are presented ordinarily.

The results of the administration of propolis extracts investigated by Buck *et al*. (2017), AlHussain *et al*. (2022) explained that they affect muscle development through the activation of genes such as myostatin. The concentration of chemical compounds in propolis, such as ketones, functional amino acids, polysaccharides, fatty acids and phenolic compounds, and flavonoids, has a positive effect on animal development. Understanding muscle growth and development processes, as well as how to include growth-promoting supplements, is important for the incorporation of knowledge and technology that is generated. Propolis has great potential in the food and medical industry due to its bioactivity, which varies depending on where the resins or plant exudates are obtained. For this reason, propolis from different geographical areas and plant species may present different biological activity.

Our results agree with Buck *et al*. (2017), Pisano *et al*. (2023), Malcangi *et al*. (2023), who observed that the effect of bee propolis on fish growth can also be influenced by factors such as temperature, dissolved oxygen, pH, water flow, the presence of microorganisms and parasites themselves that are difficult to control. Salinity was one of the parameters that had the greatest influence on the treatments in weeks 3 and 4; it remained between a range of 153.75 and 208.98 mg/L during the 4 weeks evaluated. The salinity levels in the water were adequate during the four weeks of treatment based on what was reported by Küçük *et al*. (2013), Bulusu *et al*. (2023) and Bolay *et al*. (2024) who mentions that the optimal condition for the cultivation of blue tilapia (*O. aureus*), with respect to the growth rate and metabolic parameters, is at salinities lower than 12 ppt (1200 ppm). Therefore, further research is necessary in commercial pond conditions and with smaller quantities that can be applied, such as nanoparticles of less than 100 nm that are currently under investigation.

CONCLUSION

Propolis ethanolic extracts (*Apis mellifera*) collected in the lower area of the municipality of Tapachula, Chiapas (14°48'22" N and 92°20'23" W) promote the development of tilapia (*Oreochromis niloticus*) in the juvenile stage, administered in starter rations in doses of 10 g kg^{-1} of food. Physicochemical parameters such as temperature, salinity, pH, ammonium, and dissolved oxygen have variations that influence the effect of the administration of ethanolic extracts of propolis, so these values should be considered in future work, even under controlled conditions.

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CONFLICT OF INTEREST: None

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ETHICS STATEMENT: All studies were ethically performed along the experiment.

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