PROBIOTIC-INFUSED *WITHANIA SOMNIFERA'S* EFFECTIVENESS AS A FEED INGREDIENT FOR SHRIMP DEVELOPMENT

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Abstract:

Probiotics are helpful microorganisms that help keep your body healthy, functioning correctly, and promoting growth. It is the foremost thing used to enhance the efficiency of the product for human beings, and this shrimp is one of the most popular among humans. Shrimp farming is an aquaculture enterprise that grows marine shrimp or prawns for human consumption. It is currently regarded as a critical economic and food production industry since it is an increasingly essential source of protein for human consumption. So, with the help of probiotics, we planned to estimate the growth of shrimp with the formulation of fish feed. The result shows that the feed with probiotics stimulated high growth in the shrimp.

Keywords: *Withania somnifera; Lactobacilli* sp.; *Bacillus* sp.; Probiotics; Shrimp;

Introduction

Numerous nutrients are necessary for the healthy growth of shrimp. Because the nutritional needs of shrimp alter at each step of their life cycle, nutrients for shrimp are a complicated topic (i.e., larval, nursery, juvenile, and adult). As a result, different phases of shrimp development require different types of nutrition. Compared to shrimp in other life stages, juvenile shrimp have higher

dietary needs, particularly in terms of protein. There are two elements that affect the quality of shrimp feed: ingredients and nutrients (1)

Shrimps have particular dietary needs, including those for protein, lipids, ash, and fibre. The most expensive nutrient source is protein, which comes in two varieties (crude protein and amino acid). While proteins typically contain 22 amino acids, shrimps only need ten essential amino acids (EAA), in a precise ratio, to grow to their maximum potential. Due to a paucity of knowledge regarding the quantity of total amino acids needed for juvenile shrimp to grow to their full potential, very few research have considered the importance of amino acids. While preserving optimum EAA ratios, The presence of crude protein will promote growth. Probiotics are used in pure or mixed form as a live food supplement in aquaculture to improve water quality and boost immune responses. Numerous species, including as Gram-positive and Gram-negative bacteria, bacteriophages, yeasts, and unicellular algae, being examined for their potential to be used as probiotics in aquaculture. Probiotic bacteria can outcompete other microbial populations by producing chemicals including bacteriocins, hydrogen peroxide, siderophores, lysozymes, proteases, and many others that have bactericidal or bacteriostatic effects. (2).

Live bacteria are referred to as probiotics because they can help the host's health when given in sufficient doses. Candidate probiotic microorganisms known as lactic acid bacteria (LAB) are found in nature and can be exploited in the food sector. The cytochrome system is absent from LAB, which are a category of small, aerophilic or anaerobic Gram-positive bacteria that can produce antimicrobials for biopreservation but cannot make spores or catalase (3). Some foods, such as dairy products like yoghurt, are regarded as good providers of probiotics. A relatively new idea, the use of probiotics in aquaculture refers to live bacteria that, when given in sufficient quantities, boost the host's health.

Withania somnifera (WS) also a well-known medicinal herb used to cure infertility in many nations' traditional medicine. WS has been shown to enhance reproductive system performance in a variety of ways. Due to improved semen quality, which is thought to be caused by increased enzymatic activity in seminal plasma and a decline in oxidative stress, WS extract lowered infertility in male participants. Although some animal studies had found that WS had reversible spermicidal and infertilizing effects in male individuals, WS extract also improved luteinizing hormone and follicular stimulating hormone balance, resulting to folliculo genesis and increased gonadal weight (4). The goal of this research is to develop a herbal-based probiotic formulation as a substitute for antibiotics in shrimp aquaculture.

Materials and Methods

Isolation of lactobacilli strains

Shrimp PROGUT sample commercially collected from aquaculture unit, Chennai. The probiotic present in the sample were *Lactobacilli sp*, yeast and *Bacillus sp*

Biochemical analysis

The major biochemical parameters were analyzed to the *Lactobacilli sp,* yeast and *Bacillus sp.* by using the standard protocol (5).

Plant materials:

W. somnifera roots were purchased from a reputed vendor of herbal material in big bazaar street, Tiruchirappalli. The purchased *W. somnifera* roots were cut in to small pieces and gently crushed. These roots were air dried thoroughly under shade at room temperature for 2 weeks.

Preparation of the plant extracts:

Boiling method A total of 5 g of powdered sample was taken and mixed with 50 ml distilled water in a round bottom flask and boiled for ½ hour separately. The residue was removed by filtration through Whatmann No:1 filter paper andthe aqueous extract was concentrated (6).

Refluxing method:

The air dried roots were finely powdered using an electric grinder. For conventional extraction (refluxing), 5 g of powdered plant material was mixed with 50 ml of acetone in a round bottom flask and refluxed for about 5 h at 60 °C. Liquid extracts obtained were separated from the solid residue by vacuum filtration, concentrated using a rotary evaporator (7).

Preparation of WS medium and growth of probiotic

100 ml of 5% *W.somnifera* aqueous extract was taken by ultrasonication at 380 Hz and filtered. Agar was added and plates were prepared. After solidification all the probiotics were inoculated and incubated. The growth of isolate was recorded after 48h (8).

WS fermentation

100 ml of 5% *W. somnifera* aqueous extract was taken by ultrasonication at 380 v and filtered and autoclaved. The probiotics were inoculated and kept under shaking at150rpm for 24 h and fermentation further extend to 48 h under static condition. After 48 h fermented medium is centrifuged and filtered. Cell free filtrate extracted with ethyl acetate (1:2) followed by overnight incubation under refrigeration and the solvent phase was collected, air dried and redissolved. The culture filtrate and solvent phase used to check bioactivity (9).

Antimicrobial activity assay

Antibacterial activity was determined against *Vibrio sp* on Mueller Hinton agar. Agar plates was prepared and were spread with test pathogen. 100µL of WS root extract, WS medium culture filtrate and solvent extract were used for agar well diffusion. the plates were kept under incubation for 24 h. the zone of inhibition was recorded (10).

Feed formulation

The following ingredients were mixed in a septic container and feed were prepared using sterile syringe. The concentration of sugar estimated by DNS method and protein estimated by lowry`s method (11).

Shrimp Feed Formulation

Table 1: Feed Formulation

S.No	Components	Percentage
1	*Withania somnifera*	10%
2	Egg albumin	3%
3	Wheat flour	5%
4	Corn flour	10%
5	Soya meal	20%
6	Bone meal	10%
7	Puffed rice	20%
8	Probiotic	3%

9	Yeast granules	2%
10	Fish oil	2%

Feeding test

Effect of prepared feed tested on *L. vannamei* started at an experimental with n=6. For two groups of contain 6 numbers with average mean weight of 18.6 g were introduced into fish tank. On test prepared feed 3 g per day provided on test tank three times (1g/time). Control treated with commercial feed at same quantity of feed (table 1). Weight of shrimp taken before and after 20 days treatment (12).

Growth rates and feed efficiency:

Percent Weight Gain (PWG) = Wf-Wi / Wi X 100 Specific Growth Rate (SGR) = LnWf-LnWi/t X 100

Feed Conversion Ratio (FCR) = Dry feed intake (g)/Fish live weight gain (g)Wi and Wf are initial and final weights (g) and t is time of experiment (days) (13).

Results and Discussion

Root of *Withania somnifera* (Figure 1) sample was collected and phytochemical present in root were extracted by solvent and water. The presence of phytochemical was qualitatively determined and result are given in (Table 2).data shows absence of flavonoids and saponins. The ethanol-based extract shows presence of flavonoids, terpenoids, tannins, steroids, phenols and alkaloids. Presence of alkaloid was observed only in acetone extraction similar reports were obtained by (14, 15). Lab Commercially available Shrimp PROGUT (Figure 2) obtained from aquaculture and its growth on phytochemical based Newly designed WS medium was formulated and growth of probiotics were tested (figure 3). The experiment work is the first attempt on replication of WS as a nutrient source among prebiotic cultivation. So far studies are focus on antibacterial and anticancer on *W. somnifera*. The results of growth of probiotic culture on WS medium was found to be significant. The OD at 600 nm was 0.58 compared tocontrol MRS broth it was recorded ad 0.42 OD units. The tested probiotic showed growth on luxuriant growth on WS medium and moderate on MRS medium. Further The antibacterial activity of extracted compound and cell free culture filtrate (figure4) against *V. parahaemolyticus* found that the WS fermented medium and its solvent extract gave inhibitory zone around 14 and 17 mm. no activity was found on MRS broth and WS extract alone. The probiotic inoculated WS medium increased the antibiotic production potential while permitting the growth of probiotic (16, 17).

Table 2. Phytochemical test – solvent extraction

S.No	TEST	RESULT
1	Flavonoids	Negative
2	Keller killani	Positive
3	Salkowshia	Positive
4	Terpenoids	Positive
5	Tannins	Positive
6	Carbohydrate	Positive
7	Phenols	Positive
8	Saponins	Negative

9	Alkaloids	Positive
10	Quinones	Positive
11	Phlobatannins	Positive

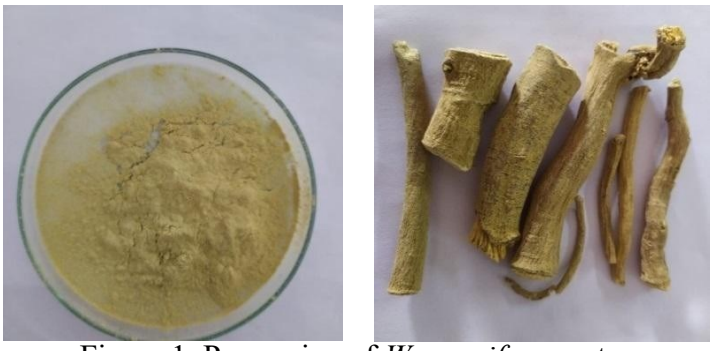


Figure 1. Processing of *W.somnifera* root

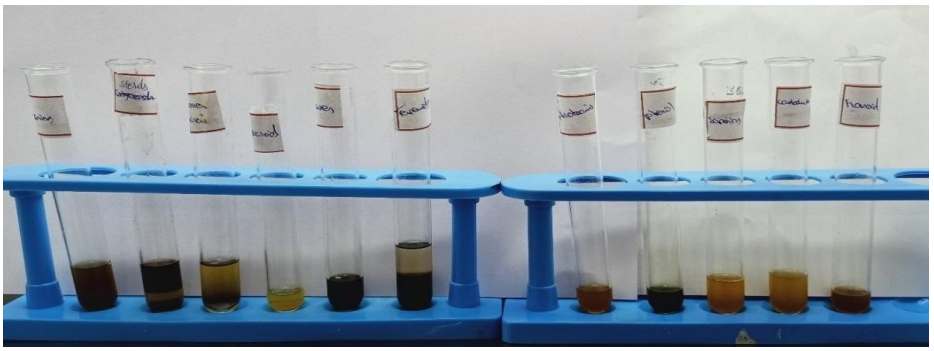
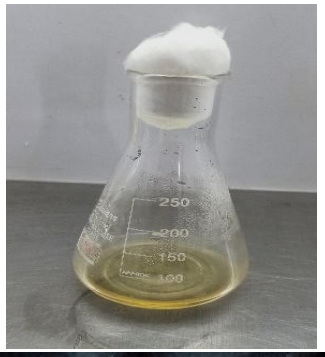


Figure 2. Qualitative phytochemical study

Figure 3. Growth of commercial probiotic on WS extracted medium

Figure 4. Antibacterial activity of fermented WS medium



Feed formulation and efficiency

Solid feed incorporated with *W. somnifera* root were prepared and tested on shrimp (figure 5). the concentration of sugar and protein content was estimated and estimated as 260 mg/g and total protein 870 mg/g comparatively lesser than commercial feed (table 3). The floating test of formulation was found to be shrinkable which is most suitable for shrimp farm (18). At the end of 20^{th} days, the treatment group showed increases in body weight from 18.6 g to 36 g ± 0.02 (93% weight gain) (table 4). Body weight gain doubled within 20 days and the protein content of shrimp was 86 mg/g. while on control weight gain was moderate reaches maximum of 22.8 ± 0.2 g has low weight gain and the average protein content was 56mg/g. Feed Conversion Ratio was 1.5 in test and 2.7 in control it was reported Symbiotic preparations with phytochemical more significantly enhanced probiotic viability and nutritional type earlier reported by many studies (19).

Table 3: concentration of sugar and protein of formulated feed

Parameter	Feed	Commercial
Total protein	870 mg/g	12 g/g
Reducing sugar	260 mg/g	300 g/g

Table 4: Feeding efficiency on Formulation

	Control	Test
Final weight	22.8 g	36 g
Initial weight	18.6 g	18.6 g
Weight Gain (%)	22	93
Feed Conversion Ratio	2.7	1.5

Figure 5. Feed formulation and its efficiency testing on shrimp

Conclusion

The feasibility of using *Withania somnifera* as herbal nutrients to promote freshwater prawn growth and survival in conjunction with a probiotic on post-larvae (PL) was investigated, followed by its antibacterial effect against *V. parahaemolyticus*. Feeds were formulated, replacing fishmeal with *W. somnifera* along with soya bean meal. Cod liver oil was added as a lipid source. Protein and sugar levels in WS feed are lower than in standard commercial feed. Diets containing *W. somnifera* were served separately as experimental feeds, while diets lacking herbal inclusion were served as controls. These feeds were fed to PL in a triplicate feeding trial conducted for a period of 20 days under laboratory conditions. Weight gain performance was found to be significant, increasing by 93 percent in excess and 22 percent in control. When PL fed WS herbal-incorporated feeds were compared to the control, changes in total protein content were also observed. The finding concludes that PL fed with *W. somnifera* and probiotic-incorporated feed showed better growth performance.

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