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## ***Cynodon dactylon* as potential anti-white spot syndrome virus agent in cultured shrimp *Penaeus vannamei***

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### **Abstract**

White spot syndrome virus (WSSV) has been listed as the most contagious pathogen causing severe mortality in farmed shrimp and substantial economic losses. A bioassay experiment was conducted to determine *Cynodon dactylon* extract's efficacy as an anti-WSSV treatment at the laboratory scale and cage level. A pond trial was performed to optimize and investigate the effects of *C. dactylon*-enriched feed. The trial's first step was performed using feed coated with 2% and 3% of ethanol-extracted *C. dactylon*. The shrimp were challenged with the virus on day 15 of feeding with the treatment feed and monitored. A total of 100% survival of shrimp was obtained in both treatment groups. The (Rajeev anti-viral) RAV-s C group had the best protection (100% survival) of three groups treated with crude extracts, including RAV-s A (10%), RAV-s B (15%) and RAV-s C (20%) mixed feed. Similar results were obtained in the cage level trial with crude extracts, where the RAV-s C group had the highest survival (100%) followed by RAV-s A (68%) and RAV-s B (85.05%). Pond trials were conducted in commercial ponds. A total of 20 units of shrimp ponds of the same size (5,000 m<sup>2</sup>) and location were selected for the trial. The treatment ponds' productivity was higher (12.7 ton/ha) than that of the control ponds (11.79 ton/ha). WSSV cases were not recorded in the control and treatment ponds. This study showed that *C. dactylon* extract has anti-WSSV properties and has no negative impact on shrimp growth.

**Keywords:** WSSV, *Cynodon dactylon*, plant extract, shrimp culture

### **Introduction**

White spot syndrome virus (WSSV) is the most severe pathogen of cultured shrimp in many countries and has been reported to cause substantial economic losses in the shrimp culture industry worldwide <sup>[1, 2, 3]</sup>. This viral disease's transmission modes mainly include consuming moribund or dead infected shrimp or contact with contaminated water <sup>[1]</sup>. The high mortality of the outbreaks of WSSV can reach 100% in 4-7 days in the laboratory and 10-14 days in the ponds <sup>[3]</sup>. The presence of circular white spots on the shrimp cephalothorax's inner side is generally considered the principal clinical sign of this disease. Other characteristics include a rapid reduction in food consumption and lethargy, and the body colour becomes pale or reddish <sup>[2, 4]</sup>.

Several strategies have been continuously applied to control the spread of this disease and reduce WSSV infection. Jha *et al.* (2006) <sup>[5]</sup> developed an envelope protein recombinant vaccine effective against WSSV in *Procambarus clarki*. Another promising disease control method includes strengthening the shrimp defence mechanisms by administering an immunostimulant <sup>[1, 6]</sup>. Various studies have been carried out to enhance the performance of natural components with antiviral properties. Jha *et al.* (2016) <sup>[3]</sup> successfully demonstrated that a formulation of an essential oil blend extracted from ten plants could use as a feed additive effective against WSSV. Some herbs are used as therapeutic agents to treat infectious diseases, including an extract from *Sargassum polycystum* that reduces the impact of WSSV in *P. monodon* <sup>[7]</sup>, the fruit of *Duriozi bethinus* <sup>[8]</sup> and the root bark of *Psidium guajava* <sup>[9]</sup>.

*Cynodon dactylon* is widely available all over the world. This herbal plant is known for its ability to treat hysteria, epilepsy and insanity. Traditional healers use *C. dactylon* to purify the blood and cure anuria, biliousness, conjunctivitis, diarrhoea and gonorrhoea <sup>[6]</sup>. Aqueous and ethanolic extracts of *Cynodon dactylon* have antiviral activity against WSSV in *Penaeus monodon* <sup>[1]</sup>. A series of trials were conducted in the present study to determine the efficacy of shrimp feed supplemented with *C. dactylon* ethanolic extract against white spot syndrome

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virus (WSSV) in *Penaeus vannamei*. The effectiveness and impact of *C. dactylon* on shrimp were assessed in the field in cages and commercial ponds.

## Materials and Methods

### Study area

The study was conducted in the Disease Research Centre, Bandar Lampung of P.T. Central Pertiwi Bahari. The bioassay and cage trials were conducted in a biosecure facility, whereas the pond trial was performed in a commercial level research farm.

### Preparation of anti-WSSV formulation from *Cynodon dactylon*

The *Cynodon dactylon* formula was prepared using two methods: the ethanolic extraction method and crude powdered extract. The ethanolic extract was top-dressed on the feed and used initially in the laboratory trials. The crude powdered extract was prepared by grinding *C. dactylon* dried in the shade and mixing in the feed as raw material during production.

### Preparation of *Cynodon dactylon* ethanolic extract

The modified method of Balasubramanian *et al.* 2008<sup>[1]</sup>, was used to extract *C. dactylon*. The *C. dactylon* (whole plant) was identified and collected from the fields of P.T. Central Pertiwi Bahari, Lampung. The clean and fresh specimens were dried in the shade and powdered using an electrical blender. The powdered plant was mixed with fresh water at a 1:10 ratio and heated at 60 °C for 20-30 minutes. A crude yellow aqueous extract was obtained by filtration of the residue, and the filtrate was diluted with absolute ethanol at a ratio of 1:4. An abundant gel-like precipitate was formed immediately after the addition of ethanol. The supernatant of the ethanol extract was recovered by filtration, and the gel pellet was discarded. The supernatant solution was subjected to distillation at 60 °C for 2-3 days to obtain a plant extract. The obtained plant extract was air-dried. The dried product was sealed in a glass container and stored in a refrigerator at 4 °C until use in the tests.

### Preparation of *Cynodon dactylon* powder

*Cynodon dactylon* (leaf and shoot) was collected from the fields of P.T. Central Pertiwi Bahari, Lampung. The clean and fresh specimens were dried in the shade for 7-10 days. The dried grass was carefully screened to discard mould and other abnormalities. The dried material was finally ground and powdered using an electrical blender. The powder was sieved, weighed, packed in sealed bags and stored in a cool place for further use. *C. dactylon* powder was used to conduct the trials on the cage and commercial pond levels.

### Feed preparation using ethanolic extract of *C. dactylon*

The ethanolic extract of *C. dactylon* was diluted and sprayed on the feed at three different concentrations, including 10 g/kg of feed or 1%, 20 g/kg of feed or 2% and 30 g/kg of feed or 3%. The top-dressed feed was kept on ice and incubated for 30 minutes to allow the absorption of the extract. Recoating of the feed was performed using squid oil to prevent the dispersion of the extract in water. The control feed was only top-dressed with squid oil. The top-dressed feed was dried in the shade and stored in a sealed bag.

### Feed preparation using crude powdered extract of *C. dactylon*

The experimental feed was produced in a feed mill, P.T.

Central Proteina Prima, Surabaya. Three doses of the powdered extract, including 100 g/kg of feed or 10%, 150 g/kg of feed or 15% and 200 g/kg of feed or 20%, were mixed. The treatment feed was named RAVs A (10%), RAVs B (15%) and RAVs C (20%). The required concentration of the extract powder was mixed with other raw materials of the feed, and all components were mixed together in a feed mill. The feed was produced at lower temperature (80 °C) to avoid degeneration of the active ingredients of *C. dactylon*.

### GCMS detection of bioactive compounds of *C. dactylon*

Detection and quantification of bioactive compounds of *C. dactylon* were performed by gas chromatography-mass spectrometry analysis by the improved method of Jebastella *et al.* 2015,<sup>[10]</sup>. GCMS was performed at P.T. Charoen Phokphand Indonesia, Jakarta. The powdered samples of *Cynodon dactylon* (prepared as described above) were mixed with the solvents (MeOH, ACN and hexane) by adding 0.1 g dried sample per 2 mL of a solvent. The mixture was sonicated at room temperature for 30 mins and injected into GCMS. The mass spectra obtained by GC-MS were assigned by comparing the result with the databased on the reports of the literature.

### Determination of the dose of *C. dactylon*

#### WSSV preparation for *per os* challenge

WSSV artificially infected healthy shrimp, and the moribund shrimp were collected and confirmed for WSSV infection by PCR. Only muscle tissue was collected by removing head, shell and gut. The muscle tissue was cut into small pieces by scissors and finely minced using a sterilized manual blender. The obtained semisolid gel-like tissue was filtered through an 80µm filter and recovered. The minced muscle containing WSSV was homogenized and checked by RT-PCR determined the virus's presence and levels. WSSV-containing minced muscle tissue stored at -20 °C before use. The tissue was thawed gradually by moving to warmer temperature (from -20 °C to 4 °C and finally to 18-20-25 °C) at the time of challenge to avoid virus damage. The complete procedure took 4-5 hours.

### Bioassay trial

The laboratory-scale trial was set up using plastic tanks for the experimental and control groups. Shrimp were collected from the Marine Research Centre, Lampung, and each tank contained 20 animals with an average weight of 1 g. The plastic tank was cleaned thoroughly, sun-dried, and disinfected with 70% alcohol. Each tank was filled with chlorinated marine water obtained from C.P.B. hatchery and provided with the required dissolved oxygen (D.O.) supply. The water temperature was maintained at 25±1 °C using a thermostat. The water salinity was 20 ppt; pH was 7.6-8, dissolved oxygen was 5.1-6 ppm, and tank water was exchanged 20% daily. The tank was siphoning, or cleaning done once daily.

### Experimental groups

The bioassay trial in tank aquaria was set up in two sets. The first set of tests proved the concept using Balasubramanian *et al.*, 2008 and Jha *et al.* 2020,<sup>[1, 11]</sup> using ethanol extract. The ethanol extract was top-dressed on the feed at 2% (treatment 1) and 3% (treatment 2) and positive control and negative control. Each group had four replicates of tanks with 15 shrimp each. The shrimp were acclimatized for three days, fed

on treatment diets for two weeks before the WSSV challenge. There was no artificial feeding on the day of the WSSV

challenge. Feeding continued from the next day of the challenge (Table 1).

**Table 1:** Bioassay trial set up details using two treatment groups i.e., 1% and 2% ethanol coated feed. No *C. dactylon* extract coating on positive and negative control groups

Serial No	Group details	Treatment	Challenge
Treatment 1	Ethanol extract 2% top dressed on feed. 4 Replicates and each replicate 15 shrimp	Shrimp fed for 14 days before challenge Water exchange 20% daily. No water exchange on challenge day	Per os challenge using WSSV infected tissue 1% of mean body weight
Treatment 2	Ethanol extract 3% top dressed on feed. 4 Replicates and each replicate 15 shrimp	Shrimp fed for 14 days before challenge Water exchange 20% daily. No water exchange on challenge day	
Positive control	Regular feed 4 Replicates and each replicate 15 shrimp	Shrimp fed for 14 days before challenge Water exchange 20% daily. No water exchange on challenge day	
Negative control	Regular feed 4 Replicates and each replicate 15 shrimp	No Challenge Water exchange 20% daily. No water exchange on challenge day	No challenge

The powdered *C. dactylon* in three different concentration (10% as Treatment 1, 15% as Treatment 2, 20% as Treatment 3) was mixed in the feed during production in the feed mill. The shrimps of positive control and negative control groups were fed on the regular feed (without *C. dactylon* mixing).

Each group had four replicates of tanks with 15 shrimp each. The shrimp were acclimatized for three days, fed on treatment diets for two weeks before the challenge. There was no artificial feeding on the day of the WSSV challenge. Feeding continued from the next day of the challenge (Table 2).

**Table 2:** Bioassay trial set up details using three treatment groups i.e., 10%, 15% and 20% powdered form of *C. dactylon* in shrimp feed. No *C. dactylon* extract mixed in positive and negative control groups

Serial No	Group details	Treatment	Challenge
Treatment 1	<i>C. dactylon</i> powder 10% mixed in feed during production. 4 Replicates and each replicate 15 shrimp	Shrimp fed for 14 days before challenge Water exchange 20% daily. No water exchange on challenge day	Per os challenge using WSSV infected tissue 1% of mean body weight
Treatment 2	<i>C. dactylon</i> powder 15% mixed in feed during production. 4 Replicates and each replicate 15 shrimp	Shrimp fed for 14 days before challenge Water exchange 20% daily. No water exchange on challenge day	
Treatment 3	<i>C. dactylon</i> powder 20% mixed in feed during production. 4 Replicates and each replicate 15 shrimp	challenge day	
Positive control	Regular feed (no mixing of <i>C. dactylon</i> powder mixing) 4 Replicates and each replicate 15 shrimp	Shrimp fed for 14 days before challenge Water exchange 20% daily. No water exchange on challenge day	No challenge
Negative control	Regular feed (no mixing of <i>C. dactylon</i> powder mixing) 4 Replicates and each replicate 15 shrimp	No Challenge Water exchange 20% daily. No water exchange on challenge day	

### Challenge method

Treatment group of shrimp and positive control shrimp were challenged *per os* with 1% WSSV-infected muscle. Negative control shrimp were not challenged.

### Post challenge observation

The shrimp behaviour, shrimp daily feeding rate and shrimp cumulative mortality after WSSV challenge were monitored on daily basis. The shrimp waste and feed waste were cleaned by siphoning. The experimental animals were examined twice daily for WSSV gross signs. The number of deaths and rate of feeding were recorded every day after WSSV challenge. The dead and dying shrimp were removed from experimental tanks and confirmed for WSSV infection by PCR method.

### Cage trial

The shrimp were divided into five groups of 40 shrimp per cage, and each group was tested in three replicates. The shrimp were reared in the cages for a week to get acclimatized

with cage life and feed. The shrimp were fed for 14 days on treatment feed before WSSV challenge. The treatment groups and positive control were challenged using *per os* WSSV challenge duration of the trial was 45 days, including 10 days of acclimatization and 14 days of feeding; at day 15, shrimp were challenged with WSSV; feeding and observation were continued for 10 days starting from day 16. The details of the method are as follows.

### Cage and water preparation

Water for the experiment was prepared by the standard procedure of culture pond preparation. Three cages in each group (1 m<sup>3</sup> size and 4 mm mesh size) were set in 150 m<sup>2</sup> ponds. The water depth was maintained at 1 m.

### Experimental groups

Specific pathogen-free (SPF) shrimp groups were stocked with juvenile shrimp (average weight of 4 g) at 40 animals/m<sup>2</sup>. The treatment and control groups were located in

different ponds. The bioassay trial in cages were set up in the following ways. The powdered *C. dactylon* in three different concentration (10% as Treatment 1, 15% as Treatment 2, 20% as Treatment 3) was mixed in the feed during production in the feed mill. The shrimps of positive control and negative control groups were fed on the regular feed (without *C.*

*dactylon* mixing). Each group had four replicates of tanks with 15 shrimp each. The shrimp were acclimatized for three days, fed on treatment diets for two weeks before the challenge. There was no artificial feeding on the day of the WSSV challenge. Feeding continued from the next day of the challenge (Table 3).

**Table 3:** Bioassay trial set up in cages with details using three treatment groups i.e., 10%, 15% and 20% powdered form of *C. dactylon* in shrimp feed. No *C. dactylon* extract mixed in positive and negative control groups

Serial No	Group details	Treatment	Challenge
Treatment 1	<i>C. dactylon</i> powder 10% mixed in feed during production. 5 Replicates and each replicate 40 shrimp	Shrimp fed for 14 days before challenge Water exchange 20% daily. No water exchange on challenge day	Per os challenge using WSSV infected tissue 1% of mean body weight
Treatment 2	<i>C. dactylon</i> powder 15% mixed in feed during production. 5 Replicates and each replicate 40 shrimp	Shrimp fed for 14 days before challenge Water exchange 20% daily. No water exchange on challenge day	
Treatment 3	<i>C. dactylon</i> powder 20% mixed in feed during production. 5 Replicates and each replicate 40 shrimp	challenge day	
Positive control	Regular feed (no mixing of <i>C. dactylon</i> powder mixing) 5 Replicates and each replicate 40 shrimp	Shrimp fed for 14 days before challenge Water exchange 20% daily. No water exchange on challenge day	
Negative control	Regular feed (no mixing of <i>C. dactylon</i> powder mixing) 5 Replicates and each replicate 40 shrimp	No Challenge Water exchange 20% daily. No water exchange on challenge day	No challenge

#### Treatment and control feed

Cage trials were conducted using powdered extract of *C. dactylon*. The powder is three concentrations, i.e., 10%, 15% and 20% were mixed in the feed at the feed mill, PT. Central Proteina Prima, Surabaya. The regular feed, i.e., standard *Vannamei* feed without *C. dactylon* mixing was used to feed control groups. All the feed of treatment groups and control groups were produced on the same day.

#### Challenge method

Experimental shrimps except negative control were challenged *per os* with 1% WSSV-infected muscle. No artificial food was provided on the day of the challenge.

#### Post challenge observation

Monitoring of the challenged shrimp was performed similarly to that in the lab experiment. The behaviour, feeding rate and mortality rate were monitored.

#### Pond trial

The commercial ponds in the WSSV-prone area in Lampung were selected for the trial and observation. The ponds had a size of 5,000 m<sup>2</sup> and full HDPE lining. P.T. Central Pertiwi Bahari's standard operating procedure (SOP) was followed in

pond preparation, water preparation, and culture. Sludge and barnacles were removed from the bottom, washed, sun-dried and disinfected with 100 ppm chlorine. Water for the experiment was treated with one ppm CuSO<sub>4</sub>, one ppm Pondfos (dichlorvos) and 20 ppm (active) chlorine.

The SPF shrimp were stocked at a density of 80 animals/m<sup>2</sup> in 10 ponds. An autotrophic system was established with low water exchange and high dissolved oxygen inputs. The pond water depth was maintained at 120 cm depth during the culture with an average 20% daily water exchange. The treatment and control feed were produced on the same day at a feed mill (PT. Central Proteina Prima, Surabaya) to avoid dissimilarities.

#### Results

##### Analysis of extract of *C. dactylon* by GCMS

Identification and quantification of individual phenolic compounds in the ethanol extract of *C. dactylon* was performed by GCMS analysis (Figure 1). The results indicated that *C. dactylon* contains 35 peaks in the acetonitrile fraction, 117 peaks in the methanol fraction, and 103 peaks in the hexane fraction (Figure 1).

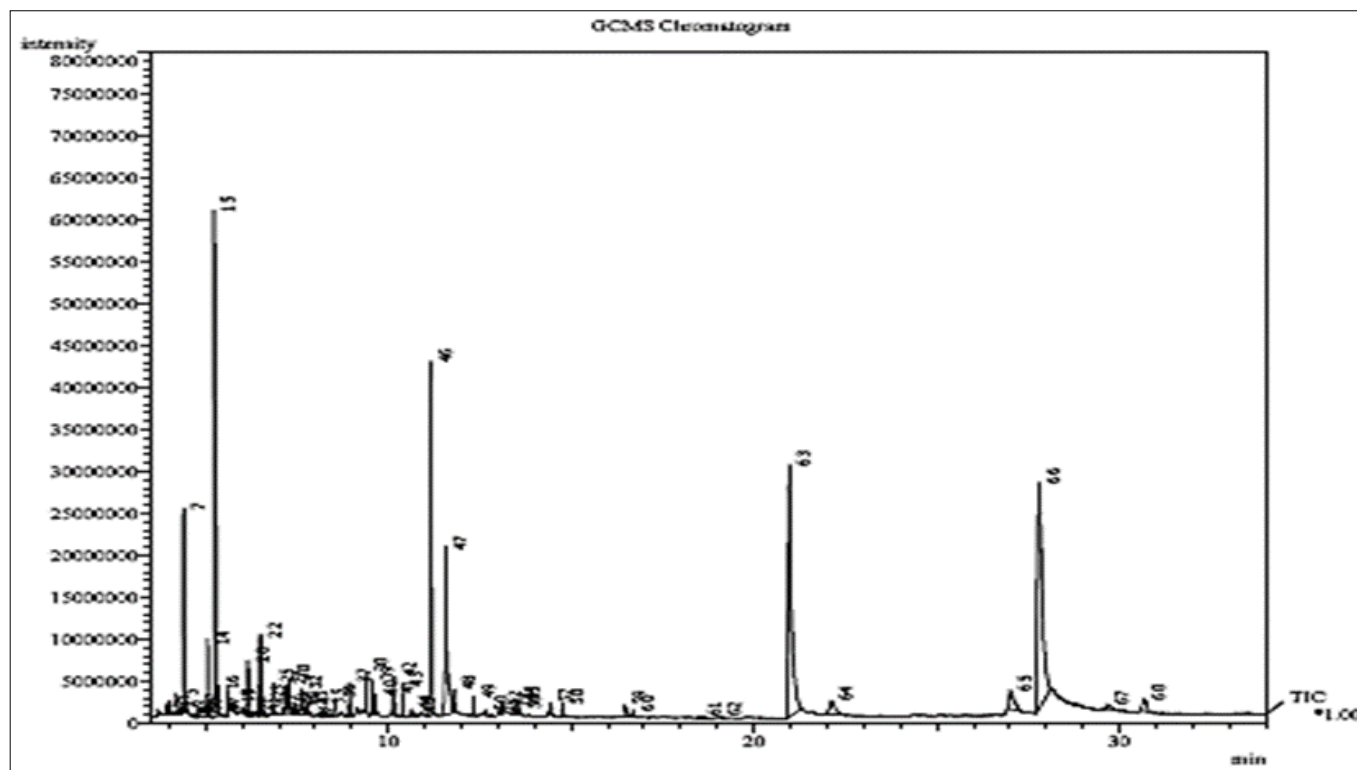


Fig 1: GCMS chromatogram of *Cynodon dactylon* extract

**Bioassay trial in tank**

The results showed that *C. dactylon* extracts at 2% and 3% concentrations are effective against white spot syndrome virus (Figure 2). Mortality started from day two post-infection (dpi) and continued to increase in the positive control. There was a

drop-in feeding on the next day after the treatment groups' challenge, but feeding returned to normal starting from day 3 of the challenge. The positive control group had a continuous drop in feed intake.

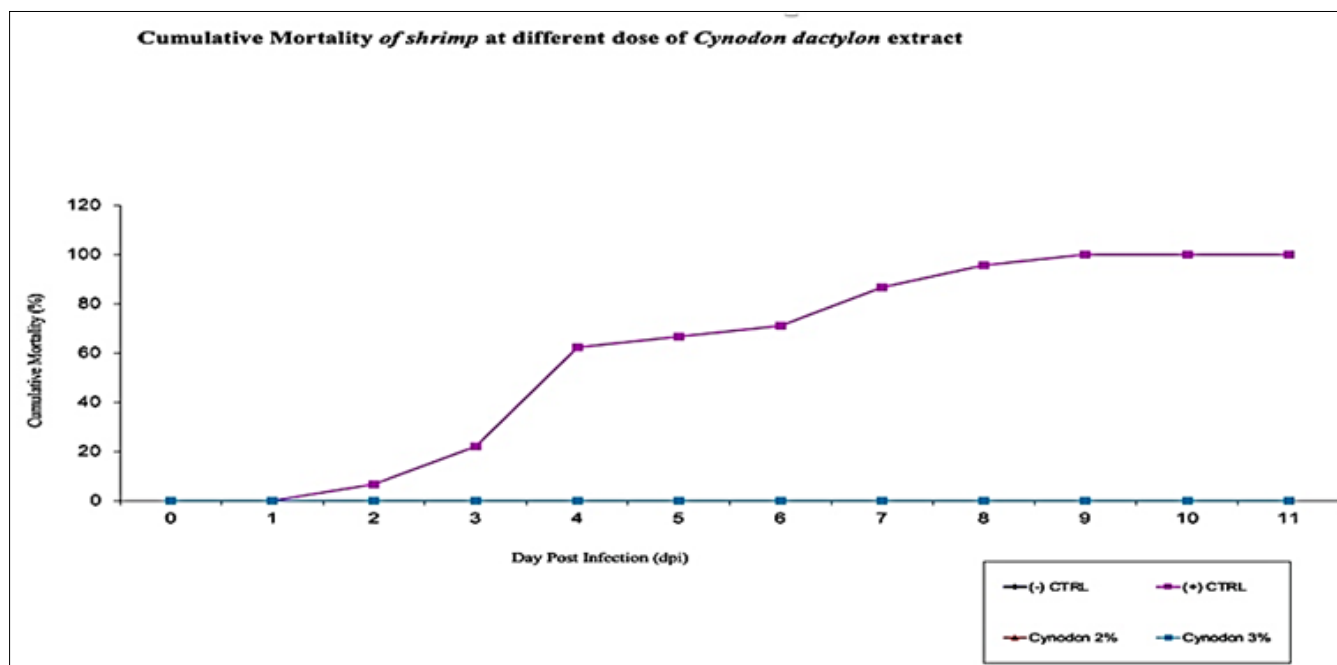
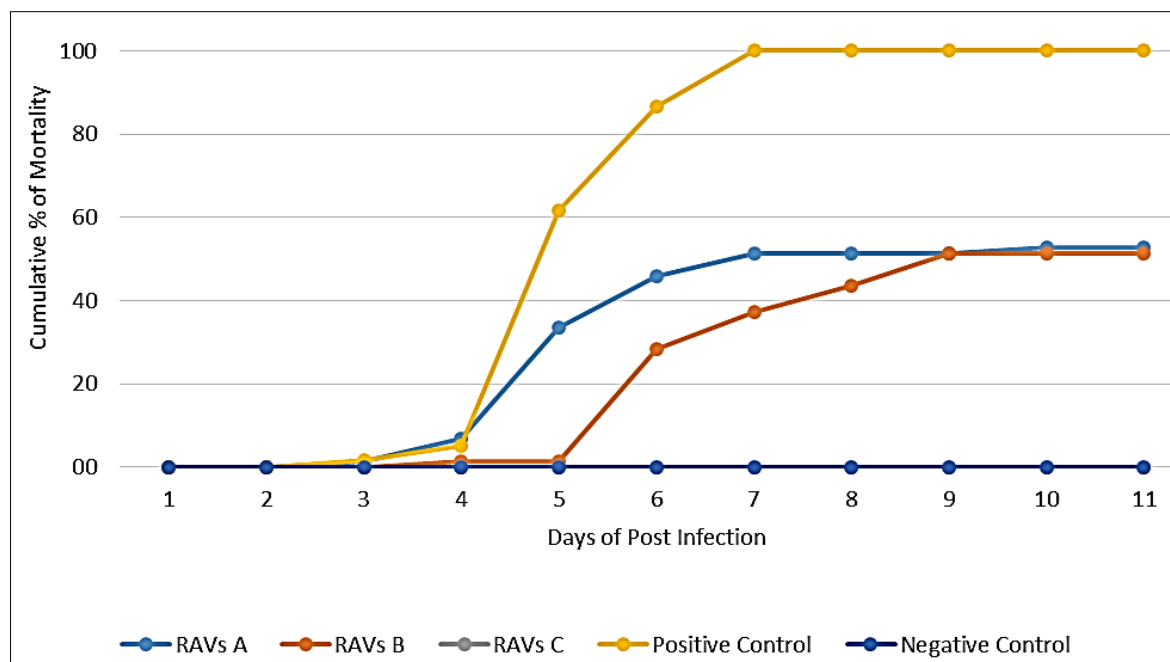


Fig 2: Percentage of cumulative mortality of *L. vannamei* in the *in vivo* challenge test with WSSV and two concentrations (2% and 3%) of *C. dactylon* ethanolic extract

The second bioassay trial was conducted using the feed containing *C. dactylon* powdered extract. The outcome was different from the results of the ethanolic extract tests. Total

(100%) cumulative mortality was detected in the positive control. Mortality in RAVs A and RAVs B groups was 51% and 51%, respectively.



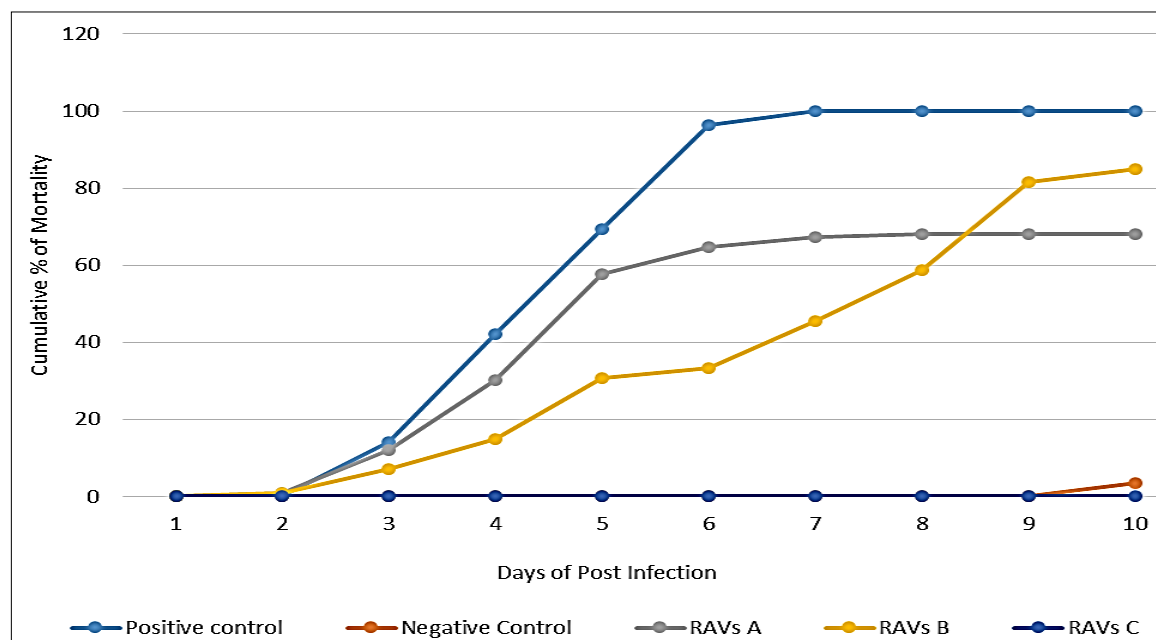
**Fig 3:** Percentage of cumulative mortality of *L. vannamei* in the *in vivo* challenge test with WSSV and three concentrations i.e., 10%, 15% and 20% (10, 15 and 20 mg/kg) of *C. dactylon* powder extract

No mortality was observed in the RAVs C (20% inclusion) and negative control groups (Figure 3).

**Anti-WSSV activity of *C. dactylon* in cage**

Then, the efficacy of *C. dactylon* was determined on a larger scale, i.e., in the small ponds with cages. In the aquaria level experiment, total mortality was detected in the positive control group six days after the infection. The 100%

cumulative mortality was observed in the positive control on day six after the challenge. The RAVs A and RAVs B treatment groups showed the cumulative mortality of 68% and 85%, respectively. No mortality was detected in the RAVs C treatment and negative control groups (Figure 4). The feed RAVs C containing 20% *C. dactylon* was effective against WSSV in the cage trial.



**Fig 4:** Percentage of cumulative mortality of *L. vannamei* in the cage experiment with WSSV and three concentrations i.e., 10%, 15% and 20% (10, 15 and 20 mg/kg) of *C. dactylon* powdered extract

**Performance of *C. dactylon* feed in commercial pond**

The final step was to determine the efficacy of *C. dactylon* in commercial ponds. The treatment feed formula RAVs C (20 mg/kg of *C. dactylon* powdered extract) was selected for the pond level trial. The trial was conducted in *Penaeus vannamei* species. The ponds in the WSSV-prone red zone area of PT.

Central Pertiwi Bahari, Lampung, were used for the trial. The results showed that *C. dactylon* has a positive effect against WSSV (Figure 4 and Table 4). The use of *C. dactylon* enhanced the productivity of the ponds (Figure 4 and Table 4).

**Table 4:** Efficacy of *C. dactylon*-enriched RAVs C (20 mg/kg) on *P. vannamei* performance in commercial ponds

Ponds	MBW	ADG	SR	FCR	BIO	Prod/Ha	Prod/HP
A. Treatment RAYS (20%) feed	19.31	0.17	84.66	1.46	3,307	12,719	551
B. Control Regular Feed	17.86	0.16	85.07	1.57	3,066	11,791	511

No WSSV cases were recorded in the control and treatment ponds. The treatment ponds yield was approximately 1,000 kg/ha higher than that of the control ponds. There was no negative impact on water quality parameters due to *C. dactylon* feed. The obtained parameters were similar in the control and treatment ponds.

#### PCR confirmation of WSSV in experimental groups

Samples from each group were collected and subjected to nested PCR test using a Genreach PCR kit (Taiwan). The WSSV band was detected in the primer sample (lane K<sup>+</sup>) under UV light after electrophoresis through a 1.5% agarose gel (Figure 5). Additionally, the WSSV band was also detected in the control group (lanes 3-5) with 100% mortality within six days post infection. In contrast, the negative control group (lanes 1-2) was negative for the WSSV band. The treatment group (lanes 6-8) was negative for WSSV.

**Fig 5:** UV visualization of the PCR test of the laboratory experiment

#### Discussion

White spot syndrome viral infection is a significant disease problem in the shrimp culture industry worldwide. The immunity level of crustaceans, such as, *P. vannamei* limits vaccines and immune boosters [1, 12, 13]. The developed and successfully reported recombinant DNA, and protein vaccines against WSSV infection [1, 5] did not achieve success due to the high production cost and adjustment. Herbal extracts with antiviral properties can play an essential role in minimizing the risk of viral diseases in shrimp. A successful effort was made to use the extracts of *Cynodon dactylon* against white spot syndrome virus of shrimp. The active ingredients were extracted using the ethanol extraction method and provided 100% protection in the bioassay trial. This result validates the concept and agrees with the data obtained previously [1]. The use of ethanol increased the extract's cost not to be utilized in shrimp culture to protect against the virus. The crude powdered extract was optimized to mix in the feed and produce shrimp feed with anti-WSSV properties. The dose of 20% *C. dactylon* powder mixed in shrimp feed as raw material provided the best protection compared to other tested

doses. The pond level trial showed no negative impact of *C. dactylon* powder on feed palatability or water quality parameters.

Various herbs have been tested for their antiviral activity against WSSV [7, 8, 9] Balasubramanian *et al.* (2006) [14] suggested three possible mechanisms of antiviral activity of the plant extracts:

1. Viral inactivation through the reaction between the extract and the envelope proteins of the virus may result in the suppression of the viral entry into the host body
2. Interference of plant extracts with the replication mechanism of the viruses, which prevents the multiplication of the viruses in the host body
3. Plant extracts may act as an immunostimulant that enhances innate immunity

Plant extracts have antioxidant properties that can protect the cells from free radicals resulting from WSSV infection [13, 14].

#### Conclusions

A successful attempt was made using the well-known herb *Cynodon dactylon* to deactivate the deadly white spot syndrome virus. This experiment developed shrimp feed supplemented with various extracts of *C. dactylon*, commonly called RAVs or Sanjivani. The results at the laboratory, cage and commercial pond levels are encouraging. Feeding of shrimp with RAVs-C (20% inclusion) provided 100% protection of *L. vannamei*. Feeding was not decreased, indicating that the virus did not multiply and did not induce shrimp stress. The results indicate that the extracts of *C. dactylon* contain chemicals with anti-WSSV properties and act independently or in combination. These chemicals can provide complete protection of the animals against WSSV. However, 15% and 10% concentrations partially protected the shrimp, indicating that a minimum quantity of antiviral substances is required for absolute protection. These findings indicate that these chemical substances need to be present at a minimum inhibitory concentration to protect shrimp from WSSV fully. The powder concentration has to be 20% or higher.

The results of this study show that *C. dactylon* has strong antiviral properties and contains many phenolic compounds. The *in vivo* challenge test results indicate that *C. dactylon* has anti-WSSV properties that protect white leg shrimp from WSSV infection. Since *C. dactylon* has potent activity against WSSV, further studies on the isolation, characterization and purification of active compounds of this plant should be carried out to develop their applications in the shrimp culture industry.

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