SURVEY OF THE EFFECTS OF NEEM LEAF EXTRACT ON THE WATER QUALITY AND PHYTOPLANKTON COMMUNITY IN FRESHWATER CATFISH POND IN BINH DUONG PROVINCE

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ABSTRACT

In Vietnam, the total area of freshwater catfish pond reached 1959 hectares in 2015 to satisfy the national and international markets. The use of synthetic chemicals in fish farms has threatened the environmental ecology and also fish quality so it is really necessary to carry out researches on alternative compounds from plants. The extract of neem Azadirachta indica has been widely applied as an environmental insecticide, especially in fish pond to control fish diseases and parasites in several countries around the world. However, there has not been widely applied in Vietnam. In this study, the effects of neem leaf extract on the water quality and phytoplankton community in freshwater catfish pond in Binh Duong province was investigated. It was shown that at a concentration of 90 mg / L, the extracted from neem leaf have a positive impact on water quality; reduce the concentration of parameters such as COD, N-NO₃, P-PO₄, SD, and SS value. pH value of water was not affected by adding extract at this concentration. In case of plankton community, the result indicated that slightly change in occurrence frequency of phytoplankton community in water was noticed and no change in phytoplankton species composition such as Anisonema sp., Euglena acus, Phacus pleuronectes, Trachelomonas armata and Trachelomonas, which means neem leaf extract might be an environmental friendly to be used in Pangasius hypophthalmus fish pond.

Keywords: neem, Azadirachta indica, water quality, phytoplankton, Pangasius hypophthalmus.

1. INTRODUCTION

Freshwater catfish Pangasius hypophthalmus is one of the main exports aquatic food products of Vietnam. According to the General Directorate of Fisheries, in just 10 years, the acreage of catfish farm has increased by 5 times nationwide (reaching 6,000 ha), production increased 36 times (about 1.35 million tons), export value increased by nearly 45 times (from 40 million in 2000 to over $ 1.8 billion in 2011 and 2012 is 1.74 billion US dollars), accounting for 34.4 % of total export value of the fisheries sector and contribute about 2.2 % the country's GDP. However, the development of the pond area has caused a great pressure on the environment. The
study by Gross et al. (1985) indicated that catfish absorb only 27-30 % nitrogen, 16-30 % of phosphorus and 25 % of organic matter from the feed, the rest was retained in the aquatic environment. This excess food affected the quality of the pond water environment as well as the growth and normal development of aquatic species, especially the rate of fish parasite infection [1].

*Azadirachta indica* is one of the most promising plants with compounds known for its insecticidal properties and perhaps the most concerned tree in the world [2]. In Vietnam, the Neem tree is also commonly known as neem and usually grown in popularity in the central coastal region, most particularly in Ninh Phuoc district, Ninh Thuan province. Significant hypoglycemic, hypolipidemic, hepatoprotective, anti-fertility and hypotensive activities were found in water soluble extract of *A. indica* leaves [3, 4]. In freshwater fish farming, losses in productivity due to fish parasites and fish predators are great and often controlled by applying toxic chemicals indiscriminately [5]. This not only leads to high levels of toxic compounds in fish but also may affect their performance [6]. In order to reduce the damage of the use of synthetic chemicals, there have been plenty of researches on pest control by substances from plants as an alternative method. According to Dunkel and Ricilards (1998), Martinez (2002), neem and products from neem have been stated to be effective in controlling fish predators and parasites in aquaculture systems [2, 6]. Currently in Vietnam, there have been many researches and application of extract from neem in varying purposes. However, the impact of neem leaf extract on the aquatic environment and ecology, environmental friendliness has not been investigated.

In this study, we assessed the impact of extracted compound from neem leaves to the water quality and protozoa community of fresh water catfish ponds in Binh Duong province in order to provide more scientific for application of neem tree in fish farming.

2. MATERIALS AND METHODS

2.1. Preparation of aqueous neem leaf extract

*Azadirachta indica* leaf from Binh Duong province were cut in to 0.5x0.5 cm pieces, washed 3 times with dH2O and dried at 60°C to constant weight. The dried samples were grounded into powder, mixed with distilled water for 30 min and sieved using 0.1mm sieve. An extract solution of 500 g dry leaf/l was achieved.

2.2. Pond treatment with aqueous neem leaf extract

Catfish pond with the size of 20m × 15m × 2m in Binh Duong province was selected for experiments. Pond water level was 1.25 m. Pond was being used for growing freshwater catfish *Pangasius hypophthalmus* with normal feeding schedule (once a day at 4 p.m.). The density of catfish was 25 fishes/ m² with average size of 500g/ one fish.

The input neem leaf extract was applied to the pond to reach final concentration of 90 mg/l once before each set of experiment (totally 6.75 l neem leaf extract for 375 m³ water). Water samples were collected during 10 weeks after treatment. The experiment was repeated three times and data was shown as average values.

2.3. Monitoring of pond water quality
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Samples from ponds were taken for analyzing the water quality and frequencies of presence of phytoplankton species at varying period of time before and after the pond was treated with neem leaf extract. Treatment of pond using distilled water instead of neem leaf extract was used as control.

The process of collecting and storing of water samples was in accordance with the regulations of the Ministry of Natural Resources and Environment (Circular of Ministry of Natural Resources and Environment on technical regulations continental surface water monitoring. 29/2011 / TT-BTNMT, 01/8/2011; ISO 6663-6: 2008; ISO 6663-3: 2008).

2.4 Sampling

Samples were taken at 4 different positions with average distance in the pond, at 8 a.m. Water samples were collected at every 2 weeks by horizontal type water collector (Wildco, USA). Water parameters analysis of pH, temperature, SD, SS, COD, N_NO3-, P_PO43- were done according to TCVN standard. The frequency of phytoplankton species was calculated as its presence in all collected samples.

3. RESULTS AND DISCUSSION

3.1. Effect of neem leaf extract to the water quality of freshwater catfish pond

3.1.1. pH

pH is one of the important parameters and often used in water quality assessment. Low or high pH values will cause harmful effects to distribution of aquatic organisms [1, 2]. The change of pH value of the pond before and after using neem leaf extract, shown in Table 1, was that pH did not change significantly during 10 weeks. The pH values were almost within the permissible limits of 44/2010/ BNNPTNT food safety rules for freshwater catfish farming. From the above results, the use of the active ingredient neem did not affect the pH value of the pond water.

Table 1. Water quality of freshwater catfish pond during the treatment of neem leaf extract.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Week 2nd</th>
<th>Week 4th</th>
<th>Week 6th</th>
<th>Week 8th</th>
<th>Week 10th</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>5.34±0.36</td>
<td>5.28±0.02</td>
<td>5.35±0.27</td>
<td>5.44±0.16</td>
<td>4.97±0.34</td>
<td>5.25±0.39</td>
</tr>
<tr>
<td>SD (m)</td>
<td>0.33±0.02</td>
<td>0.45±0.02</td>
<td>0.30±0.01</td>
<td>0.21±0.01</td>
<td>0.28±0.04</td>
<td>0.16±0.05</td>
</tr>
<tr>
<td>SS(mg/L)</td>
<td>61.00±4.08</td>
<td>66.75±2.75</td>
<td>49.00±1.41</td>
<td>40.00±1.63</td>
<td>46.25±3.50</td>
<td>39.50±4.20</td>
</tr>
<tr>
<td>COD(mg/L)</td>
<td>28.30±2.85</td>
<td>15.58±1.28</td>
<td>27.23±3.91</td>
<td>23.85±6.81</td>
<td>30.25±7.88</td>
<td>NA</td>
</tr>
</tbody>
</table>

3.1.2. Transparency (SD)

Water transparency value can affect the luminous intensity of the sun on the water hence has an impact on the photosynthesis of phytoplankton [1, 2]. The change of water transparency level increased after 2 weeks after treatment but then reduced in the following weeks, reduced 51.6 % in the 10th week as compared to initial value. It was seen that the water transparency level in the pond was improved after the addition of ingredients from neem leaf.
3.1.3. SS

SS is also one of the parameters affecting indirectly to the amount of dissolved oxygen in water and either to the amount of algae, a natural food of catfish so it needed to be investigated when adding neem extract to pond water. It was seen that SS value was reduced gradually after the addition of extract and remained 35.2% after 10 weeks as compared to initial value.

3.1.4. COD

The change of COD value of water before and after the use of Neem extract was shown in Table 1 and Figure 3. After treatment ponds with neem leaf extract, COD decreased sharply, but then gradually increased. The COD value after 8 weeks of treatment was almost the same as initial COD and was within the observed range of pond water [8]. The explanation for this phenomenon was not studied before so it needed to be well examined in further invitro experiments.

3.2. Effect of neem leaf extract to the eutrophobition index of pond (N-NO$_3^-$ and P-PO$_4^{3-}$)

Phytoplankton is natural food for many marine and freshwater fishes and crustaceans, especially rear larvae and fry [9, 10]. According to Boyd (1998), in ponds fed, remained food not assimilated by fish increases the productivity of phytoplankton [8, 11]. The existence of large amounts of organic matter in the water will cause eutrophication, affecting water quality and the growth of aquatic plants in the pond. Nitrate and phosphor are the index to analyze the eutrophobition of water.
Nitrate in pond water is non-toxic to aquatic life but high nitrate levels can cause phytoplankton blooms and hence causes harmful effect to the growth of fish. As observed in table 2, the concentration of NO$_3^-$ reduced significantly since 2 week after treatment. After 10 weeks, NO$_3^-$ value was only 23% as compared to the value before treatment.

Similarly to nitrogen, phosphorus is also the limiting factor for phytoplankton. A high level of phosphate in water will promote the algae to thrive and cause harmful effects to other aquatic lives. The analytical results in the experiment showed that P-PO$_4^{3-}$ value was unstable over the sampling times, and the overall trend was that P-PO$_4^{3-}$ value reduced slightly at the 10 weeks applying neem leaf extract as compared to initial value.

Table 2. Change of NO$_3^-$ and PO$_4^{3-}$ value in pond water during the treatment of neem leaf extract.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Week 2$^{nd}$</th>
<th>Week 4$^{th}$</th>
<th>Week 6$^{th}$</th>
<th>Week 8$^{th}$</th>
<th>Week 10$^{th}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-NO$_3$(mg/L)</td>
<td>3.39±0.18</td>
<td>1.40±0.26</td>
<td>0.99±0.40</td>
<td>1.37±0.36</td>
<td>0.62±0.07</td>
<td>0.79±0.25</td>
</tr>
<tr>
<td>P-PO$_4^{3-}$(mg/L)</td>
<td>0.68±0.08</td>
<td>0.40±0.03</td>
<td>0.83±0.04</td>
<td>0.56±0.08</td>
<td>1.19±0.31</td>
<td>0.61±0.12</td>
</tr>
</tbody>
</table>

3.3. Effect of neem leaf extract to the phytoplankton community

*Azadirachta indica* was studied in previous researches showing the inhibition to harmful bacteria in pond water and was less toxic to fish as compared to synthetic chemical [12, 13]. However the effect to pond water parameters and phytoplankton, which directly influence to food source of fish, has not been investigated before.

Table 3. Effect of Neem leaf extract to the occurrence frequency of phytoplankton species.

<table>
<thead>
<tr>
<th></th>
<th>Anisonema sp.</th>
<th>Euglena acus</th>
<th>Phacus pleuronectes</th>
<th>Trachelomonas armata</th>
<th>Trachelomonas sp.</th>
<th>Volvox</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before (%)</td>
<td>25</td>
<td>100</td>
<td>67</td>
<td>67</td>
<td>58</td>
<td>25</td>
</tr>
<tr>
<td>After (%)</td>
<td>25</td>
<td>100</td>
<td>63</td>
<td>63</td>
<td>50</td>
<td>25</td>
</tr>
</tbody>
</table>

The experiment results, according to Table 3, showed that slightly change in occurrence frequency of phytoplankton species in water samples was noticed after using the compound. Change of species composition was negligible with six species, *Anisonema sp.*, *Euglena acus*, *Phacus pleuronectes*, *Trachelomonas armata* and *Trachelomonas*. *Euglena acus* appeared with greater frequency. It indicated that the application of neem leaf extract at 90 mg/L to treat fish pond did not affect to species of phytoplankton in the pond and hence might not be harmful to the natural food of catfish.

A Nature Conservancy Council report in 1989 [14] identified impacts in the environment from mariculture including chemical pollution situations from aquaculture, feed additives, pharmaceuticals and other treatments. Alternative compounds which is environmental friendly from plants such as neem is really necessary. The application of neem leaf extract in this study showed positive impact on water quality and no affect to phytoplankton species in the pond. For that reasons, it could be used in fresh water catfish ponds as environment friendly material.
4. CONCLUSION

The use of active ingredients extracted from neem is capable of affecting the quality of the pond water. At a concentration of 90 mg/L, the extracted from neem leaf have a positive impact on water quality; reduce the concentration of these parameters: COD, N-NO₃⁻, P-PO₄³⁻, SD, and SS value. pH value was not affected by adding extract at this concentration. All analyzed water parameters were within permissible limits of Circular 44/2010 / TT-BNN provides for conditional basis, intensive fish farming to ensure food safety and NTR 02 - 20: 2014 / BNNPTNT national technical regulations on the basis of catfish (Pangasianodon hypophthalmus) in the pond - conditions to ensure veterinary hygene, environmental protection and food safety. Slightly change in occurrence frequency of phytoplankton community in water samples either before or after the use of the compound was noticed and no change in phytoplankton species composition such as Anisone sp., Euglena acus, Phacus pleuronectes, Trachelomonas armata and Trachelomonas. This result indicated that neem leaf extract applied to fresh water catfish pond was not harmful to these species in the pond. The application of neem leaf extract could be concluded that it can be used in fresh water catfish ponds as environment friendly material.

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REFERENCES

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