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ACUTE TOXICITY OF BOTANICAL PESTICIDES, NEEM OIL (*AZADIRACHTA INDICA*) AND *POLYGONUM HYDROPIPER* LEAF EXTRACTS IN FINGERLINGS OF *CATLA CATLA* (HAM.)

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Use of botanical pesticides is considered a safe and environment friendly method for controlling rice insect pests in lowland. To explore the possibility of using neem oil (*Azadirachta indica*) and the leaf extract of *Polygonum hydropiper* (a lowland weed) as botanical pesticides against rice insect pest in lowland rice-fish ecosystem, acute toxicity experiments were conducted in control condition using fingerlings of *Catla catla* (4.4±0.4 g, 7.3±0.6 cm) as the test fish. The botanicals were applied as emulsion by adding 'labolene' (a liquid detergent) as the emulsifier to neem oil and grinded leaf of *P. hydropiper*. The immediate knockdown effect in terms of fish mortality was recorded in both the botanicals within first 24 hours of application. No fish mortality was observed after 24 hours indicating faster biodegradation of these toxicants. The 24 h LC_{50} were found to be 36.6 µl/l for neem oil and 4.6 mg/l for leaf of *P. hydropiper*. These concentrations are much higher than the recommended dose applied against insect pest in rice crop. The study indicates that these two botanicals can be used in rice-fish ecosystem at their recommended dose level for control of rice insect pest without any fish mortality.

INTRODUCTION

Fish and rice are the two major components grown together in the inundated rice field of lowland rice-fish eco-system. The rice crop is subjected to the infestation of a large number of insect pests which are generally controlled by application of chemical pesticides. In spite of their efficacy for controlling insect pests, use of chemical pesticide is often restricted in lowland rice-fish eco-system due to the presence of fish in rice field, leaving the farmer behind to choose either of the crops, i.e., rice or fish. In recent years, there is a lot of concern over the indiscriminate use of synthetic pesticides, which has hazardous effect on the environment as well as human health. In this context, use of botanical pesticides is an efficient and eco-friendly alternative, which can provide ample scope to the farmers for a better fish harvest along with healthy rice crop.

Botanicals are plant derivatives and are efficient, biodegradable and fast acting with short persistence toxicity. A large range of these botanicals and their derivatives

used against rice insect pests (Roy and Dureja, 1998). Neem seed materials have been found to be promising against sucking insects like green leafhopper (*Nephotettix virescens* Distant), the brown planthopper (*Nilaparvata lugens* Stal) and whitebacked planthopper (*Sogatella furcifera* Horvath); foliage feeder like the rice leaf folder (*Cnaphalocrocis medinalis* Guenee), the ear-cutting caterpillar (*Mythimna separata,* Walker) and rice army worm (*Spodoptera mauritia acronynctoides* Boisduval) (Saxena, 2001) and yellow stem borer (Jena, 2001). Neem seed contains toxin known as azadirachtin, which affects the feeding, growth and egg-laying of these insects and controls their population and outbreak. Though less effective against parasites, application of neem oil has also been reported to control some viral diseases (Saxena, 2001). With such broad range of insecticidal and pesticidal properties, neem oil, if found non-toxic to fish at its normal dose against these insects, could be a better option for use in the lowland rice-fish ecosystem.

Leaf of *P. hydropiper* is commonly used as a piscicide in the tribal areas of Balasore and Mayurbhanj district of Orissa, India (Jena, 2000). The leaves were grinded and buried into the pond bottom that irritates and repells the mud dwelling fishes like *Clarias batrachus, Heteropneustes fossilis* and *Channa* species to come out of the bottom to be caught. Leaf of *P. hydropiper* is also reported to have effective insecticidal property against brown planthopper (BPH) and repellent action against rice case worm (Jena, 2001). Therefore, this plant was also selected for study for its acute toxicity against *Catla catla*.

MATERIAL AND METHODS

Short-term acute toxicity experiments were conducted in 50 l capacity plastic tubs filled with 30 l of water. Fingerlings of catla (4.4±0.4 g, 7.3±0.6 cm) collected from the nursery pond of Central Rice Research Institute, Cuttack were acclimatized for 15 days prior to initiation of the experiment. Feeding up to satiation, water exchange and aeration were provided in the acclimatization tank to keep the fishes in stress-free condition. Feeding was stopped 24 hours prior to the range finding as well as acute toxicity tests for both the botanicals.

Stock solutions of the botanicals were mixed with 'labolene' (a laboratory detergent) for uniform distribution in the experimental tank. The safe concentration of labolene in water against carp was found to be 0.1 ml/l without involving any fish mortality. Accordingly, the required stock solution of botanicals, for creating different concentrations, were mixed with labolene at the rate of 0.02-0.03 ml/l of test solution before application in the experimental tank.

Catla fingerlings were exposed to a series of concentration of both the botanicals to find out the lethal range. No mortality was observed in the fingerlings up to 20 µl/l for neem oil extract and 4 mg leaf/l of *P. hydropiper,* whereas 100% mortality was observed at

and above 75 µl/l for neem oil and 5.5 mg leaf/l for *P. hydropiper.* Therefore, 20-75 µl/l for neem oil and 4.0-5.5 mg leaf/l for *P. hydropiper* were taken as the range for the acute toxicity test.

Acute toxicity tests (conducted separately for the two botanicals) were designed taking six concentrations of each botanical, viz., 20, 30, 40, 50, 60 and 70 µl/l for neem oil and 4.0, 4.3, 4.5, 4.8, 5.0 and 5.5 mg leaf/l for *P. hydropiper*. Treatment without addition of botanical served as control. Three replications were maintained for each of the treatment. Six catla fingerlings with 24 hours of fasting were put in each of the replication tanks and the required amount of botanicals were added into each replication of different treatments. Continuous aeration was provided during acute toxicity test. The LC_{50} and related calculations were done as per the formula given by Reish and Oshida (1987).

RESULTS AND DISCUSSION

The acute toxicity test was conducted for 96 hours. However, the fish mortalities in both the botanicals were recorded within 10-16 hours of addition of the botanicals. The response curve for the 24 hours for both the botanicals are given in Figs. 1&2. The 24 h LC_{50} level were found to be 36.6 µl/l for neem oil extract and 4.64 mg leaf/l for *P. hydropiper*. The 24 h LC_{16} and LC_{84} were 24.3 and 55.2 µl/l for neem oil extract and 4.20 and 5.13 leaf/l of *P. hydropiper,* respectively. The study revealed faster degradation rate of these botanicals in water after their application since no mortality was recorded in any concentration beyond 24 hours exposure. Such low persistence of toxicity exerts only a short-term toxic action on the fish, thereby the long-term deleterious affect, as caused after application of synthetic pesticides on growth rate is avoided. The recommended dose of neem oil against rice insect pest is 1 ml/l of spray solution before 50 days after transplantation (DAT) and 2 ml/l after 50 DAT and applied at the rate of 500 l of spray solution/ha through hand sprayer (200 l/ha through mechanical sprayer). Considering the total 500 l of the spray solution coming to water, creates a concentration of 0.17 µl and 0.33 µl neem oil/l in water of the rice-fish field with 30 cm water depth if applied before and after 50 DAT, respectively. Recommended dose of *P. hydropiper* is 10 g leaf/l of spray solution if applied before flowering stage and also applied at the rate of 500 l spray solution/ha through hand sprayer, thereby incorporating 5 kg of leaf/ha. Considering the total leaf extract goes into water, creates a concentration of 1.67 mg leaf/l in water in a rice-fish field with 30 cm water depth. Since the botanicals are applied as foliar spray, a major portion of the applied solution remains on the canopy of rice plant and dose not go to water (drift), further reducing their concentration in water. Considering the drift value at the higher side, i.e., 50%, the concentration of the two botanicals, applied at the recommended dose, will further reduce to half on reaching to water, i.e., 0.17 µl neem oil/l and 0.84 mg leaf/l of *P. hydropiper*. These concentrations are lower than the observed

LC_{50} values (36.6 µl/l for neem oil extract and 4.64 mg leaf/l of *P. hydropiper)* in the present study and are also lower than the safe level, since no mortality was observed in catla fingerlings at 20 µl/l neem oil extract and 4.0 mg leaf/l of *P. hydropiper*. Further, due to the low specific gravity of neem oil, the contents tend to float on the upper water surface. Catla, being a surface feeder remain mostly in upper stratum of the pond, is thus the most affected species compared to the column feeders like rohu and bata including and bottom feeders like mrigal and common carp (fishes usually cultured in the low-land rice-fish system). Thus, the present study revealed that neem oil and *P. hydropiper* could be used at their recommended dose levels against rice insect pest in the rice-fish eco-system simultaneously avoiding any mortality of the cultured fish species.

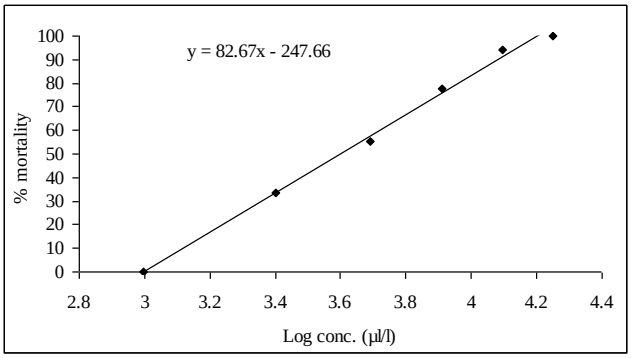

Fig. 1. Response curve for fingerlings of *Catla catla* exposed to acute toxicity of neem oil (*Azadirachta indica*)

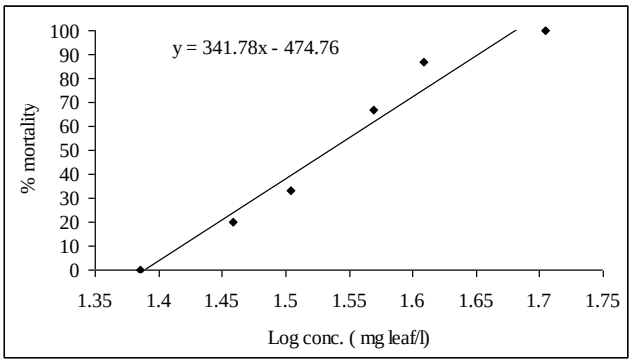

Fig. 2. Response curve for fingerlings of *Catla catla* exposed to acute toxicity of leaf extract of *Polygonum hydropiper*

The botanicals like neem oil and *P. hydropiper* are easily and plentily available with the farmer and do not involve much cost unlike the synthetic insecticides and pesticides. Further, use of the botanicals have edge over the synthetic chemicals in terms of lower risk of fish mortality, faster bio-degradation and short persistence, making it more environment-friendly. These are also efficient in controlling the targeted rice insect pest. Therefore, use of these botanicals may be encouraged to protect the rice crop in the low-land rice-fish ecosystem.

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