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Acute toxicity of water extract of *Tephrosia vogelii* Hook to species relevant in aquaculture ponds: rotifers, *Cyclops*, mosquito larvae and fish

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Summary

Rotenone is used to clear ponds of unwanted organisms and trash fish which may predate on fish when the ponds are stocked. Toxicity tests using water extract of the leaves of *Tephrosia vogelii* Hook, which contains rotenone, were conducted on rotifers (*Brachionus* species), *Cyclops*, mosquito larvae (*Culex* species) and fish (*Aphyosemion gardneri nigerianum*) in static bioassays. The 48-h LC_{50}s were derived from probit curves using the probit-analysis method, while chi-square was used to test for significant differences between observed mean mortalities and predicted mean mortality values. These showed no significant differences ($P > 0.05$). The variance ratio of the replicates in each treatment also showed no significant difference ($P > 0.05$). The 48-h LC_{50}s were found to be 2.89, 1.04, 4.48 and 0.24 mg L^{-1} for rotifers, *Cyclops*, mosquito larvae and fish, respectively. The probit mortalities were positively correlated with the log-concentration, except for the rotifers bioassay, which was negative. The fish, *A. gardneri nigerianum*, was the most sensitive; the mosquito larvae were the least sensitive.

Introduction

Prior to nursery or production pond stocking, a common practice by aquaculturists is to eliminate all unwanted species and predators. The preparation involves eradication of predatory and trash fish by the application of ichthyotoxins. Until recently, organochlorine chemicals were used (Omoregie et al., 1990).

Tephrosia vogelii Hook is an ichthyotoxic plant grown in tropical countries (Reed et al., 1967; Cox, 1979; Welcomme, 1985; Lambert et al., 1993) and used by artisanal fishermen. Apart from being extremely toxic to fish (Hassal, 1982; Ackerman and Bellwood, 2000), the active ingredient rotenone has been reported to possess insecticidal properties (Beal and Anderson, 1993; Lambert et al., 1993). It inhibits the respiratory chain (Fukami et al., 1970) and acts by blocking the oxidation of $NADH_2$ (O'Brien, 1978), which depletes the cells of ATP needed to maintain mitochondrial energization when electron transport is inhibited (Simbula et al., 1997). Rotenone degrades rapidly (Loeb and Engstrom-Heg, 1970) and bioaccumulation thereof is not a hazard (Gunther and Turrell, 1942; Hassal, 1982). Rotenoids are composed of an isoflavone nucleus with an isoprene moiety attached (Lambert et al., 1993); they are essentially extracted from the root of *Derris elliptica* (Beal and Anderson, 1993). These compounds also accumulate in the leaves of *T. vogelii* (Lambert et al., 1993) as well as in the floral parts and roots of *T. fulvinerius* and *T. pentaphylla* (Dagne et al., 1989). A great variety of rotenoids have been reported to accumulate in the leaves of this plant, wherein the total rotenoid content can reach as much as 4% of the dry matter (Lambert et al., 1993). It is also one of the commercial sources of rotenone listed by Gaskin and Stone (1971). It can also have serious impacts on aquatic ecosystems by affecting organisms other than the target species (Beal and Anderson, 1993).

In the eradication of undesirable species from a periodically reclaimed pond, Orciari (1979) reported that the 48-h LC_{50} of rotenone in golden shiners was 0.32 mg L^{-1}. Beal and Anderson (1993) reported the eradication of grass carp from a pond at 6 μl L^{-1} rotenone concentration. Hegen (1985) reported that there was a total mortality of fish at 5.00 ppm rotenone concentration, while blue crabs and brown shrimps survived at 4.00 ppm and 1.80 ppm concentrations, respectively. Early studies by Brown and Ball (1942) showed that rotenone is toxic to zooplankton at a 1 μl L^{-1} concentration, but Hooper (1948) reported rotenone toxicity to be < 1 μl L^{-1}. However, Beal and Anderson (1993) found that rotenone concentrations toxic to zooplankton were almost three times higher than those reported in Brown and Ball (1942).

The use of ichthyotoxic plants is gaining popularity, but there is a dearth of literature on the toxicity of *T. vogelii* to fish. *Aphyosemion gardneri nigerianum* Clausen 1963 (family: Cyprinodontidae) is a common trash fish inhabiting abandoned/disused ponds. This study was undertaken primarily to evaluate the toxicity of water extract of *T. vogelii* on *A. gardneri nigerianum*, mosquito larvae (*Culex* species), *Cyclops* species and rotifer (*Brachionus* species). They form part of the natural food chain of aquatic ecosystems, are the major aquatic organisms found inhabiting disused ponds, and need to be eliminated upon reactivation of such ponds for aquaculture.

Materials and methods

Test organisms

The rotifers were obtained from pure culture in the laboratory as described by Lubzens (1987). The *Cyclops* were obtained from 400-L capacity outdoor metal tanks in which productivity was stimulated by addition of poultry droppings (Wade and Stirling, 1999). Mosquito larvae were obtained by collecting their eggs from an outdoor metal tank and allowing them to hatch in 1-L capacity transparent glassware. They were allowed to grow to second instar stage before bioassay testing. The fish, *A. gardneri nigerianum* (Family: Cyprinodontidae), were collected from a disused pond and transported to the laboratory in 20-L plastic bowls. They were acclimated in 19°C dechlorinated municipal tap water [dissolved oxygen (DO):

8.20 mg L^{-1}; pH 6.7] for a 2-week period during which they were fed daily to satiation with live mosquito larvae at 08.00 and 18.00 hours prior to exposure in bioassay testing media.

Test solution

Fresh green leaves of *T. vogelii* were collected from a garden and spread on metal trays for air-drying in the laboratory. After 72 h, a constant weight was reached. The dried leaves were pounded in a mortar and sieved with a Gallenkamp sieve (90μ mesh size). The resulting powder was weighed on a Mettler MH80 balance. The test stock solution was prepared by dissolving 50 g of the powder in 1000 ml distilled water. This mixture was allowed to stand for 24 h at room temperature (19°C), decanted and filtered through a No. 1 Whatman filter paper. The filtrate (stock solution) was frozen and maintained at −20°C until required.

Bioassay

The bioassay tests to determine the 48-h acute toxicity of the *T. vogelii* extract were conducted in a renewable static system (OECD, 1981). Serial dilutions were made from the stock solution at the following concentrations: 10.0, 5.0, 2.5, 1.25, 0.625, 0.312 and 0.156 mg L^{-1}, except for the mosquito larvae bioassay, which required higher toxicant concentrations at 40.0, 20.0, 10.0, 5.0, 2.5, 1.25, and 0.625 mg L^{-1} for mortality to occur.

The rotifer bioassay was conducted in 25 ml glass specimen bottles. The media were inoculated with 1 ml of rotifers obtained from the pure culture (2400 individuals ml^{-1}) and each treatment concentration was triplicated. To determine mortality in each replicate, after thorough stirring, three 1 ml aliquots were collected with the aid of a 1 ml insulin syringe, poured into a 1 ml counting chamber and then mounted on a SWIFT microscope for observation under low power. Enumeration was undergone with a tally counter and the mean mortality per treatment was noted. The variance between mean mortality in each replicate for each treatment concentration was tested for significant difference. The subsample taken for enumeration was returned to the bioassay medium after counting. Mortality was assumed when heartbeat and cilia movement ceased.

The *Cyclops* bioassay was conducted in 100 ml capacity glass beakers. Each treatment concentration was duplicated and inoculated with 10 *Cyclops* using the 1 ml syringe. The *Cyclops* were viewed through a stereoscopic microscope and assumed dead when heartbeat stopped. The variance in mean mortality recorded in each replicate per treatment concentration was tested for significant difference. The mosquito larvae bioassay tests were carried out in 100 ml capacity glass beakers; the same procedure was repeated as for *Cyclops*. Death was confirmed when larvae heartbeat ceased.

The fish bioassay tests were conducted in 10-L glass aquaria. Ten fish (mean total length 4.5 ± 1.2 cm; mean weight 2.7 ± 0.5 g) were put into each aquarium. Each treatment concentration was duplicated and death was recorded when the opercula movement and tail beat stopped and the fish no longer responded to mechanical stimulus (touch). Any observed dead fish was removed from the medium. A variance test of the mortality per replicate for each treatment concentration was tested for significant difference.

Water quality parameters (Table 1) of the bioassay media were determined at the beginning and at the end of the experiments. The DO, total alkalinity, and total hardness were determined by the methods of APHA (1985). The pH values were determined by using a Phillips pH meter model PW9418. Water temperatures were taken with a mercury-in-glass thermometer.

Table 1
Mean values (n = 63) of bioassay media water quality parameters during the 48 h exposure period

Parameter	Mean value (SE)
Dissolved oxygen (DO, mg L^{-1})	8.17 (0.05)
Total alkalinity (mg L^{-1})	153.57 (0.53)
Total hardness (mg L^{-1})	75.57 (0.53)
Temperature (°C)	18.0
pH	6.68 (0.02)

The 48-h LC_{50}, regression analyses and correlation coefficient were determined using Microsoft Excel® Office 2000.

Results

The 48-h LC_{50} values of the test organisms exposed to the water extract bioassays of *T. vogelii* were determined from linear ($y = m + bx$) plots of the probit curves for all the test organisms. These are presented in Fig. 1a–d. The probit mortality percentages of the test organisms were plotted against log-concentrations as described by Sprague (1970, 1971).

For the rotifers, the linear equation $y = 18.29 - 3.84x$ was derived from the regression analysis of probit mortality in the test solution bioassay. Observed and predicted values are shown in Fig. 1a. The 48-h LC_{50} was calculated to be 2.89 mg L^{-1}, with a correlation coefficient of −0.833 (P = 0.0199). Chi-square test confirmed the goodness-of-fit of the trend as there was no significant difference between the observed values and the predicted values (P > 0.05). There was also no significant difference between the replicates per treatment, as the calculated variance was found to be far less than $F_{0.05}$.

For the *Cyclops* bioassay, the linear equation $y = 0.52 + 1.48x$ was derived from the regression analysis of the probit mortality with a 48-h LC_{50} of 1.06 mg L^{-1} and a correlation coefficient of 0.984 (P = 5.86E-05). The observed and predicted values are shown in Fig. 1b. Chi-square test confirmed the goodness-of-fit of the linear plot; there was no significant difference between the observed and the predicted values (P > 0.05). When the variance ratio of mortalities between replicates in each treatment concentration was calculated, there was no significant difference between the replicates, as the calculated variance was found to be far less than $F_{0.05}$.

The mosquito larvae exposed to the *T. vogelii* extract bioassay had a mortality pattern which on regression yielded the linear equation $y = -3.58 + 2.35x$. The 48-h LC_{50} derived from the equation was 4.48 mg L^{-1}. Observed and expected probit mortalities are shown in Fig. 1c, with a correlation coefficient of 0.844 (P = 0.0169). Chi-square test showed no significant difference between the observed and predicted probit values (P > 0.05). The variance test on the replicates in each treatment concentration revealed that there was no significant difference, as the calculated variance ratio was found to be far less than $F_{0.05}$.

The fish, *A. gardneri nigerianum*, on exposure to the *T. vogelii* extract yielded a mortality pattern as presented in

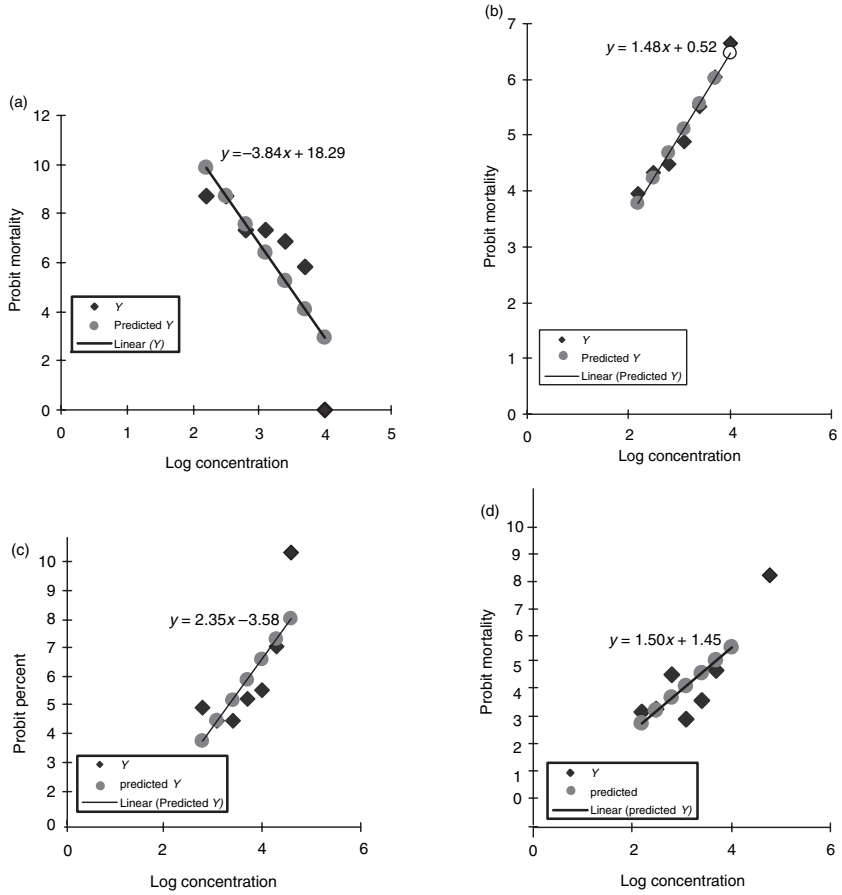

Fig. 1. Plots of probit mortality of rotifers (a), *Cyclops* (b), mosquito larvae (c) and (d) *Aphyosemion gardneri nigerianum* in *Tephrosia vogelii* Hook extract

Fig. 1d. Regression analysis gave a linear equation, $y = 1.45 + 1.50x$. Observed and predicted probit values are shown with a correlation coefficient of 0.724 ($P = 0.0659$). The 48-h LC_{50} was calculated to be 0.232 mg L^{-1}. Chi-square test found no significant difference between the observed and the predicted probit values ($P > 0.05$). There was also no significant difference between the mortalities recorded in the replicates for each treatment concentration, as the calculated variance ratio was found to be less than $F_{0.05}$.

Discussion

Ponds not in active production are normally invaded by organisms such as insects, amphibians and reptiles, as well as trash and predatory fish species. Some of these organisms could predate on the cultured fish or compete with them for food and oxygen if they are not eliminated before stocking. Some aquaculturists in Nigeria use agrochemicals for eradication; other aquaculturists use plants with ichthyotoxic properties. Artisanal fishermen use *T. vogelii* to fish in creeks, pools and isolated channels of principal rivers (Reed et al., 1967; Cox, 1979), while researchers (Hegen, 1985; Ackerman and Bellwood, 2000, 2002) and aquaculturists (Meyer et al., 1976; Orciari, 1979; Beal and Anderson, 1993) use commercial rotenone in fish biodiversity studies and in elimination of unwanted fish species from ponds, respectively.

The 48-h LC_{50} of the acute toxicity tests reveals that *T. vogelii* extract, with a value of 0.24 mg L^{-1}, is more toxic to fish than any of the other test organisms in this study. This value compares favourably with that reported for golden shiners by Orciari (1979). Beal and Anderson (1993) eradicated grass carp from a pond with a rotenone concentration of 6 μl L^{-1}. The 48-h LC_{50} for *Cyclops* and rotifers was 1.04 mg L^{-1} and 2.89 mg L^{-1}, respectively. Beal and Anderson (1993) reported eradication of zooplankton (Copepoda, Cladocera and Rotifera) at a concentration of 3 μl L^{-1}, which was almost three times higher than that reported by Brown and Ball (1942). Hegen (1985) reported that brown shrimps survived only up to a concentration of 1.8 ppm. Mosquito larvae were least sensitive to *T. vogelii* extract, with a 48-h LC_{50} value of 4.48 mg L^{-1}; this relatively high value might be due to the fact that the larvae (*Culex* species) breathe atmospheric air (Ukoli, 1984; Segun, 1989), reducing the rate of extract absorption into the organism. Rotenone is a respiratory inhibitor (Hogson et al., 1988), and exposure of rotenone to the respiratory organs is minimal in mosquito larvae when compared with the other test organisms. The observation that rotifers showed a negative correlation in the bioassay, implying that more rotifers survived at higher extract concentrations, was rather an unexpected behaviour; this observation requires further investigation to elucidate the reason(s) responsible for the pattern.

The rotenone in the *T. vogelii* extract in this study is more toxic to aquatic organisms when compared with other authors who studied plants possessing ichthyotoxic properties. Chen et al. (1996) reported the 48-h LC_{50} of saponin on juvenile *Penaeus japonicus* to be 20.82 mg L^{-1}. Saha and Kaviraj (1996) reported the 96 h LC_{50} of tannic acid obtained from spent bark cinchona to be 55 mg L^{-1} on *Oreochromis mossambicus*. Onusiriuka and Ufodike (1994) reported the 96-h LC_{50} values of spent bark extract of *Blighia sapida* (Akee apple) and *Kigelia africana* (sausage plant) on African

catfish (*Clarias gariepinus*) to be 8.32 mg L^{-1} and 26.92 mg L^{-1}, respectively.

Use of rotenone by Beal and Anderson (1993) eliminated all zooplankton from the water column in a pond. One to eight months were needed for the zooplankton community to recover to pre-rotenone application levels. Cyclopoid reappeared after 30 days, while the rotifers began to re-occur after 45 days.

The benefits of natural foods for fish culture have been reported by Little and Muir (1987). Wade and Stirling (1999) noted that Rotifera and Cyclopoids are nutritious and beneficial zooplankton to fry, juveniles and adult fish in culture systems. Rotifers and *Cyclops* form part of the natural foods for aquaculture in Nigeria. The use of *Artemia salina* by fish farmers in Nigeria is declining because it is expensive. The clearing of ponds before stocking may, therefore, pose a threat to the natural food sources for stocked fry and fingerlings. Beal and Anderson (1993) recommended a delay in restocking until natural communities were re-established. However, the practice by many farmers is usually to stimulate natural productivity of the pond ecosystem by bio-manipulation (Wade and Stirling, 1999) after treatment with ichthyotoxin.

Calow (1992) argued that the gap in relating laboratory tests to natural situations could be bridged by introducing a safety factor of dividing the estimated LC_{50} by 10. When this is applied here, the safety level of the water extract of *T. vogelii* on the test organisms becomes 0.289, 0.104, 0.445 and 0.024 mg L^{-1} for rotifers, *Cyclops*, mosquito larvae and fish, respectively. Given the estimated safety level of 0.289 mg L^{-1} for rotifers, even when concentrations above the LC_{50} value for fish (0.24 mg L^{-1}) are applied to nursery or production ponds, the Rotifera population would not be eliminated, thus providing live food for the fry.

In conclusion, this study revealed that the water extract of *T. vogelii* is very toxic to fish and other aquatic organisms when compared with other icthyotoxins of plant origin. The unregulated use of this plant by artisanal fishermen should be discouraged because of the potential risk posed to natural populations in aquatic ecosystems; concentrations lower than the safety values may exhibit different degrees of chronic effects on aquatic biocenosis and, hence, recruitment into the fisheries of natural water bodies where they are applied.

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