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ACUTE ECOTOXICITY OF AQUEOUS AND ETHANOLIC EXTRACT OF LEAVES OF KHAYA SENEGALENSIS ON CHIRONOMID LARVAE

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ABSTRACT

Adakole, J. A. & Balogun, J. B. 2011. Acute ecotoxicity of aqueous and ethanolic extract of leaves of *Khaya senegalensis* on chironomid larvae. Braz. J. Aquat. Sci. Technol. 15(2): 41-45. eISSN 1983-9057. Protection of aquatic habitat requires an understanding of both the sensitivity of invertebrates to contaminants and their ecological requirements. Phyto-chemical screening of the leaves of extract of *Khaya senegalensis* revealed the presence of active constituents. Acute ecotoxicities of aqueous and ethanolic extracts of leaves of *Khaya senegalensis* to Chironomid larvae were evaluated by static bioassay. Bioactivity of the ethanolic extract was found to be greater than the aqueous extract. The LC_{50} of aqueous and ethanolic extracts were 1.39g/l and 1.20g/l respectively. Stressful behaviors exhibited by the chironomids include deformity of mouthparts, certain body segments being filled with black particles and change in body coloration. All behaviors were dose-dependent. The larvae were not repelled by the extract, indicating that antifeedant activity was not a mortality factor. Mortality was probably due to the disappearance of the reddish coloration of the hemoglobin component. The results were discussed and compared with those of other studies.

Key words: Extracts, bioassay, chironomid larvae, phytochemicals, lethal concentration, *Khaya senegalensis*

INTRODUCTION

Both allochthonous (leaves, flowers, fruits and twigs) and autochthonous detritus support aquatic biota by providing it with an available food supply. However, some allochthonous materials constitute pollutants to receiving waters that could disrupt aquatic productivity and physiological dysfunction in aquatic organisms (Bat & Akubulut, 2001; Henriques-Oliveira et al., 2003). Ecophysiological investigation by David et al. (2000) suggest that dietary tannins and, more generally, phenolic compounds (tannins-phenolics) from leaves decaying in the water of breeding sites are involved in habitat segregation of mosquitoes and other detritus-feeder arthropods.

Alcoholic extracts of leaves and stems of Vanilla fragans were fractionated with ethyl acetate and aqueous butanol (Sun et al., 2001). The aqueous phase appeared to contain no substances that impaired mosquito larval growth. The phyto-chemical screening of the leaves of extract of *Khaya senegalensis* (Africa mahogany), revealed the presence of active constituents such as limonoids including anthraquinones, resins, saponins, tannins and alkaloids (Odebiyi & Sofowora, 1978; Olmo et al., 1997; Nathan et al., 2006). Several studies have shown the relationship between the occurrences of structural and physiological deformities in chironomid larvae and degraded aquatic environmental conditions, resulting from contamination by metals, herbicides, fungicides and insecticides (Sun et al., 2001; Bat & Akubulut, 2001; Hutchinson et al., 2006). The protection of aquatic habitat from natural and artificial damage due to contaminants requires an understanding of both the sensitivity of invertebrates to contaminants and their ecological requirements.

Ecologically, Chironomids form an important group of organisms. The greatest part of their life cycle, the larval stage, is spent in sediment. During the egg stage and the adult midge stage they live in the water and in the air respectively. They are part of different food chains. The eggs, larvae and pupae comprise an important source of food for fish. Chironomids are widespread and abundant in several freshwater systems (Campbell & Nicholas, 1994; Bat & Akubulut, 2001). Clearly, Chironomus spp. larvae have potential as a test species for sediment bioassays in Nigerian waters. Not only do they respond to contaminated sediment, but they also fulfill many bioassay criteria (APHA, 1985; Ingersoll, 1995). Because these organisms spend the majority of their life in sediment, they are continuously exposed to contaminants and they ingest sediment (and contaminants) when feeding. They often occur in high densities and tolerate a wide range of particle sizes.

In this study, the freshwater dipteran *Chironomus sp.* was evaluated as a test organism for use in sediment toxicity bioassays by adapting the standard procedures developed by the ASTM and APHA for conducting sediment toxicity tests. During the study, several experiments were carried out using clean

sediment contaminated with the aqueous and ethalonic extracts of *Khaya senegalensis* leaves and using *Chironomus spp.* as the test animal.

MATERIALS AND METHODS

All the Chironomis riparius used in these experiments were collected from the sediment along shallow pools of a slow moving stream (0.01m/sec.) in Zaria by sieving sediment through a 250 µm mesh size net. The sediments were collected to a depth of 1- 2 cm from the stream stretch known to be relatively clean (Adakole and Anunne, 2003) and free of *K. senegalensis* tree population. Large pieces of debris and other macrofauna were discarded. The animals were kept in de-chlorinated tap water in a tank, which was continually aerated. The body length and head capsule width of the larval instars of *Chironomus spp* were 8-9 mm and 450-500 µm, respectively. The mean Chironomid wet weight was 3.90 ± 0.81mg. The color of the animals was red.

The sediment was washed through a 250 µm mesh-size sieve into a tank in order to remove any macrofauna and larger sediment particles, then washed again through a 250 µm meshes to ensure a standard particle size for the sediment in all the experiments. The sediments were stirred and rinsed three times with tap water, and then allowed to stand for 24 hours. At the end of 24-hour duration, the overlying water was poured off and the sediment placed in test containers.

The leaves of *Khaya senegalensis* were collected from the university town of Samaru, Zaria Nigeria. The leaves were dried at room temperature and then powdered with a mortar and sieved to remove the fibres. One hundred grams of the fine powdered plant leaves were soaked in 300 ml of either distilled water or ethanol and sequentially extracted by shaking for 6 h on wrist action shaker. The preparations were left to stand for a further 24 h. After filtration through Whatmann's filter paper, samples were concentrated to dryness on a water bath at 40°C, packaged in waterproof polythene bags and stored in the refrigerator at 4°C until required (Sanni et al. 2005; Umar et al. 2010). 5.10g and 12.00g of the extract were obtained from 100g powdered *Khaya senegalensis* leaf by means of aqueous and ethanol extraction methods respectively.

The sediments were then treated, by shaking with solutions of *Khaya senegalensis* leaf extract. Sediments with different concentrations of *Khaya senegalensis* leaf extract were obtained through serial dilutions of the two stock (aqueous and ethanolic extracts) solutions. The mixing time was limited to 3-4 hours. The sediments used as the controls were treated as described above, but with dechlorinated tap water. Treated and control sediment supernatants were decanted and the test and control sediments were placed in transparent plastic-glass bioassay containers (10cm x 5cm x 5cm). Clean tap water was added to the containers to 1cm from the top to allow the sediment and water to equilibrate to test conditions and to allow suspended sediment to settle before the addition of the test animals. Each set-up consisted of three replicate containers for each of the 5 concentrations, plus 2 controls. After pilot bioassays following the methods described by APHA, (1985) and ASTM, (1991), the concentrations of each of the containers with aqueous extracted toxicant were 0.0 (control), 25, 75, 100, 125 and 375 mg/l while those with ethanolic extract contained 0.0 (control), 25, 75, 100, 200 and 250 mg/l respectively.

The larval instars were separated from the stock tank on the basis of body length and headcapsule width measured using a light microscope. The containers were examined daily and any dead organisms were removed. Ten fourth-instar larvae were placed in each container. After 1 hour any *Chironomus* that were dead or showed abnormal behavior were removed and replaced and the numbers of individuals that had not burrowed or were dead were noted. Dead larval instars were removed but not replaced. At 96 h, the LC_{50} was calculated by probit analysis and the individuals that had not burrowed were removed, and those remaining in the sediment were counted. Following the standard APHA (1985) and ASTM (1991), two response criteria (survival and avoidance of sediment) were examined for the two *Khaya senegalensis* leaf extract bioassays. The criteria for death were immobility and/or lack of reaction to a mechanical stimulus.

During the period of the bioassays the number of *Chironomus sp.* that had avoided the sediment, either floating on the water surface or lying on top of the sediment, was also recorded daily. All the bioassays were static and the test organisms were not fed during the test period. The pH, dissolved oxygen, temperature and electrical conductivity of the test solution were determined before and at the end of the bioassay.

RESULTS

The ethanolic extract had a lower pH (4.20 ± 0.1) than aqueous extract (5.51 ± 0.01). The mean conductivity of the ethanoloic and aqueous extracts are 383.50 ± 2.11 and 139.92 ± 0.87 µS/cm respectively. The dissolved oxygen ranged between 4.89 and 4.43mg/L for ethanoloic and aqueous extracts respectively, while the mean temperature in the test solutions was 26.78 ± 1.25°C.

There was no mortality recorded in any of the control. No chironomid avoided clean (control) sediment under bioassay conditions, whereas many animals avoided contaminated sediment during the first 24 hours of commencement of bioassay. In the sediments with the highest concentrations of ethanoloic and aqueous Khaya senegalensis extract, avoidance of sediment was not significantly different ($p > 0.05$) from the control. Overall, of the 54 survivors in the bioassay tests, 43 (79.62%) were able to nest in self-made tubes in the sediment at the end of the 4-day period, whereas *Chironomus* survival and burrowing success decreased with increasing sediment extract concentration.

The 96-h LC_{50} of aqueous extract of leaves of Khaya senegalensis' toxicant solution was estimated using probit analysis method. The calculated LC_{50} was 1.39g/L. Stressful behaviors exhibited by the chironomids include change from red to pale body coloration, deformation of mouthparts, mouthparts turning black and 5th – 8th segments being filled with black particles, and loss of antennae.

Similarly, the 96-h LC_{50} of ethanoloic extract was 1.20g/L. Stressful behaviors exhibited by the chironomids include change from red to pale greenish-yellow body coloration, shinning body cuticle becoming dull and flaccid, deformation of mouthparts, mouth parts turning black and 5th – 11th segments being filled with black particles, and loss of antennae. All behaviors were dose-dependent but were relatively more prominent with ethanoloic extract solution.

DISCUSSION

The primary criterion of toxicity tests is survival after duration of exposure to test and control sediments (Bat & Akbulut, 2001). There was no mortality in any of the controls, demonstrating that the holding facilities, water, control sediment and handling techniques were acceptable for conducting the 96-hour sediment toxicity test. Survival is also possible in conditions were there is wet sediment with no overlying water (personal observations). Published guidelines for conducting toxicity bioassays with *Chironomus spp.* recommend that first-to-second instar larvae be used (ASTM, 1991; WRC, 1994). Fourth-instar larvae were used in this study due to the ease of identification and of handling the organisms.

In other laboratory bioassays, chironomid mouthparts deformities were clearly induced after exposure to DDT (Madden et al., 1992), xylene (Janssens de Bisthoven et al., 1997) and 4-n-nonylphenol (Meregalli et al., 2001). Mouthpart deformities of chironomid larvae were also encountered after 96-h exposure to *Khaya senegalensis* extract during this present investigation. Chironomid mouthpart is often used to monitor the quality of sediments in freshwater environments. However, the mechanisms responsible for mouthpart deformity induction have to be fully understood to better interpret the existing results in this field.

The larvae exposed to both ethanolic and aqueous extracts of *Khaya senegalensis* for 96-hours become visible with certain segments being filled with black particles after death. This observation, although open to interpretation, suggest that larvae were not repelled by the extracts/compounds, indicating that antifeedant activity was not a mortality factor. According to Henriques-Oliveira (2003), there are few chironomid species that present nutritional selectivity, with the great majority being generalists and opportunistic feeders. The initial avoidance of the sediment on addition of the toxicants is a reaction by the test organism probably signaling its inability to tolerate the toxicant. Test organisms have been reported to exhibit various reactions at initial contact with toxicants (David et al. 2000; Nathan et al. 2006; Hutchinson et al. 2006). Burrowing by chironomids, according to Hoffman et al., (2007) is essentially an adaptation phenomenon, which among others enables chironomids escape from its predators.

The disappearance of the reddish coloration of the hemoglobin component of the chironomid larvae after exposure shows that mortality could had been caused by bleaching of the hemoglobin component (lost of respiratory pigment), which is required for gaseous transportation and storage purposes leading to impaired metabolism. Several investigators had also made similar conclusions (Schulz et al., 2001; Sun et al., 2001; Meregalli et al., 2001). The discoloration of the larvae from red to pale yellow in the ethalolic extracts may be due to the presence of saponins. Kritzon (2003) reported that saponin taken by fish through their gills to their blood streams acted on their respiratory organ and bleaches the reddish coloration of the gills. Saponin also has haemolytic effect when injected into the blood but not when taken orally (Sanni et al., 2005), while tannins is reputed to have a local effect (as astringent and haemostatic) and anti-inflammatory action (Awosike, 1991).

Ethanolic extract of plant materials possess higher phytoactivity against animals than aqueous extraction of plant materials (Umar et al. 2010). Thus discrepancy between aqueous and ethanolic extractions of Khaya sengalensis' 96-h LC_{50} bioassays for chironomids results, are in accordance with previous laboratory tests. The higher potency (lower LC_{50}) of ethanolic extracts was probably due to the higher extraction abilities of ethanol solvent. Thus ethanolic extract's test solution had a higher electrical conduc-

tivity than aqueous extract during this investigation. Generally, Meliaceae plant products have been shown to exert pesticidal properties against a variety of insect species (Nathan et al., 2006). Toxic bioactivity of alcoholic extract fraction of Vanilla fragrans leaves and stems against mosquito larvae was also found to be greater than that from the aqueous fraction in mosquito growth (Sun et al., 2001).

CONCLUSION

Both aqueous and ethanolic extracts of *Khaya sengalensis* have bioactivity on chironomids. Mortality of chironomid larvae was probably due to the bleaching of the reddish body coloration (lost of respiratory pigments) rather than anti-feeding activity

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