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STUDIES ON THE CHEMORECEPTION PROFILE OF *BULINUS TRUNCATUS* (AUDOUIN) AS AN AID TO THE DEVELOPMENT OF CONTROLLED RELEASE MOLLUSCICIDES.

BY

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Table of Contents:

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DEDICATION:

WITH ALL HUMILITY TO

THE LORD JESUS CHRIST

AND TO

ALL FAMILY MEMBERS WHOSE ASSISTANCE HAS BROUGHT ME THIS FAR.

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DECLARATION:

This thesis is original work produced by the author alone. Where information from other works has been used, references to those sources have been duly cited.

This work in its original form has never been submitted to this or any University for the award of a degree, or any other qualification.

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CHAPTER ONE.

1. GENERAL INTRODUCTION.

1.1 Epidemiology and geographical distribution of Schistosomiasis:

Schistosomiasis is the second most prevalent parasitic infection apart from malaria. Unlike the latter, whose prevalence tends to fluctuate with the rains in many areas of high endemicity for the year, schistosome infection prevalence may be usually high throughout the year in areas where the disease is endemic. Available WHO(1991) document indicates that the disease afflicts more than 200million people while 500-600million are considered to be at risk of infection in 76 countries. Furthermore, mortality rate is estimated to be in excess of 200,000 per year and 20million people are known to suffer from various forms of clinical morbidity and disability due to the infection each year (TDR, 1995; Hodasi, 1995).

Essentially, schistosomiasis is a tropical disease. It affects people of all countries in Africa, except Lesotho, especially those south of the sahara where the disease is endemic. In South America it is endemic in Brazil, Surinam and Venezuela. In the middle-east it occurs in Yemen, Saudi Arabia, Israel and UAR. Some countries in the Caribbean such as St. Lucia, Puerto Rico and Antigua and also the West Indies, parts of China and India have considerable numbers of schistosome infections (Rollinson and Southgate, 1987; Correa and Paraense, 1973). Out of the 76 countries affected by the disease more than 50 occur in Africa and it is estimated that 90% of all cases of schistosomiasis occur in Africa. In addition, every 3 out of 4 children may be infected (TDR, 12^{th} Program report, 1995).

One of the most important causes of the increase in prevalence of the disease in most areas has been the rapid increases in the development of freshwater resources. These developments sought to

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provide sufficient freshwater for agriculture, hydroelectric power and also for domestic use. Unfortunately, the health implications of these developments were not given any serious attention before such programs were undertaken.

1.2 WATER AND HUMAN LIFE.

Water is essential to man, animals and plants and without water life on earth would not exist. From the very beginning of human civilization people have settled close to water sources. Indeed where people live some water is normally available for drinking, for domestic use and possibly for watering animals (Hofke, 1983).

Mayer (1987) remarked that all forms of life on our planet depend on water. Water is the most precious resource on earth. Although there are microorganisms which can do without oxygen, even the most primitive unicellular life forms cannot live without water. Since it is ubiquitous, the presence of water, like that of air, is taken for granted (Ihenkoronye and Ngoddy, 1985).

In general,71% of the earth's surface is covered with water, of which only 1.9% is freshwater; less than a quarter of that occurs in the form of groundwater, lakes or springs (WHO, 1994) .

Due to the unique importance of water to human life the rapidly increasing world population calls for development of traditional water resources for increased output in agriculture , industry and also for domestic supplies. Creation of dams, ponds, and irrigation systems to boost up agriculture and to supply hydroelectric power and also freshwater for domestic use has resulted in rapid increases in prevalences of water-related diseases including schistosomiasis in most of the affected areas. However, in most of these areas no serious thought is given to the health implications of such projects. Even where scientific research gives indications of the health consequences that are bound

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to emanate from such projects no preventive measures are taken in most cases before these projects are carried out. This, in many cases, is due to lack of adequate financial resources as in the case of Ghana and most African countries.

1.3. SCHISTOSOMIASIS IN GHANA :

Available records indicate that both urinary and intestinal schistosomiasis have been in Ghana since the colonial days. Earliest reports of the disease occured in the Annual Report of the Colony of the Gold Coast in 1895. All reported cases of the disease by then, which were derived from hospital records , were only on urinary schistosomiasis . According to Odei (1961) the earliest reported case of intestinal schistosomiasis was in 1920, when Macfie reported the presence of lateral-spined eggs of *schistosoma* in the urine of a male lunatic.

Field work done by many workers since then showed that the disease was present in all the regions of the country with varying levels of prevalence. Edington (1957, as cited by Odei 1964) found that 90% of children in an area in Western Ashanti had the infection whereas in nearby villages there was little or no infection. McCullough (1965) also reported similar variations of infection rates in his work within southern Ghana. He found that more than 75% of children examined at Pokoasi and Maiyara were infected whereas the infection was less than 30% at neighbouring areas like Agbogba, Ashongman, etc. Such variations in infection, the workers noted , was largely due to factors which govern the frequency of exposure of the inhabitants to infection. In addition, it was noted that urinary schistosomiasis was more widely distributed than intestinal schistosomiasis. This was attributed, in part, to the reluctance of the inhabitants to submit stool samples for examination. A more important reason was the relative distribution of the respective intermediate host snails for the

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two *schistosome* parasites responsible for each form of the disease. *Bulinus globosus*, the then known intermediate host snail for *Schistosoma haematobium* responsible for urinary schistosomiasis was more widely distributed whereas *Biomphalaria pfeifferi*, the intermediate host for *Schistosoma mansoni* was more restricted in distribution, mainly around Tarkwa and in the Northern sector of the country (Odei, 1965).

One point worthy of note is that before 1964 (ie the pre Volta lake period) prevalence of both urinary and intestinal schistosomiasis was very low and especially along the Volta river the estimated prevalence was as low as 0.5% (Chinery, 1990). According to Hodasi (1995) the disease was virtually absent from the area during this period. Major foci then were in the Upper and Northern regions and also in large areas of Brong Ahafo, Ashanti, Eastern, Western and Central regions. Two years after construction of the Volta lake, both forms of the disease had spread rapidly to all parts of the country such that by 1967 infection rate was 37% in some villages along the Volta lake and reaching 100% by 1968 (Chinery, 1990).

At the moment, both urinary and intestinal schistosomiasis are endemic in the country but urinary schistosomiasis is more prevalent and has a more widespread distribution. The reasons for this include the following :

1. Creation of the lake gave rise to extensive aquatic vegetation due to nutrients from decaying plants from catchment area. These detached and transported the entrapped snails and their eggs to other parts of the lake downstream where they became established.

2. More extensive distribution of the two main intermediate hosts of *S. haematobium*; *B.truncatus rohlfsi* which is limited virtually to the savanna zones and *B. globosus* which has both forest and savanna distribution (McCullough, 1965).

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3. Localized distribution of *Biomphalaria pfeifferi*, the intermediate host of *S.mansoni*.

4. The fact that urinary schistosomiasis depends more on water than the intestinal type as direct contamination of water by urine is a more common occurrence than ingestion of food and water contaminated with eggs of intestinal worms.

Although the Volta lake is the largest artificial water body worldwide other smaller dams created all over the country also contributed significantly towards the increase in prevalence rate of schistosomiasis in the country. A WHO(1993) document indicated that between 1958 and 1960, 104 small dams were built in the northern parts of the country to provide enough freshwater for both domestic and agricultural purposes especially during the dry seasons. Consequently, prevalence rate of urinary schistosomiasis within 38 areas of these dams surveyed increased from 17% before the dams were constructed to 51% with some riparian communities recording 100% prevalence. Another epidemiological study carried out in settlement areas along the Weija lake near Accra also indicate prevalence rates between 34.2% and 89.4% of urinary schistosomiasis (Zuta, 1994). It can be said that, until recently, there has been a lack of serious effort at controlling the spread of the disease in the country. One reason for this is the insiduous nature of schistosomiasis in general. Unlike other parasitic infections such as malaria in which victims usually exhibit symptoms within a few days after infection, symptoms of schistosomiasis usually appear after two to three months depending on the state of immune responsiveness of the victim. At the moment, some efforts are being made towards the control of schistosomiasis along the lower Volta basin by the Volta River Authority by means of health education and chemotherapy. It is hoped that this would be extended to other parts of the country where the disease is endemic. If this is not done the desired effect of this control measure along the Volta cannot be achieved as human migrations could easily

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re-introduce the disease to the Volta basin again.

1.4 HISTORICAL ACCOUNT OF SCHISTOSOMIASIS.

Available records indicate that the recorded history of schistosomiasis dates back to the 16th century. The Ebers papyrus discovered at that time contained what was thought to be a reference to its treatment or prevention (Macpherson and Craig, 1991).

Both *Schistosoma haematobium* and *Schistosoma mansoni* are believed to originate in Africa, possibly in Central Africa, and *S. Mansoni* was spread around the world during the slave trade especially to South America (Wright, 1966,as cited by Macpherson and Craig, 1991). The first evidence of the earliest records of the disease in Africa was provided by Ruffer in 1910 when he found characteristic eggs of *S. haematobium* in mummies dating back to 1250-1000 B.C. During a post-mortem examination of a patient in Cairo, Egypt, in 1851 the German pathologist, Theodore Bilharz, recovered the first *schistosome* worms and he later linked it to the haematuria in Egyptians discharging terminally spined eggs in their urine. In 1902 Manson described the presence of laterally spined eggs in human faeces in the West Indies and suggested a possibility of there being more than one species of *schistosomes*. This view was supported by other workers and in 1907 Sambon named the worms with laterally spined eggs as *Schistosoma mansoni*. Other species which do not occur in Africa were discovered ; Katsura recovered adult *S. japonicum* from the portal sysem of a cat in 1904 . Leiper in 1915

finally demonstrated the existence of *S. haematobium* and *S. mansoni* as two distinct species which have morphologically different adult worms and eggs, different in distribution in the definitive hosts and a dependence on snails of different genera as intermediate hosts (Abdel- Wahab, 1979).

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1.5 LIFE CYCLE OF PARASITE :

Schistosomiasis is caused by the digenic trematode (commonly called blood flukes) of the genus *Schistosoma*. These flukes belong to the Phylum Platyhelminthes and Family Schistosomatidae. At the moment, nineteen species of the genus are known to cause the disease in mammals and birds but only five of them are significantly responsible for schistosomiasis in man. These include *Schistosoma haematobium* and *Schistosoma mansoni*, which have man as the principal definitive host, and also *S. japonicum*, *S. intercalatum* and *S. mekongi* which are zoonotic in nature infecting mainly herbivorous mammals (sheep, water buffalo, etc.)

1.6 LOCATION OF ADULT WORMS IN DEFINITIVE HOSTS:

The adult female *schistosome* is permanently held in the gynaecophoric canal of the male and the paired worms inhabit various anatomical locations of the definitive hosts depending on which species of the worms are involved. Adults of *S. haematobium* are found in the vesical plexus and sometimes in the portal veins and its mesenteric tributaries. Adults of *S. mansoni* are commonly found in the inferior mesenteric veins and its tributaries. Copulation and oviposition occur at these locations.

1.7 EGGS, MIRACIDIA AND SPOROCYSTS:

The eggs, which are oval in shape and non-operculate are characterised by either lateral (those of *S.mansoni*) or terminal (*S.haematobium*) spines. They secrete enzymes which enable them to move into the lumen of the intestine or the urinary bladder by passing through the venules and tissues.

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Approximately half of the eggs produced are voided 'normally'. The other half go astray and end up in ectopic sites where they embryonate in six days and live for another 15 days. During this period they secrete antigenic materials which are toxic to the surrounding tissues. This invokes immune reactions which lead to granuloma formation. The unshed (unexcreted) eggs are eventually destroyed by macrophages through phagocytosis over a period of months.

Under favourable conditions of temperature, light and osmotic pressure the eggs deposited in the external environment, on reaching a freshwater body, hatch into free-swimming ciliated larvae, the miracidia. These seek and infect by penetration the appropriate snail intermediate host a few hours after hatching to proceed with development. Lytic secretions as well as muscular action are thought to be involved in the mechanism of penetration which usually occurs along the tentacles, mantle collar or the head-foot regions of the snail. There is development into a mother sporocyst which later gives rise to numerous daughter sporocysts. These migrate to the hepatopancreas of the snail and continue development there for several weeks to form cercariae which are later shed by the snail into the water.

1.8 CERCARIA.

This larval stage of the parasite is infective to the definitive hosts including man. When shed from the snail host into the water it remains infective for approximately 20 hours. Infection is by direct penetration of the unbroken skin and mucus membrane of the host. During the penetration process, the cercaria loses the tail, external layer and penetration glands and transforms into a *schistosomulum* which is known to be capable of surviving only in salt water (Warren, 1973). The *schistosomula* move through the tissues of the portal vein into the lymph and blood vessels and eventually reach

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the lungs where they remain for several days. They then migrate to the liver via the blood stream or directly through the tissues. In the liver, they develop into adult male and female worms, move back to either the blood vessels of the bladder or the mesenteric veins of the intestines, depending on the type of worm involved. They then mate and start a new cycle (Warren, 1973).

1.9 MORBIDITY OF INFECTION.

Morbidity of schistosomiasis infection depends on the intensity of the infection. Only a small proportion of the victims have high intensity of infection (Sturrock et al., 1987). The majority of the victims without urgent need for clinical attention remain untreated and act as reservoir hosts of the disease and thus maintain the transmission cycle.

Generally, schistosomiasis morbidity is due to the massive egg output by the adult worms of both *S. haematobium* and *S. mansoni* (Cheever,1968, as cited by Kpikpi, 1990). Only a few of these eggs are expelled from the victim. The rest cause various organomegalies in their victims, the most common ones being splenomegaly and hepatomegaly (Friis and Byskov, 1987). Other notable effects include ureteric strictures, irregularities of the contour of the bladder, hydronephrosis as well as carcinoma of the urinary bladder (Honey and Gelfand, 1960). Most often, some of the eggs may be transported to the brain and spinal cord forming various granulomas which may need surgical operation to remove (Cosnnet and Van Dellen, 1986).

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1.10 SCHISTOSOMIASIS CONTROL.

Considering the increasing incidence of schistosomiasis the world over it is apparent that measures which have been put in place to check the spread of the disease have not been quite successful. At the moment, the emphasis is on reduction of transmission to levels at which the more serious morbidity associated with the advanced stages of the disease are unlikely to occur (WHO,1983a). The various control methods in use at the moment are discussed below.

1.10.1 CHEMOTHERAPY.

Over the years, schistosomiasis control has relied on chemotherapy as the method of choice. The expectation of this method is that by using drugs, in the correct dose, the adult worm load of the victim would be reduced and thereby reducing egg output and hence the disease transmission rate. Among the several drugs that have been tried the most outstanding ones include thioxanthone, antimony compounds (Astiban) (Gothe et al.,1965), Niridazole and Hycanthone. In recent times the drugs have included Metrifonate which is an organophosphorous cholinesterase, Oxamniquine and Praziquantel (WHO, 1993). Most of these drugs, when taken in the correct doses, are able to destroy all adult worms within the victim. Chemotherapy is thus thought to achieve rapid success in schistosomiasis control.

However, the rapid re-infection that follows drug administration tends to obliterate the perceived successes. It has been reported that in order to contain transmission, drug administration needs to be carried out annually or biennually (Sturrock et al.,1987; Polderman and Mashande, 1981; Bensted-Smith et al.,1987;Tingley et al.,1988). It has been argued that schistosomiasis transmission would

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be broken if drugs were used to kill the adult parasites. However, the high cost, logistics and difficulty in getting the full coorperation of all the people makes chemotherapy a less feasible option in schistosomiasis transmission control. It should be seen as a way of offering some relief to victims with serious clinical complications due to high intensity of infection (Kpikpi,1990).

1.10.2 MODIFICATION OF HUMAN BEHAVIOUR .

Many aspects of human behavior patterns tend to encourage the transmission of schistosomiasis. These include ignorance of the transmission patterns of the disease due to lack of appropriate education . A number of surveys conducted in areas where the disease is endemic indicates that there is lack of basic knowledge of the disease (Agudogo, unpublished; Stephenson et al.,1986). Beliefs and customary practices also contribute to increased schistosomiasis transmission. Due to lack of knowledge the disease is considered a natural phenomenon associated with some traditional practices. For example, haematuria in males among the Gongola people in Nigeria is taken to signify the coming of age of the victim (Akogun, 1991) .

Some occupations such as agriculture and also recreational activities that draw humans to water bodies also promote schistosomiasis transmission. Furthermore, efforts to increase water supplies and provide irrigation facilities have encouraged the spread of the disease.

Education of the people in areas where the disease is endemic is normally by means of speech and visual aids to desist from practices that promote and bring them in close contact with infected waters and those which result in more snails becoming infected. Coupled with this is the provision of alternative sources of water for domestic use and toilet facilities for hygienic disposal of human excreta and urine .

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Unfortunately, poor implementation and the lack of adequate logistics have so far resulted in very little being achieved by this control method (WHO/UNDP,1979; el Kholy et al., 1989). In addition, the difficulty of getting people to change from certain normal practices, such as fishing, that requires constant contact with water and the absence of toilet facilities in canoes have all been contributory factors against modification of human behavior (Jordan and Webbe,1982).

1.10.3 SNAIL CONTROL.

The intermediate host occupies an important part of the life cycle of the *Schistosome* parasites. Their control is of vital importance towards the successful control of schistosomiasis. Some features of the snails, however, tend to make their control quite difficult. These include their hermaphroditic nature that enables only a few of them to repopulate new water bodies, their ability to survive out of water over a relatively long period (Olivier,1955; Chu et al.,1967a) and their ability to survive long periods of aestivation in sediments, detritus or vegetation (Thomas and Tait,1984).

Notwithstanding these factors, there have been various strategies for manipulating the biological, chemical and physical environments of the snails with a view to controlling their population dynamics. These strategies are discussed below .

1. *Manipulation of the physical environment* :

This can be done by measures aimed at increasing the mean velocity of moving water in channels beyond certain thresholds. Such measures include stream canalisation, canal relocation with deep burial of snails, removal of vegetation, earth filling, improved agricultural practices and proper drainage in irrigation schemes . All these methods have been observed to increase water velocity and

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thereby decreasing snail populations in them especially when these methods are combined with biological control (Jordan, 1975; Jobin & Michelson,1969; Madsen and Christensen,1992; Ayeh-Kumi,1996 ; Hicklin, 1988;Thomas and Compston, 1980; Jobin et al.,1984).

2. *Manipulating the Biological Environment* :

This involves measures directed at using the biotic components of the environment to control the population of the snails . This may be done by

a) Using competitor snails:

This involves competitive interactions between host and non-host snails. There have been a lot of evidence indicating the elimination of host snails from ponds upon the introduction of competitor species . Barbosa (1973) reported the exclusion of *Biomphalaria glabrata* from a pond when *Marisa conuarieties* were introduced. Also, *Helisoma duryi* has been shown to compete successfully with *Bulinus* and *Biomphalaria* under laboratory conditions (Frandsen and Madsen, 1979; Madsen, 1982, 1983). Other intra molluscan competition tested and proved to be successful recently involved *Melanoides tuberculata* against *Bulinus truncatus* (Ayeh-Kumi, 1996). These competitor snails have been found to also adversely affect miracidial penetration into the snail host by acting as a miracidial sponge (Frandsen,1987). Most of this work is, however, at the experimental stage at the moment.

Much work still needs to be done on this aspect of biological control of the schistosome host snails before large scale field application can be feasible .

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b) Using Microbial Pathogens, Parasites, Parasitiods and Predators :

Parasites and predators are biotic agents that could be used to control population of host snails. Predators such as sciomyzid flies, malacophagous fishes such as *Geophagus brasiliensis*, *Serranochromis* sp. , *Tilapia melanopluera* , some prawns, as well as aquatic birds have long been considered as good biological control agents for *schistosome* host snails (McCullough, 1981; Slootweg, 1989; Weinzettl and Jurberg, 1990) . Recently it has also been found in Kenya, that the American crayfish, *Procambarus clarkii*, is capable of preying on *Bulinus africanus* in large permanent pools of water and thereby reducing schistosomiasis transmission for at least two years (Mkoji, 1997). Micropathogens such as *Bacillus thuringiensis* as well as viruses have been used in biological control of host snails (Osman and Mohammed,1991).

As with the use of competitor snails, most of this work is still at the laboratory stage and a lot more work is needed to be done before field testing can be feasible.

3 *Manipulating the Chemical Environment*:

This involves the use of molluscicides in the control of the host snails. Although there are a large number of plants with molluscicidal activity such as *Phytolacca dodecandra*, *Ambrosia maritima* *Balanites aegytiaca* and *Anacardium* sp.(Webbe and Lambert,1983; Appleton,1985) synthetic varieties such as Bayluscide are most preferred at the moment. This is due to their availability and the rapid results they give in bringing down the snail population. Also apart from 'Endod' which has been given a few field trials, studies on most of these plant molluscicides are still in the laboratory stage of research and development .

Despite their quick action, molluscicide use gives rise to some problems which can best be considered

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as more serious than the snail problem. Notable among these are their adverse effects on non-target organisms due to their broad-spectrum activity and the high cost of the chemicals in the areas where they are needed (Okunji and Iwu,1988).

Owing to these problems it has been suggested that cheaper, species-specific molluscicides should be developed which could selectively kill snail hosts without much effect on non-target organisms (WHO, 1977). To enhance the efficiency of such molluscicides the use of a controlled release system has been proposed (Cardarelli,1977). Thomas and Assefa (1979) suggested that such slow release molluscicides incorporated into specific snail attractants, arrestants and phagostimulants in the form of ingestible and digestible capsules would result in the release of the toxic component in the digestive system of the host snails only after ingestion . This would ensure a higher selective destruction of target snails without adversely affecting non-target organisms.

Since then, much research effort has been directed towards the search for specific attractants, arrestants and phagostimulants for schistosome host snails. Although some successes have been achieved in the identification drive (Thomas and Assefa 1978; Thomas et al., 1980,1983, 1985, 1986, 1989; Thomas,1982, 1986, 1989, Kpikpi, 1990, 1991; Kpikpi and Thomas, 1992, 1993 ;), all the materials found have been pure synthetic chemicals which are more expensive than the synthetic molluscicides in current use. Furthermore, such molecules may possess only one active factor for attracting and arresting the snail hosts and may therefore not be ideal materials.

To this end, Kpikpi (1990) suggested that it should be possible to obtain naturally occuring bioactive materials that could be used as attractants, arrestants and phagostimulants for use in the development of controlled release formulations. The potency of such materials is thought to be due to the possesssion of complex chemical factors. Refinement or processing of some of these materials may

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remove any repellent factors and thereby increasing their attractant, arrestant and phagostimulant properties .

1.11 SCOPE OF THE PRESENT STUDY:

The present study had three major objectives .

First, to identify naturally occurring bioactive materials which act as attractants and arrestant for *B. truncatus* snails under laboratory conditions using diffusion olfactometers . The findings for this are reported in chapter 2 .

Secondly, to explore the possibility of improving upon the bioactive effects of naturally occurring materials by subjecting them to various processing regimes . This is reported in chapter 3 .

In chapter 4, a comparison between the effectiveness of processed and unprocessed naturally occuring bioactive materials used in chapters 2 and 3 is made .

Thirdly, to determine the applicability of the bioactive materials for field conditions by investigating their effects under simulated natural environmental conditions . This is reported in chapter 5 .

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CHAPTER TWO.

CHEMORECEPTION RESPONSE OF *BULINUS TRUNCATUS* TO SOME UNPROCESSED BIOACTIVE PLANT MATERIALS.

2.1 INTRODUCTION :

The control of the intermediate host snail of schistosome parasites forms a major aspect of any integrated program towards control of schistosomiasis. Currently, the method of choice for snail control is the use of synthetic molluscicides due to the rapid kills they tend to produce. The rising costs of these chemicals and their ecologically unacceptable effects are, however, major drawbacks that require a search for more cost-effective and ecologically acceptable alternatives.

Owing to these problems associated with synthetic molluscicides, the Scientific Working Group of the World Health Organization in 1993 recommended the development of cheaper, environmentally compatible and more species-specific molluscicides for control of schistosome host snails WHO (1977). In response to these recommendations, Thomas and Assefa (1978) suggested that known snail toxicants incorporated into specific attractants, arrestants and phagostimulants could be used to selectively remove the schistosome host snails from their natural environments.

Since then some work has been done towards the identification of the species-specific attractants, arrestants and phagostimulants of the main intermediate hosts of the schistosome parasites, *Bulinus* and *Biomphalaria* species (Daldorph and Thomas, 1988; Kpikpi, 1990; Kpikpi and Thomas, 1992, 1993; Thomas and Assefa, 1979; Thomas, 1986, 1989; Thomas et al, 1985a,b) by means of a

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bioassay technique developed by Thomas and Assefa (1979).

Most of the substances so far identified as attractants, arrestants and phagostimulants have been pure synthetic chemicals that are not easily available in the areas where schistosomiasis is endemic. Furthermore, the acquisition of these chemicals may even be more expensive than the molluscicides in current use.

In response to a proposal by Kpikpi (1990) that it should be possible to obtain naturally occurring bioactive substances which could serve as more potent attractants, arrestants and phagostimulants, Kpikpi et al (1995) assayed 26 naturally occurring substances of plant origin using a modified form of the diffusion olfactometer, of these 14 (ie 53.85%) were in their raw unprocessed forms. Among the 5 bioactive materials identified as strong attractants and arrestants, 3 (ie 60%) were unprocessed. This work indicated the need for expansion of the search for even more potent, naturally occurring bioactive materials with multiple chemical factors that can be used as attractants, arrestants and phagostimulants for selective removal of Schistosome intermediate host snails.

In line with these findings, 35 naturally occurring substances in their crude unprocessed forms were obtained from common plants found in schistosomiasis endemic areas in Ghana. By means of similar bioassay technique, these materials were tested for their bioactive effects on both adult and juvenile *Bulinus truncatus*, the intermediate host of *Schistosoma haematobium*, the causative agent of urinary schistosomiasis in Africa.

The results of this study and its possible implications for future snail control are discussed.

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2.2 MATERIALS AND METHODS:

2.2.1 The Snails:

Healthy *Bulinus truncatus* snails were selected from laboratory stocks (plate 2.2.1) originally obtained from the Weija lake, a Schistosomiasis endemic area near Accra(plate 2.2.2). These were fed on fresh lettuce leaves (*Latuca sativa*) over a period of four months. Eggs laid by these snails during the period were separated and allowed to hatch. The freshly hatched snails were fed on fresh lettuce leaves till they attained the desired age for the experiments. Two groups of snails were selected for the assay; the juveniles and the adults. The juveniles were obtained by allowing freshly hatched snails to grow up to 4-6 weeks after hatching. The adults were at least 10 weeks old.

2.2.2 Pre treatment of Snails:

For the bioassay, 25 snails from each age group were carefully selected on the basis of health (mainly those observed to be feeding normally) and weight. Weight of the juveniles was 20- 60mg while that of the adults was 400mg. The snails were weighed just before each assay. Those whose weights fell outside the range were discarded. Individual juvenile snails used for the bioassay were placed in a separate 150ml plastic cylindrical container with 100ml of tap water. The adults were similarly placed in a 250ml container with 150ml of tap water. This was to minimise variations in behavior of the snails (plate 2.2.3). They were kept under a temperature of 26±1°C and photoperiod of 12 hours light and 12 hours darkness and were allowed to acclimatize for 3 days.

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PLATE 2.2.1

LABORATORY SETUP FOR BREEDING OF BULINUS TRUNCATUS

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PLATE 2.2.2

SITE WHERE SNAILS WERE COLLECTED FOR BREEDING.

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PLATE 2.2.3

EXPERIMENTAL SET UP FOR PRETREATMENT OF SNAILS BEFORE BIOASSAY.

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The snails were handled with plastic spoons due to their fragile nature.

Each adult snail was fed daily with a disc of lettuce leaf of diameter 2.0cm while each juvenile snail was given a leaf of diameter 1.1cm. The water medium was changed every other day. In order to reduce response differences among the snails that might be due to their individual differences in physiology, each of the selected snails was deprived of food 20-24 hours prior to the bioassay.

2.2.3 Preparation of Materials:

Thirty-five fresh, unprocessed materials obtained from 26 different common plant species were used for the experiment (Table 1). These were obtained 3 days prior to the test in order to use them in the fresh state. To ensure their cleanliness each sample was washed with tap water and kept in the refrigerator. Ripe fruits used were those that have naturally ripened on the plant.

2.2.4 The Olfactometers:

Diffusion or gradient olfactometers were used for the test. These were similar to those used by Kpikpi et al (1995). Each olfactometer consisted of a central chamber measuring $7.8 \times 1.9 \times 2.0$ cm joined at each end to a cylindrical end chamber measuring 2.4cm in diameter and 2.0cm in depth. A smaller version of the olfactometer used for the assay response of the juvenile snails measured $3.9 \times 0.9 \times 1.0$cm with an end chamber of 1.2cm diameter and 1.0cm depth.

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PLATE 2.2.4

ADULT BULINUS TRUNCATUS FEEDING ON LETTUCE LEAVES.

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Table 1 **Plants and the various parts used as Test Materials.**

Plant Name	Part(s) Used			
	Fruits	Leaves	Stems	Roots
Anacardium occidentalis	*			
Annona muricata	*, (*)			
Azadirachta indica	(*)	*		
Borassus aethiopium	*			
Calotropis procera	*			
Carica papaya		*		
Chrysophyllum albicum	*			
Citrulus vulgaris	*			
Citrus sinensis	*,@			
Citrus aurantifolia	*			
Dialium guinensis	*			
Duranta plumieri	*			
Eleais guinenesis	*			
Ipomea batatas			*	
Latuca sativa		*		
Mangifera indica	*, (*)			
Manihot esculentus		*		*
Musa paradisiaca	@			
Parlaa clappertoniana	*			
Phoenix dactylifera	*			
Saccharum officinarum			*	
Synsepalum dulcilicum	*			

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Plant Name	Part(s) Used			
	Fruits	Leaves	Stem	Roots
Theobroma cacao	*			
Thevetia neriifolia	(*)			
Xanthosoma maffafa		*	*	
Zingiber officinale			*	

Note : (*) denotes unripe form of fruits used.
@ denotes peels of fruits used.

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One perspex block of 20 olfactometers was used (plate 2.25). This made it possible to replicate each test material twenty times. Since the snails have been shown to respond to gravity (Lever and Geuze, 1965), care was taken to ensure that the olfactometers were level. Prior to the experiment each olfactometer was thoroughly cleaned with tap water and kept at the laboratory temperature of 26± 1degree Celsius.

2.2.5 The Bioassay :

The test materials were cut into smaller sizes such as can fit into the olfactometer chambers. The solid test materials such as fruits were cut into small discs of sizes between 7.5×1.5×0.7 and 6.8×1.0×0.4mm using a knife.

The test material was placed at one chamber of the olfactometer while a polystyrene material, which served as the control, was in the opposite chamber. For the non solid test materials which were mainly in powdered form, a small quantity of the sample was placed into the chamber of the olfactometer and covered with a disc of No. 1 Whatman filter paper or cotton wool. In order to counteract any directional bias due to gradients, the test material and the control were alternated in successive olfactometers. 25ml and 5ml of tap water were pipetted into each larger and smaller olfactometer respectively and one appropriate aged snail was placed into the centre of each olfactometer 5minutes later. The time lapse was to allow for the establishment of a diffusion gradient between the test and control chambers.

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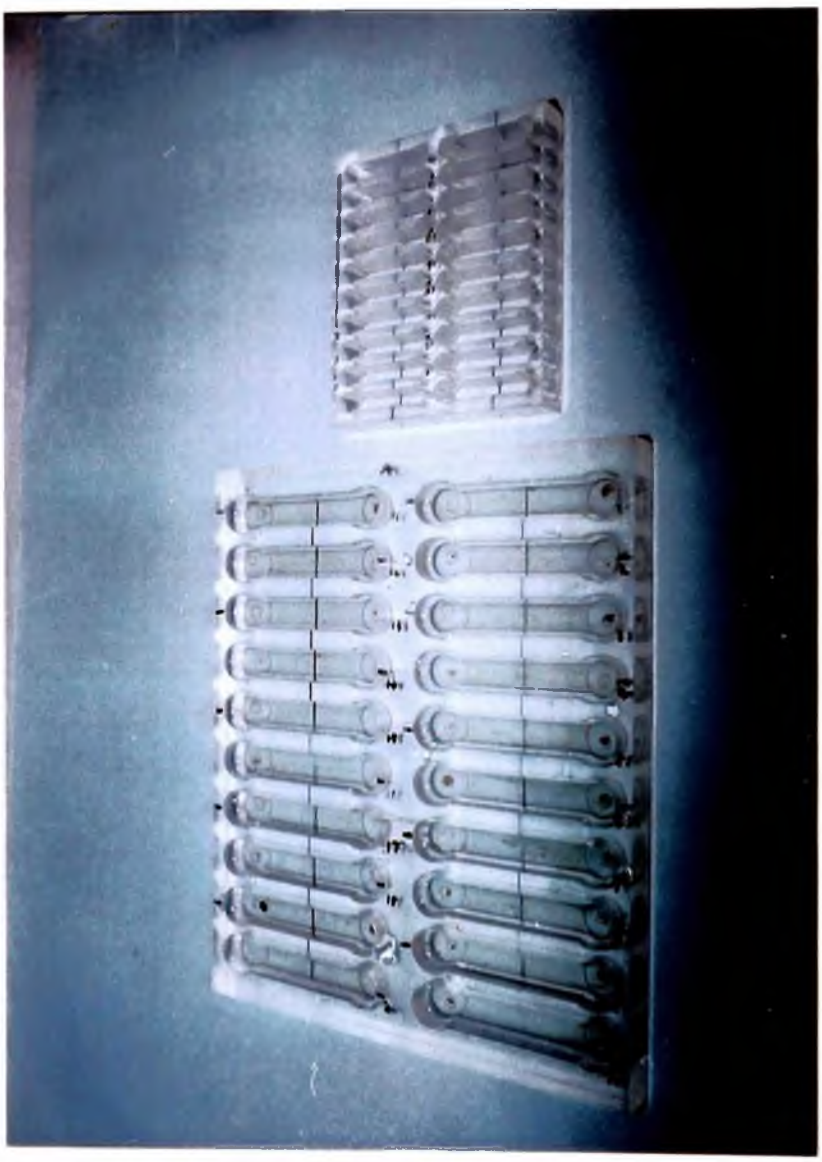
PLATE 2.2.5.

DIFFUSION OLFACTOMETERS FOR BIOASSAY TESTS



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2.2.6 Scoring Snail Positions:

The position of each assay snail in the olfactometer relative to the test material was recorded at $2^1/_2$ minutes interval on a specially designed chart for a period of 30minutes. They were scored positive if found on the test side +, or on test disc (+), and negative if found on the control side −, or on control discs (−) .

Difference between the + scores and − scores was calculated for each olfactometer and the mean difference computed for the 20 replicates to give the attractant index for the test material. Similarly, the mean difference between (+) and (−) was calculated to give the arrestant index for the material. The student t-test (Bailey, 1981) was used to determine the level of significance of the attractant and arrestant effects recorded.

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2.3 RESULTS:

2.3 1 ATTRACTANT EFFECTS:

The present results show that 15 (ie 42.86%) of the unprocessed materials tested emerged as statistically significant attractants to adult *Bulinus truncatus* snails at either $p<0.05$, 0.01 or 0.001 significant levels (Table 2.3.1) These include mango > water melon > fresh sugar cane > ripe date palm > fresh pawpaw leaves > locust beans > fresh cocoyam leaves > ripe cashew, fresh cassava root tuber, lettuce leaves > sweet potato stem >ripe miraculin > sweet potato + lettuce leaves > sodom apple > showers of gold . With response index ranging between 6.7 - 8.8, the five most potent attractants for the adult *B.truncatus* include ripe mango > water melon > sugar cane > date palm > fresh pawpaw leaves (Fig. 2.3.1).

Only 6 (ie 17.14%) of the materials showed significant attractant effects on the juvenile snails at either $p< 0.05$, 0.01 or 0.001 . These include fresh sugarcane > sweet potato stem tuber > locust beans >fresh lettuce leaves >showers of gold > ripe cashew . Of these, fresh sugarcane > sweet potato > locust beans emerged as the 3 strongest attractants with response indices of 10.0, 6.3, and 4.6 respectively.

Since all 6 significant attractants for the juvenile snails are a subset of the attractants for the adult snails, it means that 6 (ie 17.14%) of the 35 unprocessed materials assayed proved to be statistically significant attractants to both adult and juvenile *B.truncatus* at either $p< 0.05$, 0.01 or 0.001 significant levels.

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Table 2.3.1

ATTRACTANT RESPONSES TO UNPROCESSED MATERIALS.

TEST MATERIAL	SCIENTIFIC NAME	ADULTS		JUVENILES.	
		MEAN INDEX	t -value (p< 0.05)	MEAN INDEX	t-value (p<0.05)
FRUITS:					
Ripe cashew	*Anacardium occidentalis*	5.2	4.7720*	3.6	5.9142*
Mango:	*Mangifera indica*				
ripe		8.8	9.9173*	-1.6	-0.8619
unripe		-2.0	-1.1608	-4.9	-2.3390
Water melon	*Citrulus vulgaris*	8.7	15.3412*	0.7	0.3251
Ripe lime	*Citrus aurantifolia*	-3.9	-2.3442	-7.9	-4.2846**
Ripe miraculin	*Synsepalum dulcilicum*	4.8	4.1223*	3.0	1.9407
Orange :	*Citrus sinensis*				
ripe		2.4	1.6250	0.4	0.1819
peels		1.1	0.5165	3.0	1.9407
Ripe date palm	*Phoenix dactylifera*	7.7	8.6917*	1.9	0.8444
Long banana peels	*Musa paradisiaca*	3.0	2.2712	0.6	0.9112
Ripe cocoa :	*Theobroma cacao*				
pod		2.8	2.0881	0.2	0.1966
beans		5.0	4.1943*	1.5	1.5607
Ripe fan palm	*Borassus aethiopium*	0.6	0.5011	1.1	0.9280
Ripe milk bush	*Thevetia nerifolia*	2.9	1.9462	0.1	0.0594
Ripe royal palm	*Eleais guinensis*	2.4	1.2972	2.8	1.3028
Showers of gold	*Duranta plumieri*	3.8	2.5470*	4.0	4.1558*
Sodom apple	*Calotropis procera*	3.9	2.4590*	-2.6	-2.2739

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TEST MATERIAL	SCIENTIFIC NAME	ADULTS		JUVENILES	
		MEAN INDEX	t-value ($p<0.05$)	MEAN INDEX	t-value ($p<0.05$)
Unripe neem	*Azadirachta indica*	-4.9	-2.6252**	-5.0	-2.4837**
Locust beans	*Parlaa clappertoniana*	6.2	5.3880*	6.0	6.5381*
Sweet potato + locust beans	*Ipomea batatas* + *Parlaa clappertoniana*	-1.5	-0.7069	1.1	0.5806
Velvet tamarind	*Dialium guinensis*	0.5	0.2425	-4.0	-2.5379
Star apple	*Chrysophyllum albicum*	-5.2	-3.1628**	-8.2	-4.6628**
Sour sop :	*Annona muricata*				
ripe		2.1	1.2211	0.2	0.1297
unripe		-2.1	-1.5411	1.3	0.9038
LEAVES :					
Cocoyam	*Xanthosoma maffafa*	5.6	3.9268*	1.4	0.7510
Lettuce	*Latuca sativa*	5.2	3.4296*	4.6	3.4544*
Neem	*Azadirachta indica*	2.3	1.3197	0.0	0.0000
Pawpaw	*Carica papaya*	6.7	5.9476*	3.5	1.4935
Cassava	*Manihot esculenta*	2.2	1.1953	2.3	1.8150
STEMS :					
Cocoyam tuber	*Xanthosoma maffafa*	0.4	0.3043	0.4	0.2670
Ginger	*Zingiber officinale*	-7.2	-8.5868**	-9.3	-10.5000**

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TEST MATERIAL	SCIENTIFIC NAME	ADULTS		JUVENILES	
		MEAN INDEX	t-value ($p<0.05$)	MEAN INDEX	t-value ($p<0.05$)
Sugar cane	*Saccharum officinarum*	8.5	13.1477*	10	20.7802*
Sweet potato	*Ipomea batatas*	5.0	5.5624*	63	6.9000*
Sweet potato + lettuce leaves	*Ipomea batatas* + *Latuca sativa*	4.4	3.5501	0.9	0.8112
ROOTS: Cassava tubers	*Manihot esculenta*	5.2	5.6387*	-2.9	-2.1371

Note: * denotes significant attractants at 0.05 confidence limit
** denotes significant repellents at 0.05 confidence limit.

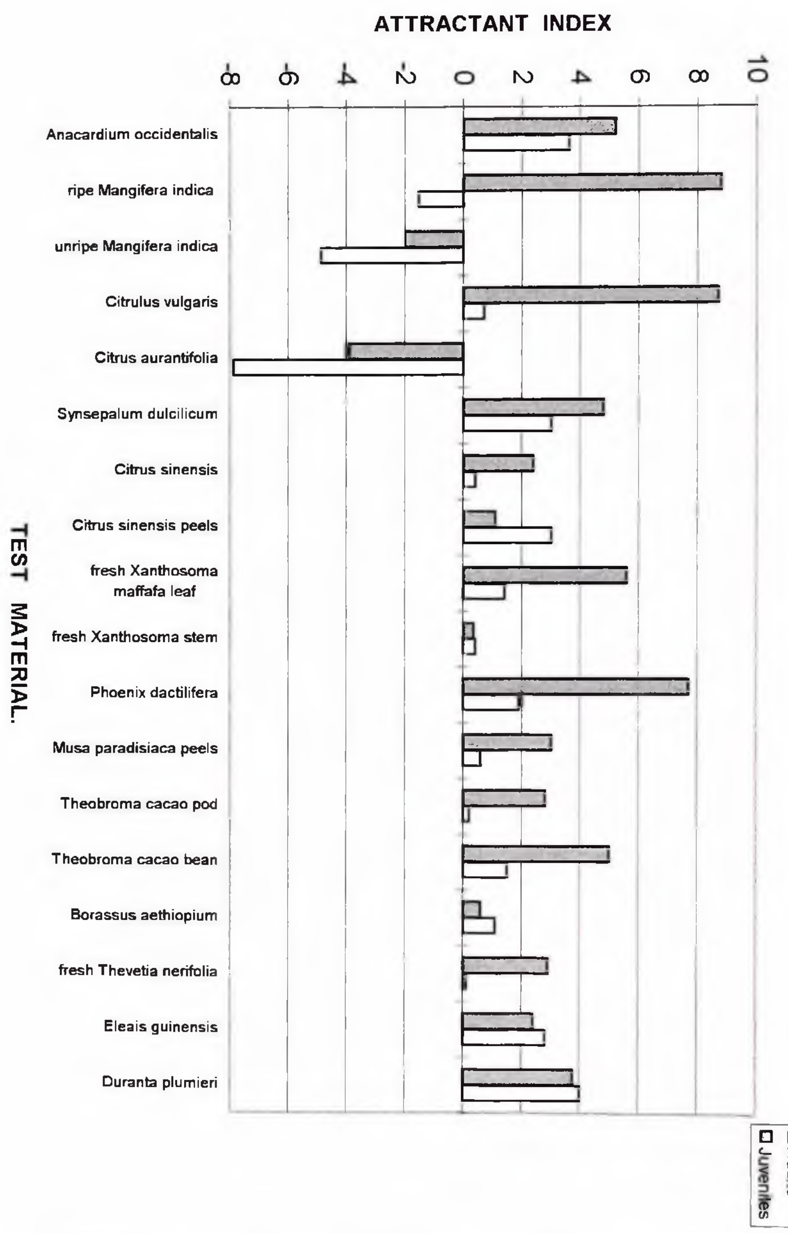

Fig. 2.3.1 ATTRACTANT RESPONSES OF *BULINUS TRUNCATUS* TO SOME UNPROCESSED BIOACTIVE PLANT MATERIALS.

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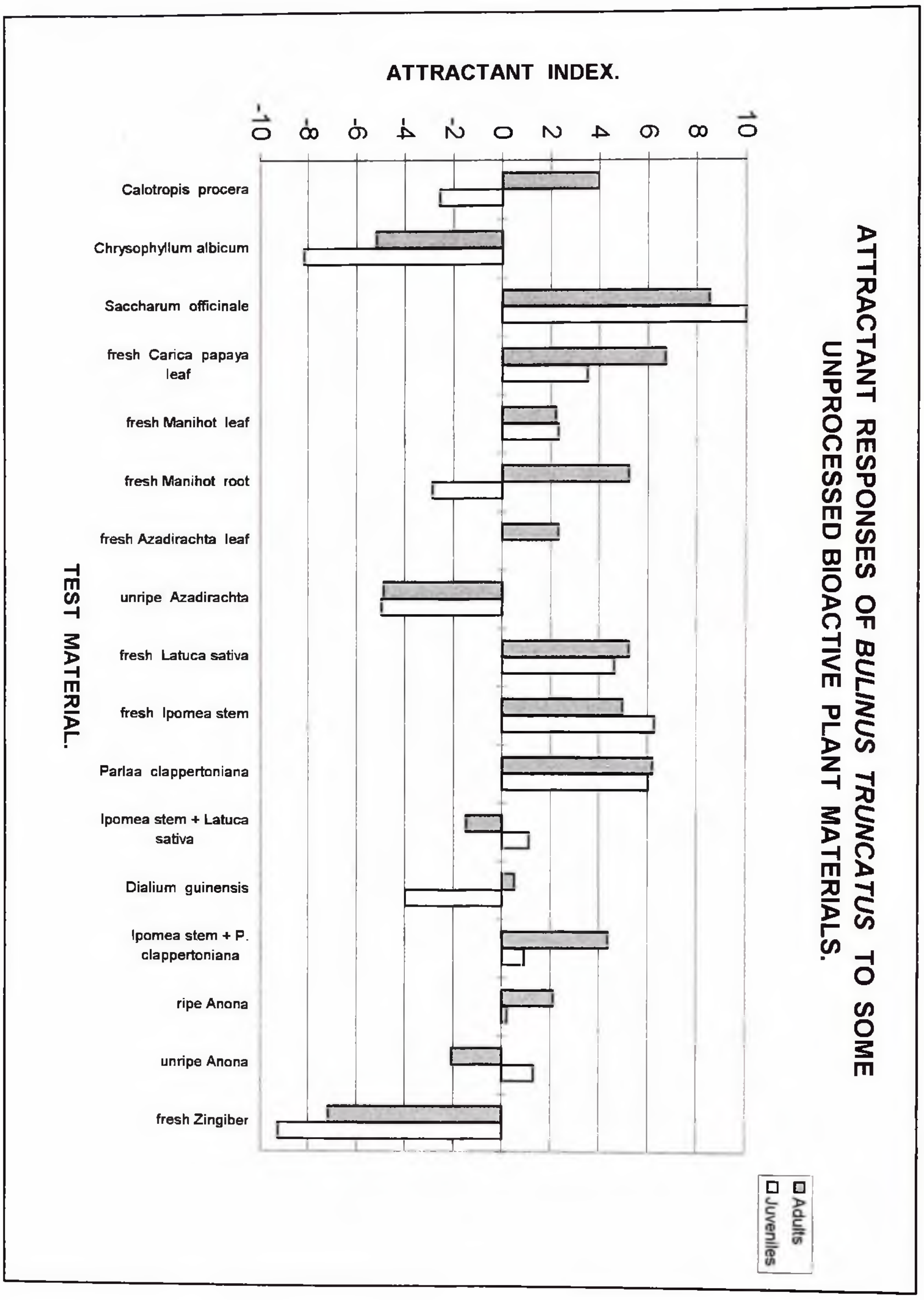

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2.3 2 ARRESTANT EFFECTS:

Only 5 (ie 14.29%) of the unprocessed materials tested emerged as statistically significant arrestants to both adult and juvenile snails at either $p< 0.05$, 0.01 or 0.001 significant levels (Table 2.3.2). These include sugarcane, lettuce leaves, showers of gold, sweet potato and cashew. In addition, 8 other materials also proved statistically significant as arrestants to the adult conspecifics alone while 2 were similarly significant only to the juveniles. These were ripe mango > water melon > fresh pawpaw leaves > date palm > fresh cocoyam leaves > fresh lettuce leaves > sweet potato + lettuce leaves > ripe cocoa beans > fresh cassava root tuber.

In the case of the juveniles, ripe miraculin and fresh cassava leaves with respective response indices of 3.6 and 2.5 were significant arrestants at $p<0.05$ (Fig. 2.3.2).

2.3.3 REPELLENT EFFECTS:

The results also show that 3 materials emerged as statistically significant repellents to *B. truncatus* of both ages at $p<0.05$, 0.01 and 0.001 confidence limits. These were fresh ginger stem > star apple > unripe neem fruit. In addition, lime fruits, ripe cocoa pod and fruits of sodom apple were significant repellents to the juvenile conspecifics alone at $p<0.05$,0.01 and 0.001 significant levels.

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Table 2.3.2 **ARRESTANT RESPONSES TO UNPROCESSED MATERIALS.**

TEST MATERIAL	SCIENTIFIC NAME	ADULTS.		JUVENILES.	
		MEAN INDEX	t - value ($p<0.05$)	MEAN INDEX	t - value ($p<0.05$)
FRUITS :					
Ripe cashew	*Anacardium occidentalis*	2.4	3.1722*	1.9	3.3838*
Mango :	*Mangifera indca*				
ripe		7.3	8.5104*	0.9	0.9873
unripe		0.3	0.6882	-1.9	-2.2549
Water melon	*Citrulus vulgaris*	6.8	9.6372*	2.0	1.5744
Ripe lime	*Citrus aurantifolia*	-0.5	-1.0000	-2.8	-3.2432**
Ripe miraculin	*Synsepalum dulcilicum*	1.6	2.0101	3.6	2.4956*
Orange :	*Citrus sinensis*				
ripe		0.4	0.5674	-0.3	-0.4483
peels		-0.4	-0.4163	2.2	1.9530
Ripe date palm	*Phoenix dactylifera*	5.0	5.6844*	1.8	1.7832
Long banana peels	*Musa paradisiaca*	0.6	0.9112	0.2	0.1482
Ripe cocoa :	*Theobroma cacao*				
pod		0.2	0.1966	-4.9	-4.7840**
Beans		3.2	3.3672*	0.6	0.9524
Ripe fan palm	*Borassus aethiopium*	2.5	1.9493	0.4	0.4963
Ripe milk bush	*Thevetia nerifolia*	2.4	1.5800	1.8	1.5340
Ripe royal palm	*Eleais guinensis*	2.4	1.2972	2.5	1.9493
Showers of gold	*Duranta plumieri*	3.4	4.5888*	3.2	5.1069*
Sodom apple	*Calotropis procera*	3.6	2.2272	-2.3	-2.6706**

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TEST MATERIAL	SCIENTIFIC NAME	ADULTS		JUVENILES	
		MEAN INDEX	t -value (p<0.05)	MEAN INDEX	t- value (p<0.05)
Unripe neem	*Azadirachta indica*	-3.2	-	-3.6	-3.0869**
Locust beans	*Parlaa clappertoniana*	3.9516**		2.2	0.7021
	Ipomea batatas	0.2	0.2912		
Sweet potato	+				
+	*Parlaa clappertoniana*			1.6	1.4220
locust beans	*Dialium guinensis*	-1.7	-1.9450		
	Chrysophyllum				
Velvet tamarind	*albicum*			-0.5	-1.2538
Star apple	*Annona muricata*	0.2	1.2952	-1.0	-1.4001
		0.1	0.4382		
Sour sop :					
ripe				1.9	2.3818*
unripe		2.1	1.7653	0.8	0.6000
	Xanthosoma maffafa	-0.7	-0.8812		
LEAVES :	*Manihot esculenta*				
Cocoyam	*Latuca sativa*			1.1	0.6408
Cassava	*Azadirachta indica*	4.5		2.5	3.5668*
Lettuce	*Carica papaya*	4.3700*		2.4	2.8710*
Neem		2.0	1.7900	0.0	0.0000
Pawpaw	*Xanthosoma maffafa*	4.6		1.7	1.1759

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TEST MATERIAL	SCIENTIFIC NAME	ADULTS		JUVENILES	
		MEAN INDEX	t-value ($p<0.05$)	MEAN INDEX	t-value ($p<0.05$)
STEMS :					
Cocoyam tuber	*Xanthosoma maffafa*	-0.3	-0.2855	1.2	1.1982
Ginger	*Zingiber officinale*	-4.7	-7.4261**	-2.2	-2.9794
Sugar cane	*Saccharum officinarum*	5.0	6.3444*	6.7	9.1393*
Sweet potato	*Ipomea batatas*	2.9	3.9612*	2.7	3.7037*
Sweet potato + lettuce leaves	*Ipomea batatas* + *Latuca sativa*	3.5	3.9294*	1.3	1.4163
ROOTS :					
Cassava tubers	*Manihot esculenta*	3.2	4.0097*	0.1	0.1275

Note : * denotes significant arrestants at 0.05 confidence limit .
** denotes significant repellents at 0.05 confidence limit .

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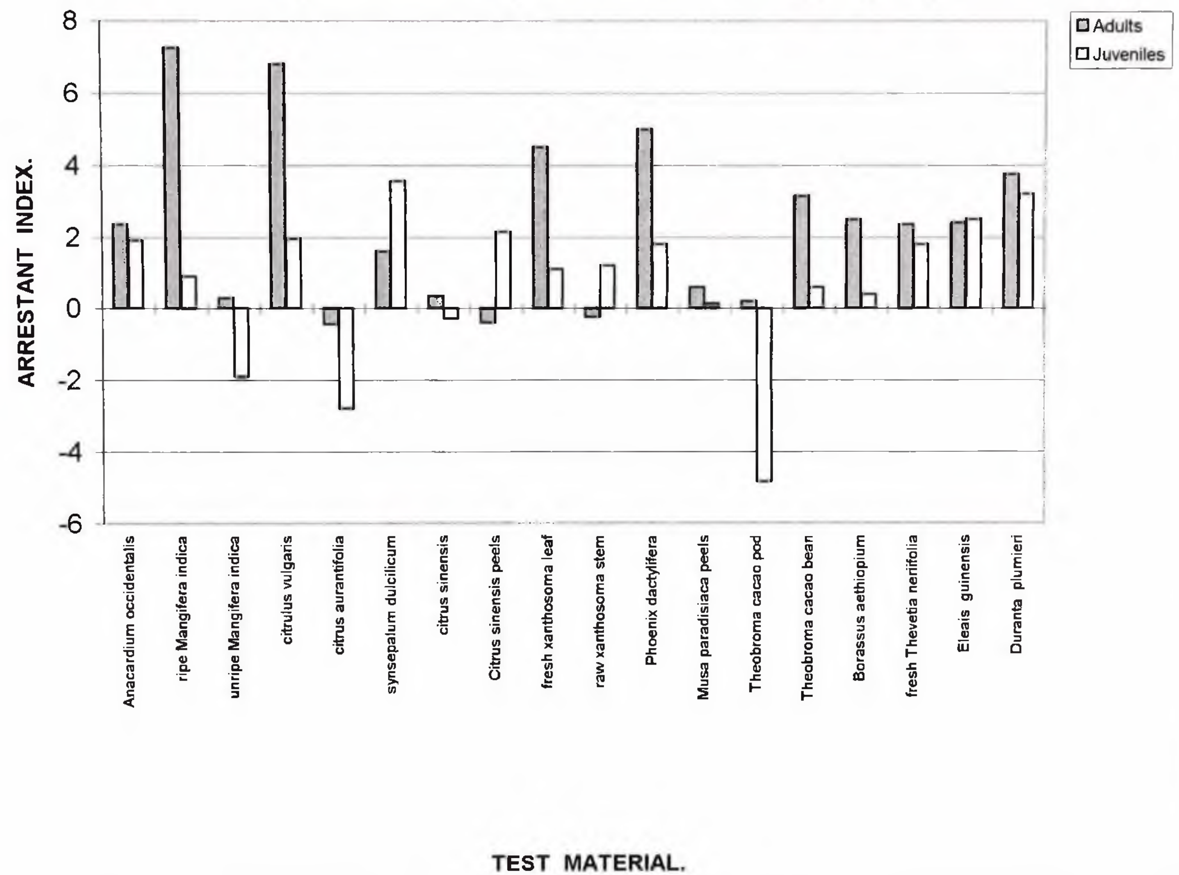
Fig.2.3.2 ARRESTANT RESPONSES OF *BULINUS TRUNCATUS* TO SOME UNPROCESSED BIOACTIVE PLANT MATERIALS.



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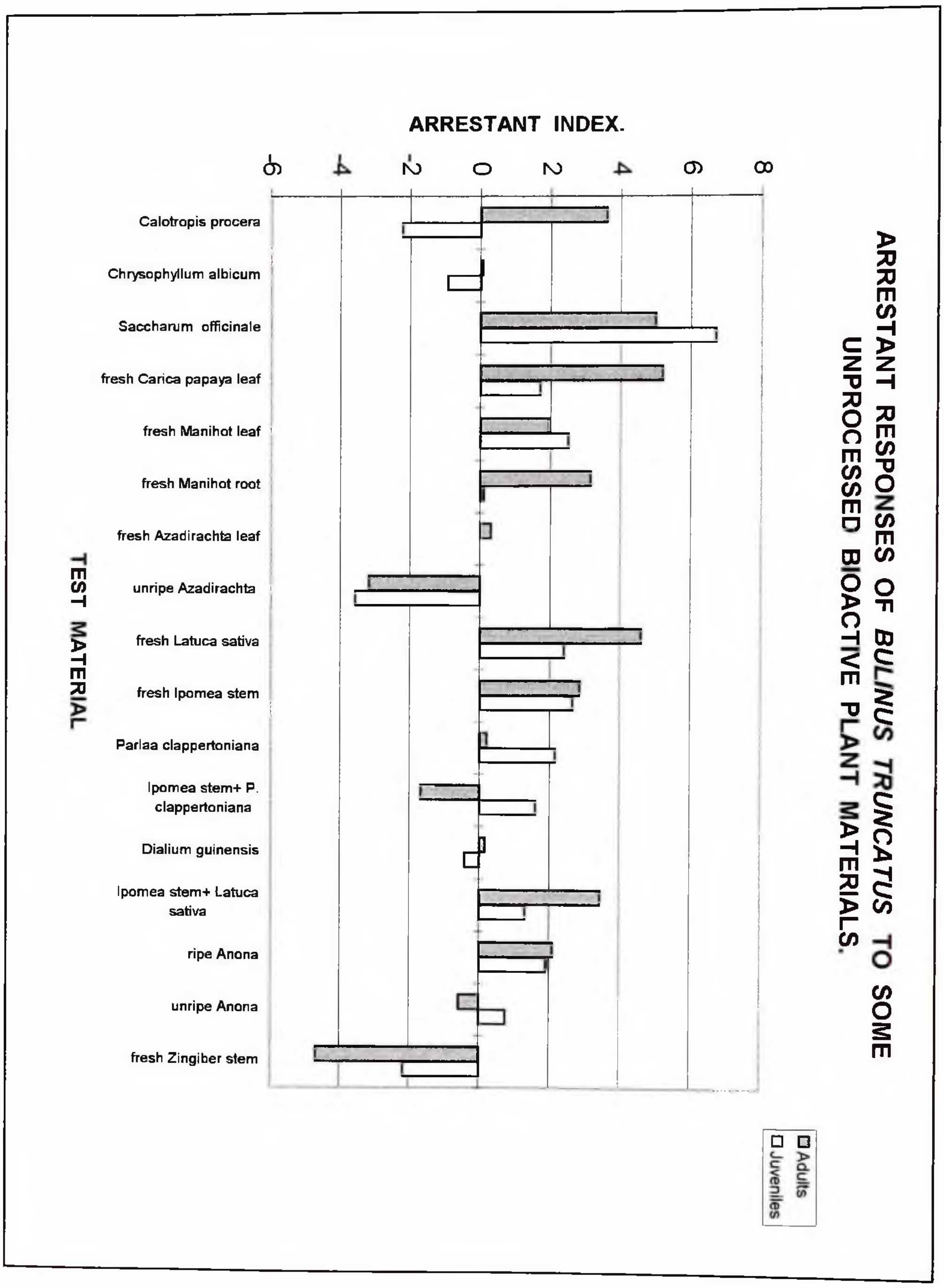

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2.4 DISCUSSION:

2.4 1 Discriminatory responses:

The results indicate that both adult and juvenile *Bulinus truncatus* exhibit significant levels of discriminatory responses towards the materials tested. Thus whereas the adults responded significantly towards 51.43% of the materials juvenile responses were significant towards only 28.57 % of these materials.

This selective chemoreception response is not unique to Buliniid snails alone but has long been observed and documented among other freshwater Planorbid snails and some invertebrates as well as vertebrates (Thomas and Assefa, 1978; Thomas et al, 1980a,b; Thomas, 1982; Kpikpi and Thomas, 1992, 1993). Using pure chemical, these workers have demonstrated that the adult and juvenile *Bulinus rohlfsi* and the South American schistosome intermediate host snail, *Biomphalaria glabrata*, are discriminatory to different extents in their chemoreception responses. Furthermore, the juveniles of both species of snails were found to exhibit a much wider response pattern than the adults.

The pattern of chemoreception niches of *Bulinus truncatus* as shown by the results of this present work seems to be in contrast to those found by earlier workers in that the adults rather than the juvenile exhibited a wider chemoreception niche. A number of factors may be responsible for this observation.

First, unlike in previous works in which single chemical species were used as test materials, the materials assayed in the present work are crude forms (unprocessed) of naturally

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occurring plant materials. These are thought to consist of an array of complex chemical factors (Kpikpi,1990) some of which may be stimulatory, inhibitory or repelling to these snails to different extents. The summation of these different chemical factors determines the direction of response of the snails towards the test material in question.

Secondly, the narrower chemoreception niche showed by the juvenile snails of *B. truncatus* could be attributed to their ages. A juvenile bulinid snail such as *B. truncatus*, unlike the adult, has a relatively immature olfactory sense to be able to significantly respond towards materials with such complex chemical factors. Similar observations were made by Kpikpi et al (1995) on the chemoreception niche of *B. rohlfsi* on some forms of natural bioactive materials with similar complex chemical factors.

2.4 2 RESPONSE TO FRUIT MATERIALS:

One of the important features of the chemoreception niche of adult and juvenile *B. truncatus* as shown by the present results is found in their responses towards the fruit materials assayed (Figs 2.4.1 and 2.4.2). Whereas 9 (ie 39.13 %) of the 23 materials emerged as significant attractants and arrestants to the adults only 3(ie 13.04 %) proved to be significant attractants and arrestants to the juvenile snails. Also, 4 (ie17.39 %) of these fruits were found to be statistically significant repellents to adults while 7(ie 30.43 %) were similarly repellent to the juveniles .

All the fruit materials that emerged as statistically significant attractants and arrestants to these snails are in their ripened states. In addition, with the exception of the date palm fruits these ripe fruits are very succulent and juicy (eg ripe mango, water melon). In contrast, those fruits that

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emerged as significant repellents were unripened and generally hard in texture.

It is known that all green unripe fruits contain protopectins and large stores of carboxylic acids. During ripening, the protopectins are converted to pectins which are complex polysaccharides similar to starches. A large proportion of the acids are also converted to sugars and other flavours . The ripe fruits thus contain far greater amounts of sugars and pectins than the unripe fruits. The observed preference for ripe fruits by these snails could therefore be due to the presence of the large amounts of sugars and other polysaccharides in them. These observations resemble those described by Kpikpi (1991) for sugar chemoreception niche of *B. rohlfsi* and *B. globosus*, although in that case the materials used were pure chemicals. He found that 29.4 % and 23.5% of various polysaccharides and oligosaccharides assayed by means of the diffusion olfactometer were statistically significant as attractants or arrestants to *B. rohlfsi* and *B. globosus* respectively. Thomas et al (1985a) also found that 27.8% of various carboxylic acids were statistically significant as repellents to *Biomphalaria glabrata*. It is possible that the unripe fruits acted as repellents because of the high concentrations of carboxylic acids they contain which may therefore give rise to high H^+ concentrations. As pulmonate snails are generally known to be intolerant to waters of low ph values (Boycott, 1936) the probable cause of the repellent effects of the unripe fruits may be the presence of the H^+ concentrations in them.

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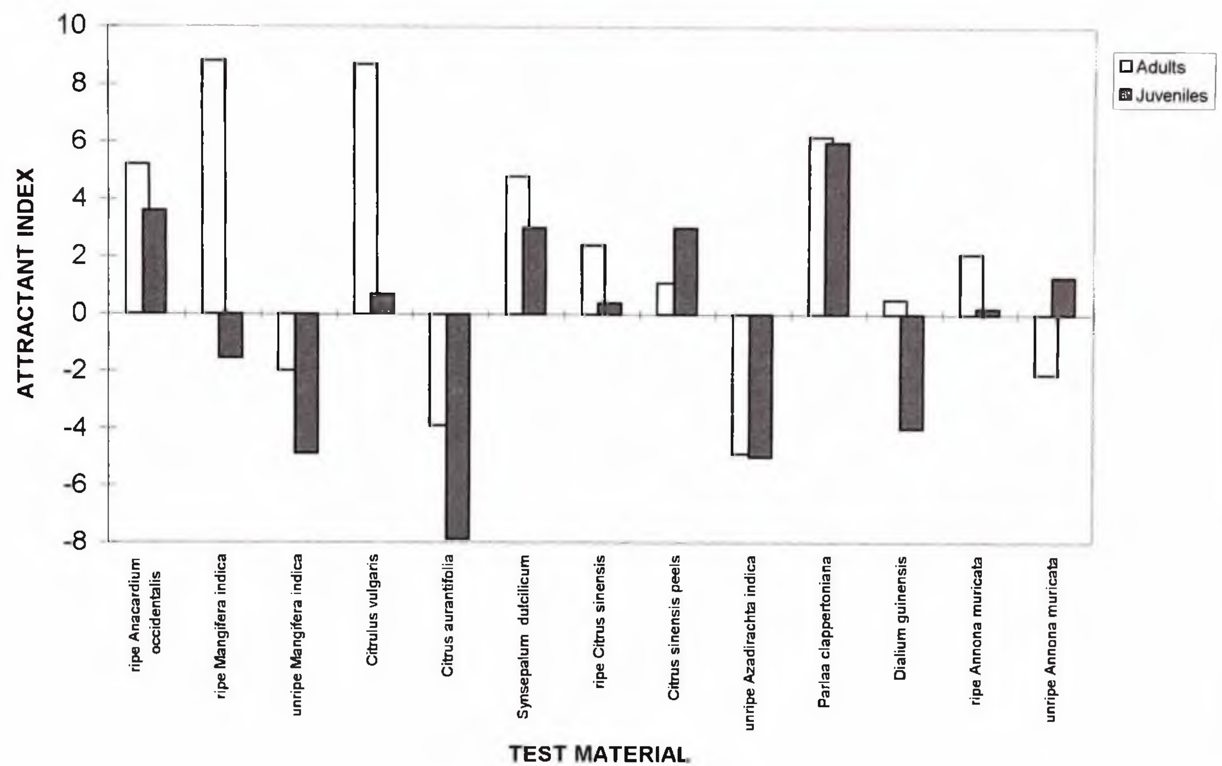
Fig.2.4.1 ATTRACTANT EFFECT OF SOME COMMON BIOACTIVE FRUITS ON *BULINUS TRUNCATUS*.



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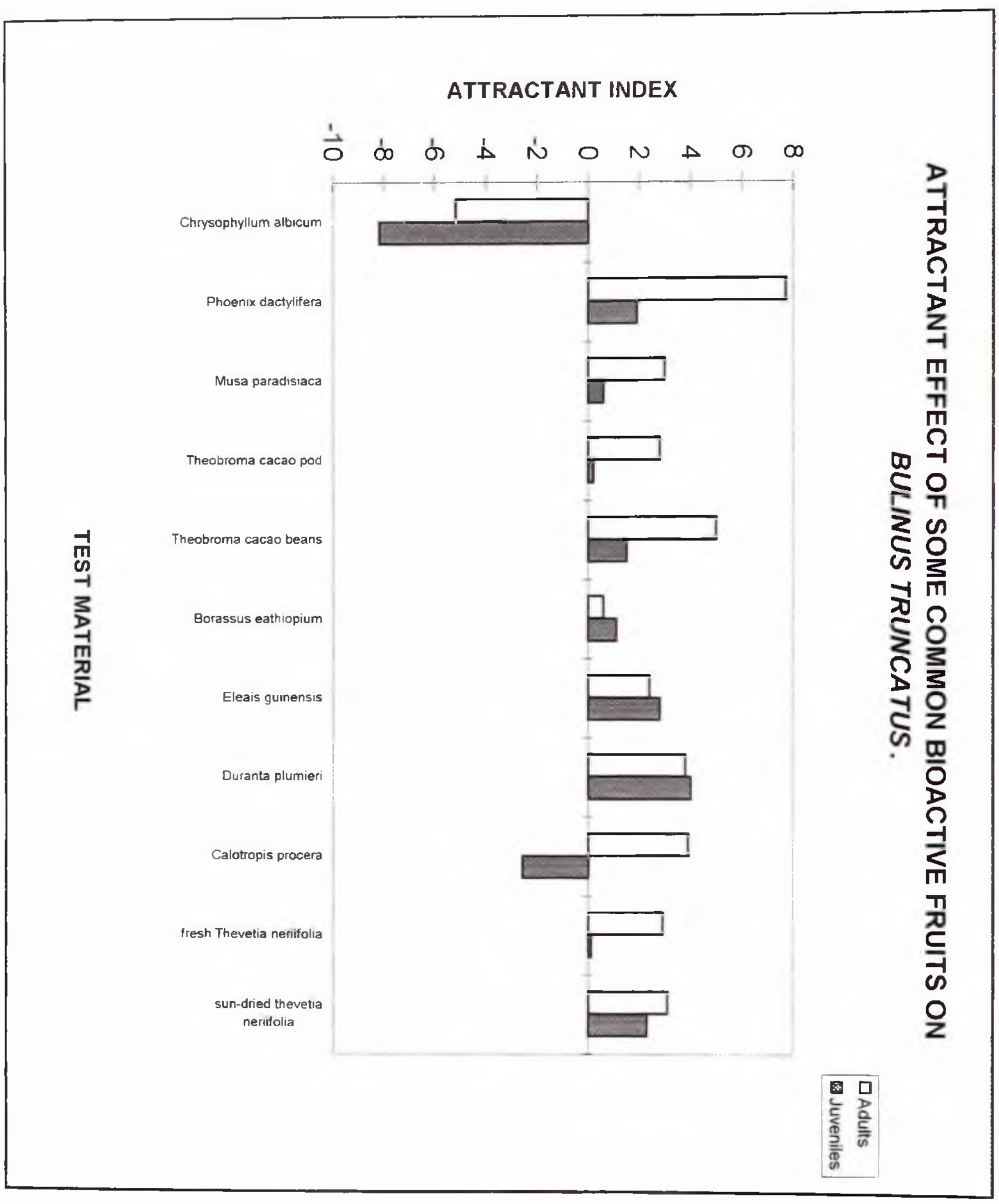

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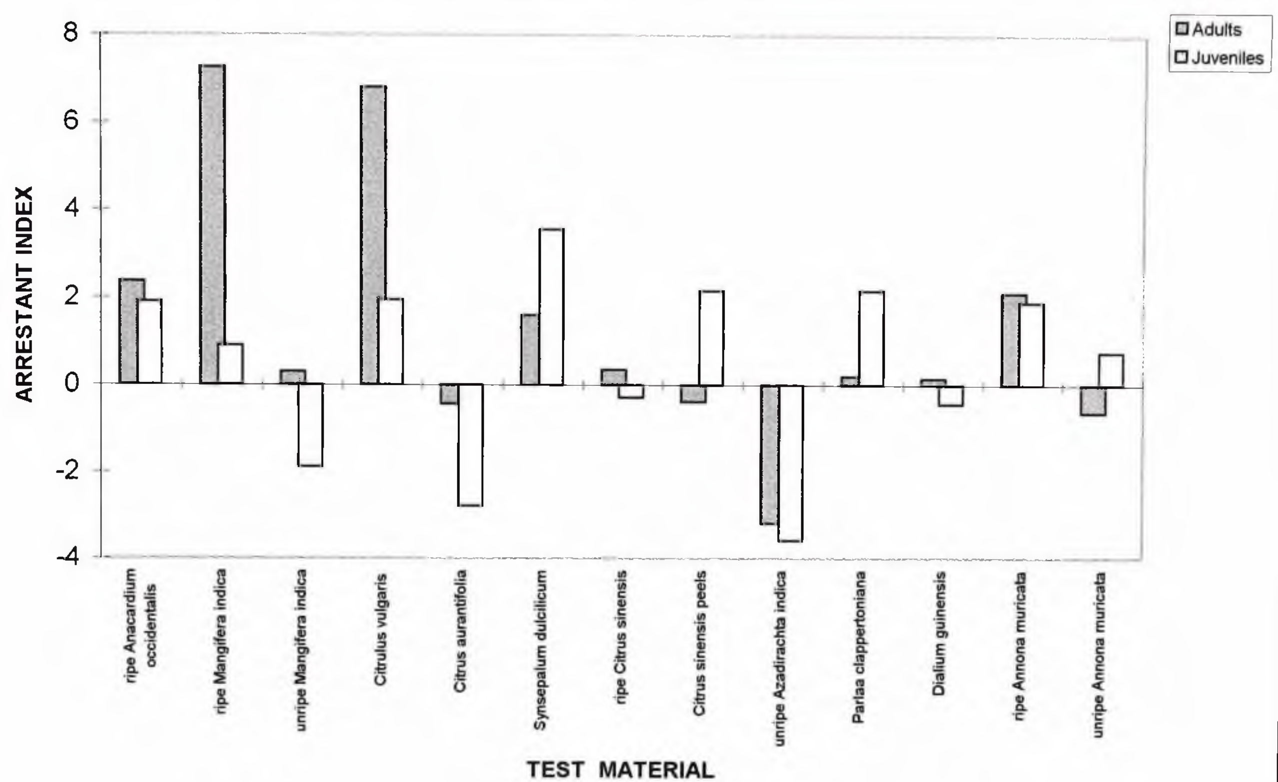
Fig. 2.4.2 ARRESTANT EFFECT OF SOME BIOACTIVE FRUITS ON *BULINUS TRUNCATUS*.



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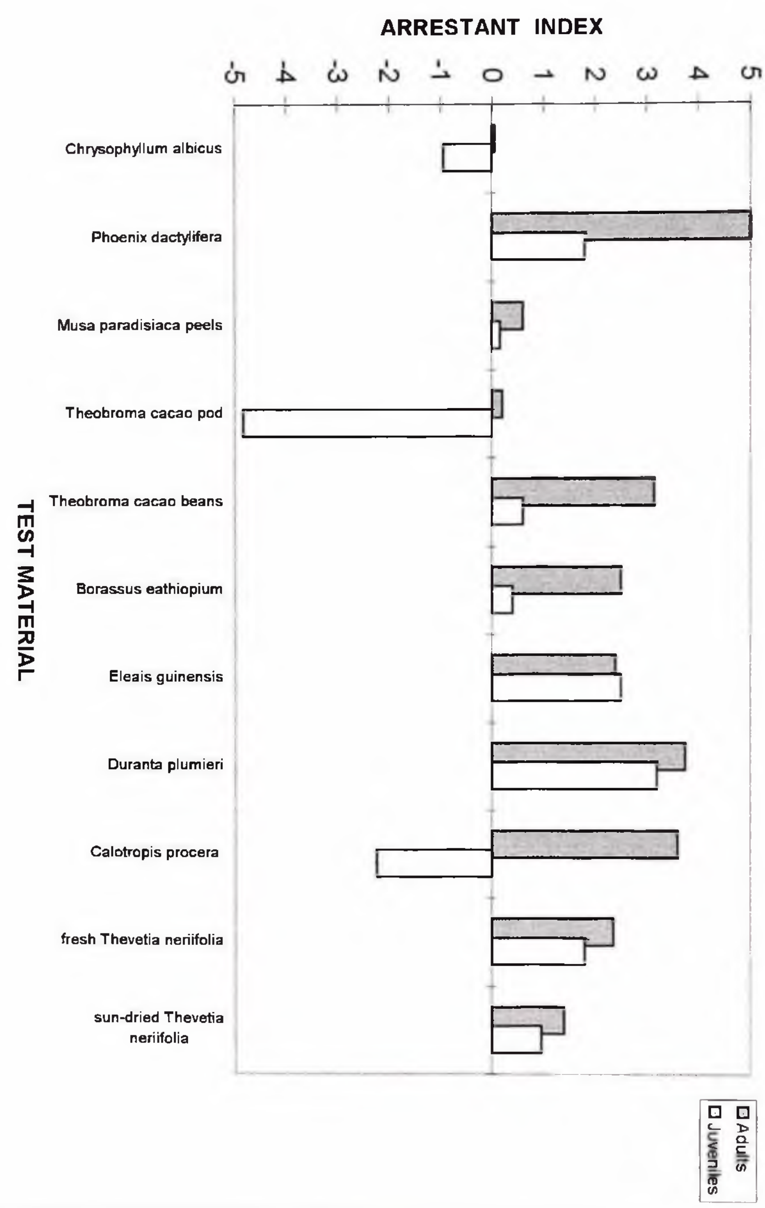

ARRESTANT EFFECT OF SOME BIOACTIVE FRUITS ON *BULINUS TRUNCATUS*.

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CHAPTER 3.

CHEMORECEPTION RESPONSE OF *BULINUS TRUNCATUS* TO SOME PROCESSED BIOACTIVE MATERIALS.

3.1 INTRODUCTION:

The search for potent, naturally occurring bioactive substances to serve as attractants, arrestants or phagostimulants for incorporation with known snail toxicants is an important step towards the development of a more cost-effective environmentally friendly molluscicide for schistosomiasis control.

Since Markowski(1955) demonstrated the possibility of using baits such as decaying palm leaves for the selective removal of snails under field conditions in Africa, it has been shown that plant and animal materials in their early stages of decomposition are generally attractive to pulmonate snails (Pimentel and White, 1959; Sterry et al, 1983; Thomas et al, 1985b; Thomas and Tait, 1984). These materials are known to release various oligosaccharides which form important components of dissolved organic matter (DOM) on which various aquatic organisms such as bacteria (Lodge,1985), freshwater molluscs such as *Pisidium* sp.(Efford and Tsumura,1973), bulinid snails (Kpikpi and Thomas,1992) rely for cues and nutrition.

These observations point to the fact that there exist some chemical factors in these decomposing natural materials that could be utilised as strong attractants, arrestants and

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phagostimulants. This view was demonstrated when Ndifon (1979) observed that the Prosobranch molluscs, *Lanistes lybicus* tend to congregate around a soaked cassava tuber which is known to contain high levels of starch.

Earlier work by Kpikpi et al (1995) in which 12 of the 26 naturally occurring materials assayed were processed by boiling, only 2 of them emerged as significant attractants to the *B. truncatus* snails. This work suggested the need for other methods of processing natural plant materials for the bioassay towards the identification of bioactive natural materials for use in snail control.

Accordingly, naturally occurring materials from 7 different common plants were processed by either fermentation, sun-drying or oven-drying methods to obtain 19 different test materials. Using the bioassay technique, these were tested for their bioactive effects separately on the adults and juveniles of *B. truncatus*. The results and the implications for development of cheaper and target specific molluscicides for snail control are discussed.

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3.2 MATERIALS AND METHODS:

3.2.1 PROCESSING OF MATERIALS :

Oven-drying, sun-drying and fermentation were used to process the materials for the bioassay. The objective of this processing is to alter the form and texture of the materials to find out if a higher chemoresponse could be elicited from these snails under the experimental conditions. In all the processing procedures yielded 19 different materials for the bioassay.

3.2.2. CHOICE OF MATERIALS FOR PROCESSING:

Certain factors were considered in selecting the materials for processing in the present experiments. These include the chemoreception effect of the unprocessed form of the materials on the snails as well as availability and cost of the materials.

a) *Chemoreception effect:*

Some of the materials selected for processing were those that elicited significant chemoresponses from either the adult or juvenile snails in their unprocessed forms. This was to investigate the possibility of obtaining higher response indices from their processed forms.

Similarly those unprocessed materials that proved to be significant repellents were also selected for processing . It was expected that the processed forms could be much stronger repellents.

Based on this criterion leaves of pawpaw (*Carica papaya*), cocoyam (*Xanthosoma maffafa*) and neem (*Azadirachta indica*) as well as stem of ginger (*Zingiber officinale*) were selected for processing .

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b) Availability and cost:

Although some of the materials selected for processing were weakly stimulating in their unprocessed forms their abundance and cheaper cost qualify them for use in the present test. It was envisaged that in case their processed forms prove to be significant attractants or arrestants (or both) it would be much easier and cheaper to obtain them for use in snail control programs.

Based on these criteria, materials selected for processing include milk bush fruits (*Thevetia nerifolia*), cassava root tuber and leaves (*Manihot esculentus*), cocoyam stem tuber (*Xanthosoma maffafa*) and sweet potato stem tuber (*Ipomea batatas*).

3.2.3 PROCESSING PROCEDURES:

a) SUN-DRYING:

Materials processed under this method include leaves of cocoyam, pawpaw, and fruits of milk bush. Fresh samples of each material were placed in a clean open tray and left on a table under sunlight for a period of 7 hours (8am to 3pm) each sunny day for 3 successive days.

After the period the leaves were observed to be changing colour from green to brown and softer whereas the fruits were turning black and softer. These changes were indications of possible onset of decaying due to the action of atmospheric microbes.

b) OVEN-DRYING:

Stems of ginger, root tubers of sweet potato and leaves of cassava were processed under this method. Samples of each material were placed in an oven maintained at a temperature of 70°C for 48 hours.

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c) FERMENTATION:

Materials processed under this method include root tubers of cassava, sweet potato, stem tubers of cocoyam and leaves of pawpaw, cocoyam and cassava.

Fermentation procedure:

Fresh sample of each material was cut into smaller discs of size 1.0 x 1.2 x 1.0cm suitable for the bioassay of the juvenile snails. Larger forms of size 1.5 x 1.4 x 1.2cm were also cut for the adult snail bioassay. Each group of materials was placed in a clean 300ml plastic container, covered completely with 250ml of tap water and closed with a tight fitting lid.

The set up was left under laboratory conditions and allowed to ferment at a temperature of 29± 1°C. For one day fermentation, the samples were allowed to remain in the water for 24 hours whereas for 3 days fermentation they remained in the water for 72 hours. In the case of 7 days fermentation, 168 hours of fermentation was allowed before the bioassay.

In the case of the leaves, each sample was placed in a clean plastic container and closed with a tight-fitting lid. No water was added in this case. This was to slow down the rate of decomposition of the leaves.

3.3.4 THE BIOASSAY:

Diffusion or gradient olfactometers used were same as described in section 2.2.4 of chapter 2. By means of a plastic spoon each fermented material was transfered into the olfactometer chamber while an inert material made of polystyrene was placed in the opposite chamber. 25ml and 5ml of tap water was added to each large and smaller olfactometer respectively as discussed in section 2.2.5.

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The position of each snail was noted at intervals of $2^1/_2$ minutes for 30 minutes.

The scores were computed after the period to generate the attractant and arrestant indices for each processed material used as described in section 2.2.6.

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3.3 RESULTS.

3.3.1 *Attractant effects:*

The results show that 4 (21.05%) of the 19 processed materials proved to be statistically significant attractants to both adults and juvenile *B. truncatus* snails at either $p< 0.05, 0.01$ or 0.001. These include one-day fermented sweet potato >3 days fermented sweet potato >1day fermented cassava root tuber > 3 days fermented cocoyam stem (Table 3.3.1, Fig. 3.3.1) In addition, oven-dried sweet potato stem, sun-dried milk bush fruits, sun-dried cocoyam leaves and 1 day fermented cocoyam stem are also significant attractants to only the adult snails. For the juveniles,7 days fermented sweet potato, 3 days fermented cassava, 7days fermented cassava, 3days fermented cocoyam leaves and 7 days fermented cocoyam stem tuber also proved to be significant attractants. The most potent processed bioactive attractants as found from this experiment for both the adults and juvenile snails were respectively 1 day fermented sweet potato root tuber and 7 days fermented cocoyam stem tuber.

3.3.2 *Arrestant effects:*

Unlike the attractant responses, there were no overlaps in the arrestant response patterns of the adult and juvenile snails as shown by the present results (Table 3.32, Fig 3.32). Whereas 9(ie 47.4%) of the 19 processed materials emerged as statistically significant arrestants to the juveniles, only 3 (ie 15. 8%) showed similar significant effects on the adults at either $p< 0.05, 0.01$ or 0.001 confidence limits. For the adults, oven-dried sweet potato root, sun-dried

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Table 3.3.1

ATTRACTANT RESPONSE OF *BULINUS TRUNCATUS* TO SOME PROCESSED BIOACTIVE MATERIALS

TEST MATERIAL	SCIENTIFIC NAME	ADULTS		JUVENILES	
		MEAN INDEX	t-value ($p<0.05$)	MEAN INDEX	t-value ($p<0.05$)
FRUITS: Sun-dried milk bush	*Thevetia nerifolia*	3.1	2.4042*	2.3	1.6326
LEAVES					
Pawpaw;	*Carica papaya*				
sun-dried		-0.6	-0.3639	-1.7	-0.9892
3 days fermented		3.1	2.1750	2.1	1.5052
Cassava;	*Manihot esculenta*				
oven-dried		-2.4	-2.2592	0.0	0.0000
3 days fermented		3.5	3.3201	4.3	4.3442*
Cocoyam;	*Xanthosoma maffafa*				
sun-dried		3.8	3.4124*	0.1	0.0601
3 days fermented		1.6	1.8973	3.6	3.4112*
Neem; sun-dried	*Azadirachta indica*	-3.2	-2.4596**	-2.0	-1.0603
STEMS					
cocoyam;	*Xanthosoma maffafa*				
1day fermented		3.8	2.3068*	2.8	1.7742
3 days fermented		3.4	2.3421*	8.2	9.8534*
7 days fermented		2.0	1.3942	9.7	5.1805*
Oven-dried ginger	*Zingiber officinale*	-4.2	-3.3008**	-2.1	-0.9617
Sweet potato;	*Ipomea batatas*				
oven- dried		2.8	3.1236*	1.3	0.6729
1 day fermented		5.0	3.7268*	6.5	3.8573*
3 days fermented		3.9	3.5455*	5.1	6.0750*
7 days fermented		2.4	1.7983	5.7	4.5894*
ROOT					
cassava	*Manihot esculenta*				
1 day fermented		4.6	3.2172*	4.1	3.2966*
3 days fermented		-1.8	-1.3878	5.3	3.2505*
7 days fermented		0.7	0.4017	8.5	9.9195*

Note: * = Significant attractants ** = Significant repellents.

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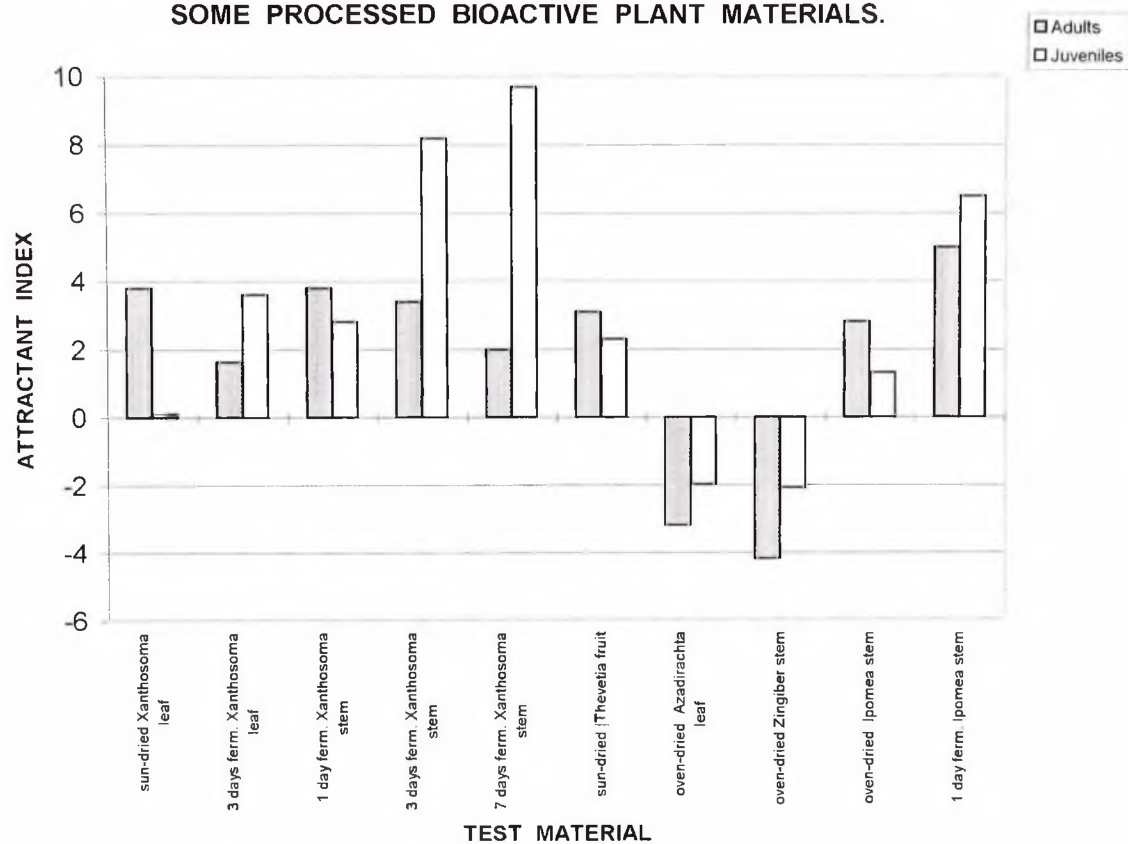
Fig.3.3.1 ATTRACTANT RESPONSES OF *BULINUS TRUNCATUS* TO SOME PROCESSED BIOACTIVE PLANT MATERIALS.



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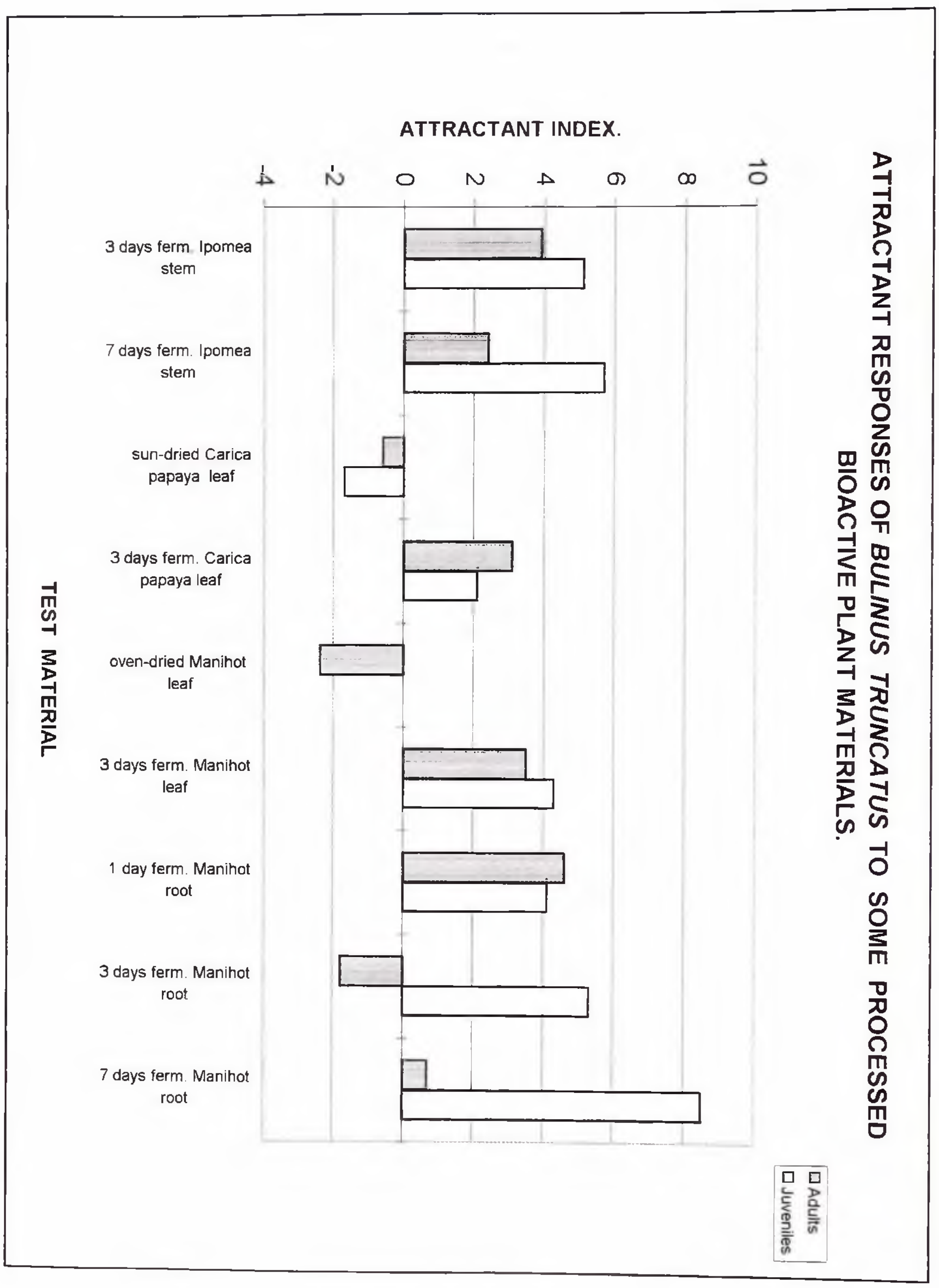

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Table 3.3.2

ARRESTANT RESPONSE OF *BULINUS TRUNCATUS* TO SOME PROCESSED BIOACTIVE MATERIALS.

TEST MATERIAL	SCIENTIFIC NAME	ADULTS MEAN INDEX	ADULTS t-value (p<0.05)	JUVENILES MEAN INDEX	JUVENILES t-value (p<0.05)
FRUITS:					
Sun-dried milk bush	*Thevetia nerifolia*	1.4	1.3500	1.0	1.0402
LEAVES:					
Cassava;	*Manihot esculenta*				
oven-dried		-2.2	-2.5946	0.0	0.0000
3 days fermented		1.8	2.0641	2.8	3.4112*
Cocoyam;	*Xanthosoma maffafa*				
sun-dreid		4.0	4.0000*	1.1	0.9638
3 days fermented		1.1	1.2334	2.3	2.9672*
Neem; oven-dried	*Azadirachta indica*	-3.3	-3.0419**	-0.4	-0.4474
Pawpaw;	*Carica papaya*				
sun-dried		-1.8	-1.4684	-2.1	-2.0243
3 days fermented		2.6	2.1794*	1.0	0.8587
STEMS:					
Cocoyam;	*Xanthosoma maffafa*				
1 day fermented		3.6	3.1887*	0.3	0.2629
3 days fermented		-0.5	-0.4051	4.3	5.5736*
7 days fermented		-0.3	-0.3243	9.5	4.1977*
Ginger; oven-dried	*Zingiber officinale*	-2.6	-2.9620**	-3.7	-4.3744**
Sweet potato;	*Ipomea batatas*				
oven-dried		2.1	2.5071*	0.6	0.4381
1day fermented		1.8	2.1000	2.4	3.4478*
3 days fermented		0.8	1.7098	1.9	2.4852*
7 days fermented		-0.4	-1.1648	4.0	4.0306*
ROOTS:					
Cassava;	*Manihot esculenta*				
1 day fermented		1.0	1.0731	0.4	0.6308
3 days fermented		-1.8	-2.3077	4.3	3.7330*
7 days fermented		-0.6	-0.5901	6.4	7.4453*

Note : * = Significant arrestants ** = Significant repellents.

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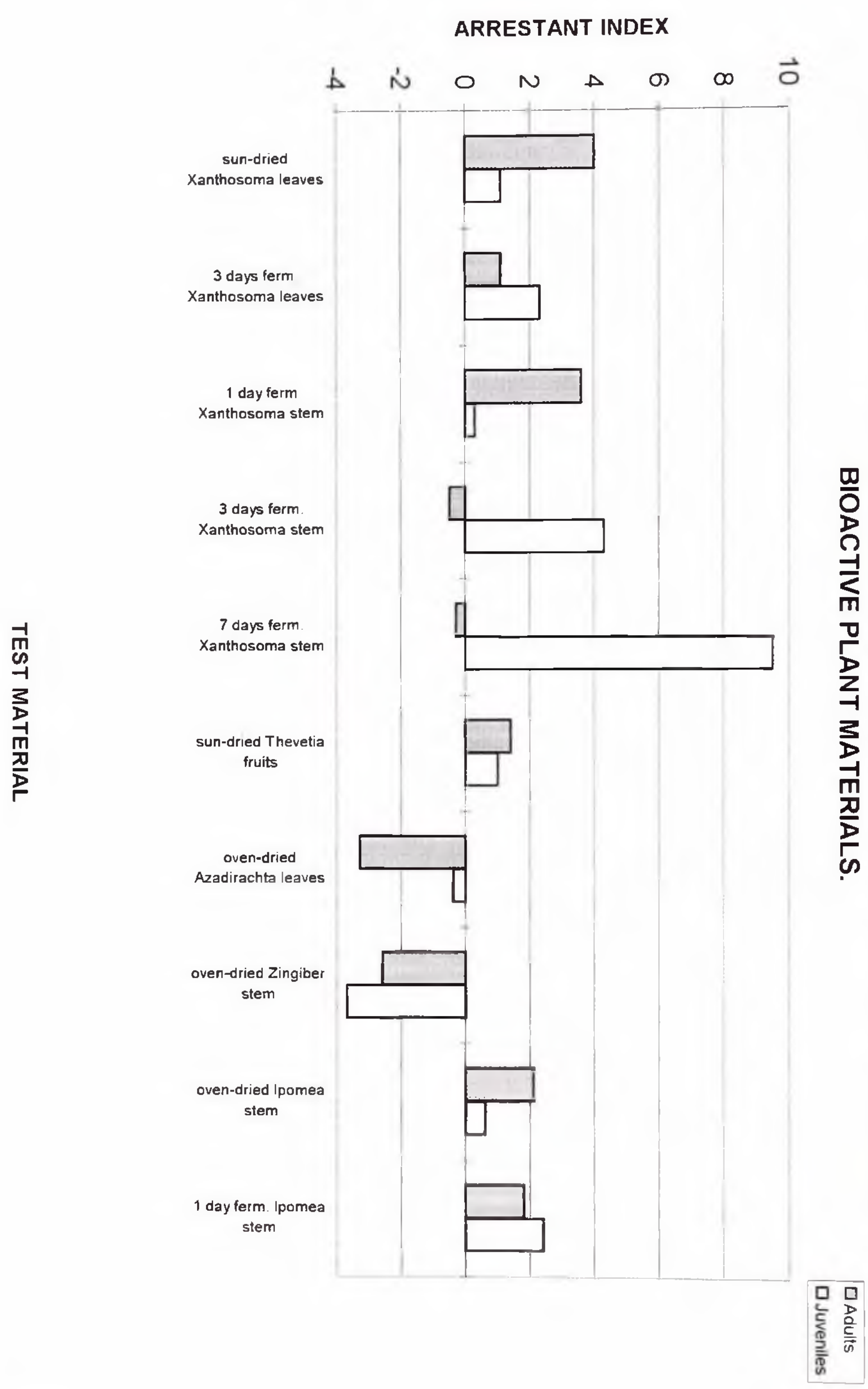
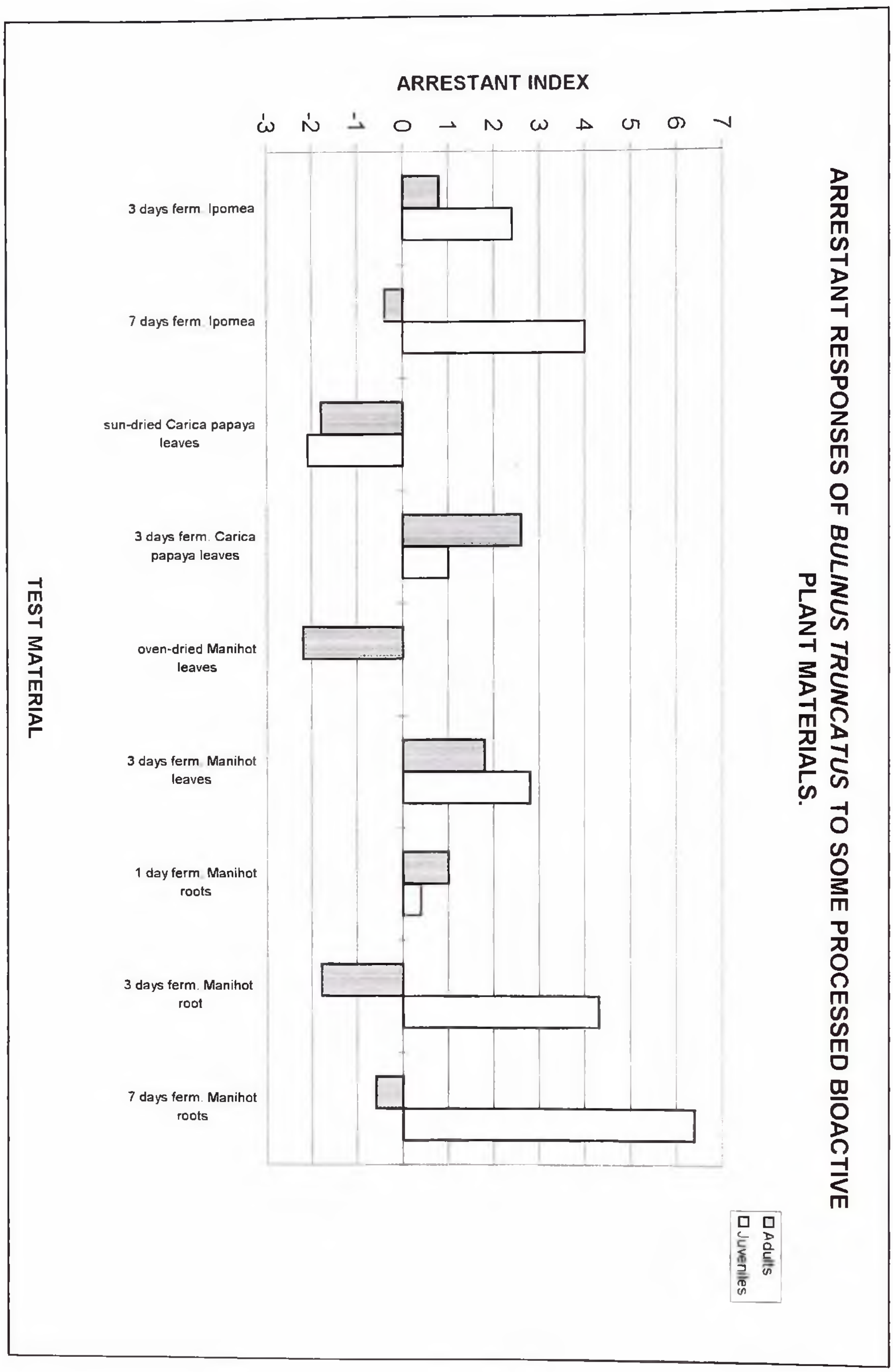


fig. 3.3.2 ARRESTANT RESPONSES OF *BULINUS TRUNCATUS* TO SOME PROCESSED BIOACTIVE PLANT MATERIALS.


ARRESTANT RESPONSES OF *BULINUS TRUNCATUS* TO SOME PROCESSED BIOACTIVE PLANT MATERIALS.
ARRESTANT INDEX
-3
-2
-1
0
1
2
3
4
5
6
7
3 days ferm. Ipomea
7 days ferm. Ipomea
sun-dried Carica papaya leaves
3 days ferm. Carica papaya leaves
oven-dried Manihot leaves
3 days ferm. Manihot leaves
1 day ferm. Manihot roots
3 days ferm. Manihot root
7 days ferm. Manihot roots
TEST MATERIAL
Adults
Juveniles

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cocoyam leaves and 1 day fermented cocoyam stem were found to be statistically significant arrestants with respective response indices of 2.1,4.0 and 3.6. In the case of the juveniles, 7days fermented cocoyam was found to be the strongest statistically significant arrestant with a response index of 9.5. Other significant arrestants for the juvenile snails were sweet potato root tuber (1 day, 3 and 7 days fermented), 3 days fermented cassava leaves, cassava root tubers (3 and 7 days fermented) and 3 days fermented cocoyam stem tuber.

3.3.3 *Repellent effects:*

The present results show that although some of the processed materials elicited negative responses from both adult and juvenile *B. truncatus*, most of these responses gave no statistically significant indices to qualify as repellents. Only oven-dried ginger stem was found to be a significant repellent to snails of both ages. The repellent effect of this material was, however, much stronger on the adults than on the juveniles as shown by their respective response indices (Table 3.3.2). Oven-dried neem leaves were also strong repellent to the adults while on the juveniles the effect was very weak.

3.3.4 *Response to fermented materials:*

With the exception of 3 days fermented cassava root tuber which gave a statistically insignificant negative attractant index for the adult snails, the rest of 12 fermented materials assayed elicited positive attractant indices from both adults and juvenile *B. truncatus*. However, only 5 (ie 41.7%) of these proved to be statistically significant attractants to the adult snails whereas 9 (ie 75%) were

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similarly significant as attractants to the juveniles at either $p < 0.05$, 0.01 or 0.001 confidence limits. More importantly, responses elicited from the juveniles by the processed materials were generally stronger compared to the unprocessed forms as shown by their corresponding response indices (Table 3.3.4, Fig. 3.3.4). None of these fermented materials was able to elicit statistically significant arrestant responses from the adult snails. In fact 5 (41.7%) of these responses were repellent in nature (negative) although their respective indices were not statistically significant enough. Unlike the adult snails, all 12 fermented materials elicited positive arrestant responses from the juvenile snails out of which 9 (ie 75%) were statistically significant (Table 3.3.4).

The most potent, statistically significant processed material with strong attractant and arrestant effect on the adult *B. truncatus* is sun-dried cocoyam leaves (attractant index= 3.8; arrestant index = 4.0). In the case of the juveniles, 7days fermented cocoyam stem tuber, with respective attractant and arrestant indices of 9.7 and 9.5, emerged as the strongest fermented bioactive material.

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Table 3.3.4

RESPONSE OF *BULINUS TRUNCATUS* TO SOME FERMENTED BIOACTIVE MATERIALS.

FERMENTED MATERIAL	RESPONSES							
	ADULTS				*JUVENILES*			
	Attr. Ind.	t- value	Arrs. Ind	t-value	Attr Ind	t-value	Arrs Ind	t-value
LEAVES:								
Cassava, 3 days fermented	3.5	3.3201	1.8	2.0641	4.3	4.3442*	2.8	3.4112*
Cocoyam, 3 days fermented	1.6	1.8973	1.1	1.2334	3.6	3.4112*	2.3	2.9672*
Pawpaw ; 3 days fermented	3.1	2.1750	2.6	2.1794	2.1	1.5052	1.0	0.8587
STEMS:								
Cocoyam tuber;								
1day fermented	3.8	2.3068*	3.6	3.1887*	2.8	1.7742	0.3	0.2629
3days fermented	3.4	2.3421*	-0.5	-0.4051	8.2	9.8534*	4.3	5.5736*
7days fermented	2.0	1.3942	-0.3	-0.3243	9.7	5.1805*	9.5	4.1977*
Potato tuber;								
1day fermented	5.0	3.7268*	1.8	2.1000	6.5	3.8573*	2.4	3.4478*
3days fermented	3.9	3.5455*	0.8	1.7098	5.1	6.0750*	1.9	2.4852*
7days fermented	2.4	1.7983	-0.4	-1.1648	5.7	4.5894*	4.0	4.0306*
ROOTS:								
Cassava tuber;								
1 day fermented	4.6	3.2172*	1.0	1.0731	4.1	3.2966*	0.4	0.6308
3 days fermented	-1.8	-1.3878	-1.8	-2.3077	5.3	3.2505*	4.3	3.7330*
7 days fermented	0.7	0.4017	-0.6	-0.5901	8.5	9.9195*	6.4	7.4453*

* = significant attractants and arrestants. Attr. Ind. = Attractant index. Arrs. Ind.= Arrestant Index

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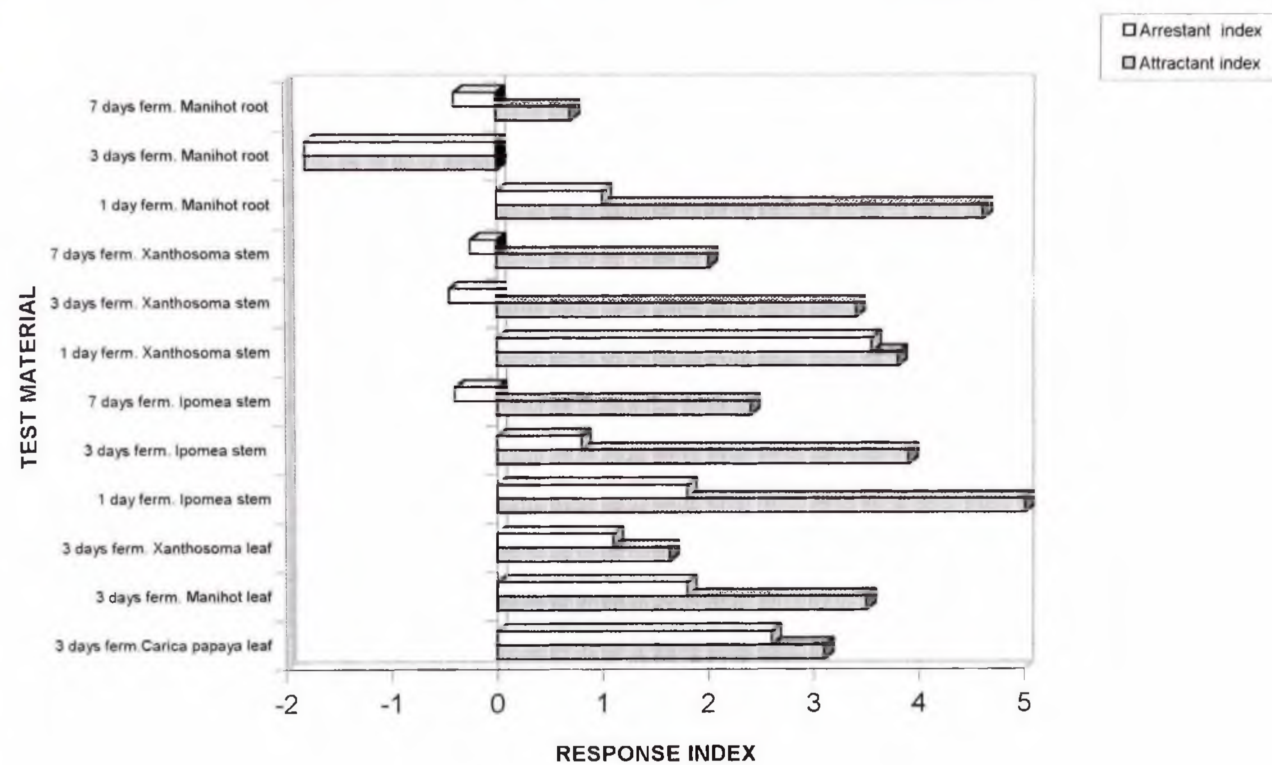
Fig. 3.3.4 CHEMORECEPTION RESPONSE OF ADULT *BULINUS TRUNCATUS* TO SOME FERMENTED BIOACTIVE PLANT MATERIALS.



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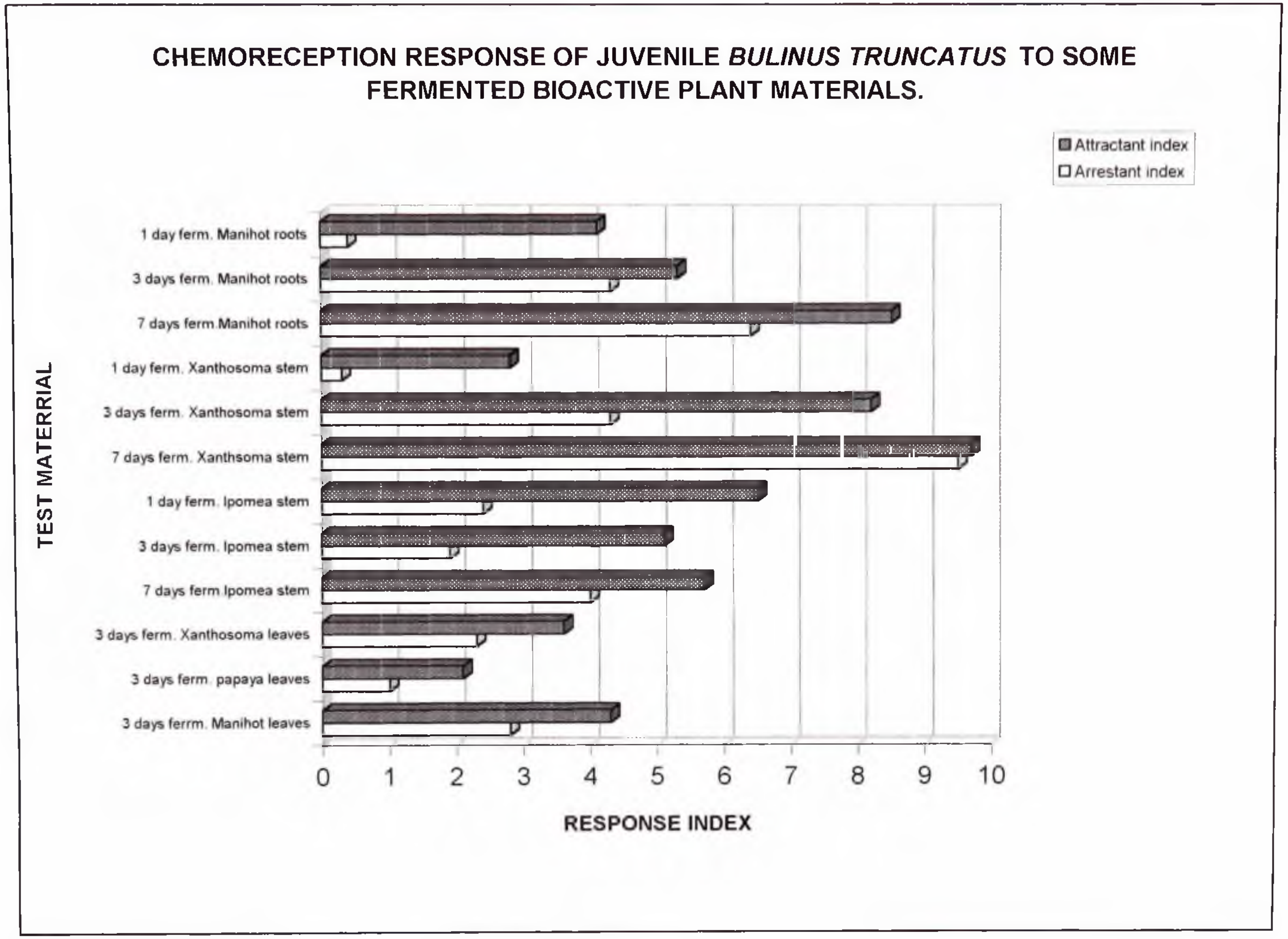

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3.4 Discussion.

3.4.1 Age- specific responses :

The present results clearly indicate that a large number of processed materials are highly stimulatory to both adult and juvenile *B. truncatus* (Fig.3.4.1). The extent of stimulation, however, tends to vary with the age of the snails. Thus whereas 66.7% of the processed materials were found to either attract and/or arrest the adults to a statistically significant extent, 83.3% had similar effects on the juveniles (Table 3.3.1).

With respect to their behavioural responses to processed materials, it is apparent that juvenile *B. truncatus* snails differ from their adults to some extent. First, the levels of stimulation elicited by these materials as indicated by their respective response indices were significantly higher for the juveniles than for the adults (Fig. 3.4.2). Thus, for example, attractant and arrestant indices of 3 days fermented cocoyam tuber for the juvenile snails were 8.2 and 4.3 respectively whereas those for the adults were 3.4 and -0.45 . Furthermore, some of the materials such as 7 days fermented sweet potato and 3 days fermented cassava leaves that significantly attracted and arrested the juvenile snails had very insignificant effects on the adults. The difference in chemoreception niches between adult and juvenile *B. truncatus* snails as found in the present study on processed materials appears similar to those found by Thomas and Assefa (1978) and Thomas et al (1983, 1989) for *Biomphalaria glabrata*, the main South American intermediate host snail, although different snail genera are involved and more importantly pure materials with single chemical factors were used .

These workers found that the juveniles of *Biomphalaria glabrata* exhibited a much wider chemoreception niche in their reponse towards pure amino acids such as asparagine, glutamine and

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citrulline and some short chain carboxylic acids like propanoic acid, butanoic acid and chloroacetic acid. To explain these intraspecific differences in chemoreception niche among these snails, they postulated that niche diversification has evolved among snails to reduce the intensity of intraspecific competition for food, shelter,etc. Furthermore, these observed differences in niche size may be related to differences in feeding habits. As noted by Thomas and Assefa (1979), and Ndifon (1989), juvenile snails tend to feed on a variety of microorganisms such as bacteria, epilithic and epiphytic algae and appear to be more euryphagous than the adult conspecifics that may often subsist on only a few species of aquatic macrophytes. In the natural freshwater environment therefore, juveniles of planorbid snails such as *B. truncatus* and other related molluscs are stimulated by a much wider range of chemical factors to which they are attracted and utilized as food energy sources than their adult conspecifics. This niche diversification is not peculiar to freshwater molluscs alone but it occurs at the interspecific level in various aquatic invertebrates (Lenhoff and Lindstedt, 1974; Bardach, 1975). There is some indication, however, that the behavioural niche of most aquatic invertebrates may be much narrower than those of freshwater planorbid snails including *B. truncatus*.

3.4.2 *Effect of fermented materials :*

One other important feature of the present study is the significantly high attractant and arrestant effects of the fermented materials on the movement of *B. truncatus*. The results indicate that, with the exception of fermented pawpaw leaves, all the fermented materials were capable of significantly attracting and arresting either adult and/or juvenile *B. truncatus* (Table 3.4.2). This result is not unexpected. Fermentation of materials from plant origin is a biochemical process leading to glycolytic production of various chemical species by coccoid bacteria which invade these materials

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at the onset of the process. Prominent among these products are short chain carboxylic acids, especially the C_2 - C_5 groups (Patience et al., 1983; Sterry et al., 1985; Daldorph, 1988). In addition, other chemicals like amino acids such as threonine, serine, glutamine, asparagine and tryptophan known to be present in plant materials (Jabbar-Muztar et al., 1978) are also likely to be released into the external medium during the fermentation process. These materials in their right combinations can attract or arrest the snails since they serve either as sources of nutrient or information in their natural environment (Thomas et al., 1989). An important component of the diet for pulmonate snails such as *Physella acuta*, *Helisoma duryi*, *Lymnaea natalensis*, *L. stagnalis* as well as those that serve as hosts to schistosome parasites are decaying plant materials and associated short chain carboxylic acids released during anaerobic decomposition (Thomas et al., 1982).

It is important to investigate the chemoresponses of other planorbid snails such as *Bulinus globosus* and also *Melanoides tuberculate*(of the family Melaniidae) towards these fermented bioactive natural products. This would help in characterising their chemoreception niches and thereby identifying some of the chemical factors that could be involved in stimulating these snails. At the moment, it has been shown that African planorbid snails such as *Bulinus globosus* are relatively unresponsive to some pure carboxylic acids (Thomas et al., 1982). It is also not yet known, at the moment, which chemical factors within these fermented natural materials that are responsible for stimulating the snails under the present experimental conditions. It is possible that, unlike the pure carboxylic acids with a single chemical factor, a medley of different chemical species present in these natural materials may have a combined effect in attracting and arresting these snails. It may also be that the fermenting process simply decomposes the crude material to release the various chemical

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components (eg C_2-C_5 carboxylic acids, amino acids, etc.) into the water medium. Thus these different chemical species, in the appropriate combinations, would then elicit significant attractant and arrestant responses from the snails.

The present finding that fermenting materials have a stimulatory effect on *B. truncatus* snails also lends credence to the observation by Ndifon (1979) that the prosobranch mollusc, *Lanistes lybicus*, and the planorbid, *B. globosus*, tend to congregate on soaked cassava tubers, a food crop known to contain high amounts of starch and short chain carboxylic acids. It is possible that these snails are stimulated not only by the amino and carboxylic acids but also the monosaccharide and disaccharide units that are also likely products of plant material fermentation (Ihekoronye and Ngoddy, 1985). Many sugars such as maltose, maltotriose, xylose have been observed to attract and arrest some planorbid snails such as *Bulinus rohlfsi*, *B. globosus*, *Biomphalaria glabrata* to various extents (Kpikpi and Thomas, 1993; Thomas, 1986, 1989).

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CHAPTER 4.

RELATIVE EFFECTIVENESS OF UNPROCESSED AND PROCESSED BIOACTIVE MATERIALS AS ATTRACTANTS, ARRESTANTS AND REPELLENTS.

An important feature of the materials used in the present study as indicated by their response indices is the change in their stimulatory effects on *B. truncatus* after processing. With the exception of three test materials, ie oven-dried sweet potato tuber, 7 days fermented cocoyam tuber and fresh cocoyam leaves, where some processed states had the same effects on the snails as the unprocessed forms, the effectiveness of all the other test materials was altered to a large extent by the various processing methods.

4.1 *Effect of processed Leaves :*

The present results show that adult *B. truncatus* are less responsive towards processed leaves. Thus both attractant and arrestant response indices for the adult snails decreased by at least 11.1% when the cocoyam, cassava, and pawpaw leaves were processed by either sun-drying, oven-drying or fermentation (Tables 4.1.1 and 4.1.2). This argument is, however, weakened by the fact that both attractant and arrestant responses of the adult snails towards cassava leaves increased by at least 33.3% when these leaves were fermented for 3 days. Although responses of juvenile conspecifics towards unprocessed leaves is relatively weak, this further reduces by at least 92% when these leaves were either sun-dried or oven-dried. Sun-dried cassava leaves could neither attract nor repel juvenile *B. truncatus* snails although in the unprocessed form it proved to be a significant

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Table 4.11 **Attractant Response of Adult *Bulinus truncatus* to some Unprocessed and Processed Bioactive Materials.**

TEST MATERIAL	RESPONSE INDEX						% CHANGE IN RESPONSE
	UNPROCESSED	*PROCESSED*					
		Sun-dry	Oven-dry	Fermentation			
				1day	3days	7days	
COCOYAM : Leaves	5.6	3.8					-32.14
	5.6				1.6		-71.43
Stem tuber	0.4			3.8			+1166.67
	0.4				3.4		+871.43
	0.4					2.0	+471.43
CASSAVA : Leaves	2.2		-2.4				-209.09
	2.2				3.5		+59.09
Root tuber	5.2			4.6			-11.54
	5.2				-1.8		-134.62
	5.2					0.7	-86.54
SWEET POTATO Stem tuber	5.0		2.8				-44.00
	5.0			5.0			0.00
	5.0				3.9		-22.00
	5.0					2.4	-52.00
PAWPAW : Leaves	6.7	-0.6					-108.96
	6.7				3.1		-53.73
MiILK BUSH : Fruits	2.9	3.1					+6.90
GINGER : Stem tuber	-7.2		-4.2				-41.67
NEEM : Leaves	2.3	-3.3					-239.13

$$\% \text{ increase in response} = \left(\frac{\text{response index of processed}}{\text{response index of unprocessed}} \times 100 \right) - 100$$

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Table 4.12 Attractant Response of Juvenile *Bulinus truncatus* to some Unprocessed and Processed Bioactive Natural Materials

TEST MATERIAL	RESPONSE INDEX						% CHANGE IN RESPONSE
	UNPROCESSED	*PROCESSED*					
		Sun-dry	Oven-dry	Fermentation			
				1day	3days	7days	
COCOYAM : Leaves	1.4	0.1					-92.86
	1.4				3.6		+157.14
Stem tuber	0.4			2.8			+600.00
	0.4				8.2		+1950.00
	0.4					9.7	+2325.00
CASSAVA : Leaves	2.3		0.0				-100.0
	2.3				4.3		+86.96
Root tuber	2.9			4.1			+141.38
	2.9				5.3		+182.76
	2.9					8.5	+293.10
SWEET POTATO: Stem tuber	6.4		1.3				-79.20
	6.4			6.5			+4.00
	6.4				5.1		-18.40
	6.4					5.7	-8.80
PAWPAW : Leaves	3.5	-1.7					-148.57
	3.5				2.1		-40.00
MILK BUSH : Fruits	0.1	2.3					+2200
GINGER : Stem	-9.3		-2.1				-77.42
NEEM : Fruits	0.0		-2.0				-200.0

$$\% \text{ increase in response} = \left(\frac{\text{response index of Processed} \times 100}{\text{response index of Unprocessed}}\right) - 100$$

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attractant and arrestant (Tables 4.1.2 and 4.2.2). Pawpaw leaves, which were potent attractants and moderate arrestants in the unprocessed state tend to become repellents after sun-drying though this repelling property seems to be mild towards juvenile conspecifics. Fermenting the leaves of cassava and cocoyam for 3 days appears to increase their attractant and arrestant effects on the juveniles tremendously. Thus response towards fermented cassava leaves for snails of both ages increased by about 12-87% while in the case of cocoyam leaves, fermenting increased the effectiveness to about 109-157%. Carbohydrate, and hence sugar, content of most leaves including cassava, cocoyam and pawpaw are known to be as low as 6-7mg per 100mg weight whereas the level of mineral salts such as calcium and some amino acids are about 6-60mg 100mg weight (Ihenkoronye and Ngoddy, 1985).

In the natural freshwater environment, snails are known to actively take up calcium and some other ions such as iron and magnesium (Thomas, 1973; Bovaird, 1959). Calcium forms an important component of snail shells and are also essential in reproduction (Jahan-Parwar,1975). Thus the presence of calcium in these leaves may be responsible for the observed responses of the snails towards them. It is also possible that these mineral ions in the unprocessed leaf may be relatively inacessible to the snails as they are more likely to form complexes with other compounds. Processing of these leaves by drying and fermentation leads to breakdown of some of these complexes (Nalewajko & Schindler, 1976) and thus making the calcium ions more available and hence the increased movement towards these processed leaves. It would be of considerable interest to investigate these hypotheses further.

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Table 4.21 **Arrestant Response of Adult *Bulinus truncatus* to some Unprocessed and Processed Bioactive Materials.**

TEST MATERIAL	RESPONSE INDEX: UNPROCESSED	PROCESSED: Sun-dry	PROCESSED: Oven-dry	Fermentation: 1day	Fermentation: 3days	Fermentation: 7days	% CHANGE IN RESPONSE
COCOYAM : Leaves	4.5	4.0					-11.11
	4.5				1.1		-75.56
Stem tuber	-0.3			3.6			+142.20
	-0.3				-0.5		+80.00
	-0.3					-0.3	0.00
CASSAVA : Leaves	2.0		-2.2				-212.82
	2.0				2.6		+33.33
Root tuber	3.2			1.0			-68.75
	3.2				-1.8		-157.14
	3.2					-0.6	-119.05
SWEET POTATO : Stem tuber	2.9		2.1				-26.32
	2.9			1.8			-36.84
	2.9				0.8		-71.93
	2.9					-0.4	-114.04
PAWPAW : Leaves	5.2	-1.8					-133.65
	5.2				2.6		-50.00
MILK BUSH : Fruits	2.4	1.4					-40.43
GINGER : Stem	-4.7		-2.6				-44.68
NEEM : Leaves	0.3		-3.3				-1183.33

$$\% \text{ increase in response} = \left(\frac{\text{response index of Processed} \times 100}{\text{response index of Unprocessed}}\right) - 100$$

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Table 4.22 Arrestant Response of Juvenile *Bulinus truncatus* to some Unprocessed and Processed Bioactive Natural Materials.

TEST MATERIALS	RESPONSE INDEX						% CHANGE IN RESPONSE
	UNPROCESSED	*PROCESSED*					
		Sun-dry	Oven-dry	Fermentation			
				1day	3days	7days	
COCOYAM : Leaves	1.1	1.1					0.00
	1.1				2.3		+109.09
Stem tuber	1.2			0.3			-79.17
	1.2				4.3		+258.33
	1.2					9.5	+691.67
CASSAVA : Leaves	2.5		0.0				-100.00
	2.5				2.8		+12.00
Root tuber	0.1			0.4			+300.00
	0.1				4.3		+4200.00
	0.1					6.4	+6300.00
SWEET POTATO : Stem tuber	2.7		0.6				-79.25
	2.7			2.4			-11.32
	2.7				1.9		-30.19
	2.7					4.0	+49.06
PAWPAW : Leaves	1.7	-2.1					-223.53
	1.7				1.0		-44.12
MILK BUSH : Fruits	1.8	1.0					-47.22
GINGER : Stem	-2.2		-3.65				+65.91
NEEM : Leaves	0.0		-0.4				-35.00

$$\%\ \text{increase in response} = \left(\frac{\text{response index of Processed}}{\text{response index of Unprocessed}} \times 100\right) - 100$$

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4.2 *Effects of processed stem tubers :*

Attractant and arrestant responses of both adult and juvenile *B. truncatus* towards oven-dried sweet potato stem tubers appears to have been reduced relative to those of the unprocessed form. Extent of reduction, however, is much greater for the juveniles (-79.17 - 79.20%) than for the adults (-26.32 - 44.00%). Responses of these snails towards cocoyam greatly improved after fermentation. In the case of the adult snails, attractant response increased by at least 471% while for the juveniles it was 600%. There is an exception to this trend; the stem of ginger in both the processed and unprocessed forms proved to be strong repellent ($p<0.05$) for snails of both ages. The effectiveness of this repelling property however, decreased by about 50% for the adult snails while it increased by 77% in the case of the juvenile conspecifics after oven-drying.

4.3 *Effects of processed roots :*

Unprocessed cassava root tubers appear to be highly stimulatory to juvenile *B. truncatus*. Attractant responses to cassava roots tubers increased tremendously for adult snails after fermentation by about 140-293% while there was a 69-120% decrease in arrestant response after processing. In the case of the juveniles, an even greater increase in arrestant response of about 300-6300% was observed when the cassava tubers were processed by fermentation. Attractant response also increased although to a much lower extent than that of the arrestant.

4.4 *Processed Repellents:*

The present results show that the ability of bioactive materials to repel *B. truncatus* snails appear to

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have been lost or significantly reduced after processing. Ginger stem, a strong repellent in the unprocessed state, had its repellent effects reduced by about 42% when processed by oven-drying. Furthermore, some materials such as cocoyam tuber, which proved to be a weak repellent for the adult conspecifics in the unprocessed form, had this property reduced by at least 142% and become a significant arrestant ($p<0.05$) to the adult snails after 1 day fermentation (Table 4.2.1). On the other hand, some bioactive materials such as cassava and pawpaw leaves, became weak repellents ($p<0.05$) to snails of both ages after processing whereas in the unprocessed state they proved to be strong attractants and arrestants.

The present results also point to neem leaves as possessing some repellent effects on both adult and juvenile *B. truncatus*. In the unprocessed form these leaves appear to be neutral to the juvenile snails but weak attractants and arrestants to the adults at $p< 0.05$ confidence limit. After oven-drying, they showed some significant repellent effects ($p<0.05$) on snails of both ages with these effects being much lower on the juvenile conspecifics.

The implication of neem plant in the present work as possessing some repelling effect on *B. truncatus* is of interest. The repellent properties of all parts of the neem plant against terrestrial organisms especially insects is well known (Arnason et al.,1988; Ketkar et al.,1976). The most important active ingredients in the leaves of neem plant responsible for the observed repellent effects on most terrestrial organisms are terpenoids and some other complex oils which are known to be inactive at higher temperatures due to structural decomposition (Olaifa et al., 1986). Further work needs to be done towards identification of the compounds responsible for the observed repellent activity of the neem leaves against these freshwater bulinid snails. It is possible that

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some other unknown compounds that are not affected by high temperatures may be responsible for the repellent action of these leaves.

4.5 *Comparing processed and unprocessed Materials :*

An interesting observation is apparent when the response indices of the processed materials are examined vis a vis those of the unprocessed (Tables 4.1.1 and 4.1.2). The results show that processed materials tend to be much less stimulatory as attractants and arrestants for adult *B. truncatus* snails whereas they elicit very strong responses from the juveniles. Thus the unprocessed materials are more strongly stimulatory to the adult snails than the processed forms. In effect, in the present result, the highest attractant and arrestant indices (8.8 and 7.3 respectively) are generated by ripe mango (unprocessed) when tested on the adult conspecifics. On the other hand, processed materials were found to elicit the highest responses from the juvenile snails. In fact the fermented forms of these materials have been found to be most strongly stimulating. Thus 7 days fermented cocoyam tuber, which generated attractant and arrestant indices of 9.7 and 9.5 respectively, proved to be the strongest processed bioactive natural product for juvenile *B. truncatus* in the present bioassay study. Fresh sugar cane was, however, an exception. Although it was tested in the unprocessed form only, it proved to be the most potent attractant and arrestant ($p<0.001$) for the juvenile snails.

These findings lend credence to the observation by Dogbey (1995) that boiled pawpaw fruits (a processed bioactive natural material) elicited the highest significant attractant response from juvenile *B. truncatus* whereas the unprocessed materials such as sugarcane and sweet potato tubers

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were the most significant stimulants for the adult conspecifics in a similar bioassay study. It is quite interesting to note that in other chemoreception studies involving other planorbid snail responses towards organic molecules like carbohydrates and amino acids (Thomas and Assefa,1979; Thomas et al., 1989; Kpikpi and Thomas,1991,1993; Kpikpi et al.,1995) the juveniles most often exhibit much wider chemoreception niche for these materials than the adults. Sugars such as maltose, fructose, sucrose and also short-chain carboxylic acids like lactate, acetate and propanoate are known to be normal intermediate and end-products of anaerobic fermentation. This occurs when anaerobic microbes, especially bacteria, utilise carbohydrate sources in glycolytic energy production (Ihenkoronye and Ngoddy, 1985). In the normal freshwater environment of the snails these products are known to be released by senescing and decaying macrophytes such as *Lemna paucicostata* and *Ceratophylum demersum* (Patience et al., 1983a ; Sterry et al., 1985 ; Daldorph, 1988) into the medium. There is also evidence that some amino acids such as glutamate, aspartate, proline and hydroxyproline are released from living freshwater macrophytes into the external medium (Watt, 1966; Watzel and Manny, 1972 ; Bardach,1975). These organic molecules, most of which form a normal component of the dissolved organic matter (DOM), serve as chemical cues which may function as kairomones by serving as sources of information for food and shelter for these snails and other aquatic invertebrates and some vertebrates (Carefoot, 1982; Thomas et al.,1982, 1985; Croll, 1983; Kemenes et al., 1986). It was also suggested that sugars and carboxylic acids released from fermenting and decaying materials in the freshwater habitat of snails as well as amino acids normally present in the medium may also function as pheromones for the location of conspecifics. Another

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function of these molecules is that they facilitate the uptake of heavy metals such as iron or copper which are needed for the synthesis of respiratory pigments (Jørgenson,1976; Thomas et al.,1983). Thus exogenous molecules of plant origin occur commonly in the aquatic environment of both marine and freshwater ecosystems (Degens,1970;) influencing the behavior and physiology of many aquatic organisms including pulmonate snails (Potts, 1967; Stephens, 1972 ; Gilbertson & Jones, 1972). DOM and their sugar components are more likely to serve as food to the juvenile planorbid snails since their radulae may not be as well developed for scraping the surfaces of macrophytes and other floating plants for food as in the case of the adults (Thomas et al., 1983, 1989). This could probably account for the observation that juvenile *B. truncatus* were more responsive towards processed materials since these were already in a state of decomposition. It is possible that this wider response niche exhibited by the juveniles could be an early neonatal behavior that has been retained, when they used to feed entirely on DOM. Also, adult chemoreception may become reduced due to high degree of discrimination in their response towards chemical factors as a result of learning (Kpikpi & Thomas,1992). Audesirk and Audesirk (1985) gave evidence which suggests that pulmonate snails and other gastropods are capable of classical Pavlovian learning after relatively few trials.

4.6 *Effectiveness of processing techniques :*

Response indices from the present study and those found by other workers (Agudogo, 1992; Dogbey, 1995) for some processed natural materials suggest that the effectiveness of bioactive natural products to attract and/or arrest *B. truncatus* depends on the techniques used in processing

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them. Thus one form of a given processed material may prove to be a significant attractant and/ or arrestant whereas another form of it may be a significant repellent.

The various processing methods employed in the present work and those used by previous workers towards the search for significant bioactive natural materials for use as attractants and arrestants are discussed below.

4.6.1 *Sun-drying and fermentation :*

It appears that bioactive materials processed by sun-drying are more effective attractants to the adult *B. truncatus* snails than the fermented forms. Thus in the present results, sun-dried cocoyam leaves proved to be stronger attractants to the adult snails than the 3 days fermented cocoyam leaves (response indices were 3.8 and 1.6 respectively). On the other hand, fermented bioactive materials tend to be more potent attractants to the juvenile conspecifics than the sun-dried forms. Whereas the sun-dried cocoyam leaves generated attractant index of 0.1 for the juvenile snails 3 days fermented cocoyam generated 3.6 (Table 4.6).

4.6.2 *Oven-drying and Fermentation :*

Response indices of oven-drying and fermented forms of bioactive materials as found in the present study suggest that the latter forms are more potent attractants to *B. truncatus* snails of all ages than the former ones. Thus whereas oven-dried cassava leaves elicited a negative response from the adult snails and no response from the juveniles, response indices of the 3 days fermented

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forms were 3.5 and 4.3 for the adult and juvenile snails respectively. Similarly, fermented sweet potato tubers stimulated both adult and juvenile *B. truncatus* snails more strongly than the oven-dried forms (Table 4.6). However, response index of 7 days fermented sweet potato tubers for the adult snails was much lower than that of the oven-dried form.

It is not clear at the moment, why sun-dried cocoyam leaves, for example, tend to stimulate adult *B. truncatus* snails to a significantly greater extent ($p<0.05$) than the fermented forms. It may be that the large amounts of minerals such as calcium and iron in these leaves (Ihenkoronye & Ngoddy, 1985) may be responsible for attracting these adult snails even in the sun-dried state.

Fermented forms of bioactive plant materials used in the present work proved to be most strongly stimulating, especially to the juvenile snails than the sun-dried and oven-dried forms. This is not unexpected. Fermentation of materials from plant origin gives rise to anaerobic decomposition to release a medley of chemical factors such as sugars, carboxylic and amino acids most of which could attract and/ or arrest the snails to various extents (see Chapter 3.4 of present study).

4.6.3 *Boiling and Fermentation:*

A comparison of the results of the present study and those of previous workers (Agudogo,1992; Dogbey,1995) shows that of the two processing techniques, fermentation enhances the attractant effects of bioactive natural materials on the juvenile *B. truncatus* than boiling. Furthermore, the extent of these effects tends to increase with the period of fermentation. Thus whereas no significant response was elicited from juvenile snails by boiled cassava tubers, the fermented forms of this material generated significant response indices of 4.1, 5.3 and 8.5 respectively for 1, 3 and 7 days fermentation at $p<0.05$ confidence limit. In the case of cocoyam tubers, whereas the boiled

Table 4.6.

Relative efficacy of different forms of bioactive materials under various processing techniques as shown by their respective response indices.

TEST MATERIAL	RESPONSE INDICES											
	ADULTS						*JUVENILES*					
	S.d	O.d	B.	Fermentation			S.d	O.d	B.	Fermentation		
				1D	3D	7D				1D	3D	7D
Cassava leaves	-	-2.4	-		3.5*			- 0.0	-		4.3*	
Cassava tubers	-	-	4.6*(@) 3.1**(!)	4.6*	-1.8	0.7	-		2.3	4.1*	5.3*	8.5*
Cocoyam leaves	3.8*				1.6		0.1				3.6*	
Cocoyam tubers			2.1(@) 4.0(!)	3.8*	3.4*	2.0			-7.4(**) (!)	2.8	8.2***	9.7 ***
Sweet potato tubers		2.8	1.8(@)	5.0*	3.9*	2.4		1.3	-0.3(@)	6.5*	5.1*	5.7*

Note: S.d = Sun-dried; O.d = Oven-dried; B.= Boiled.

*, **, *** = Significant response indices at p< 0.05, 0.01 and 0.001 confidence limits respectively.

(**) = Significant repellent index at p<0.01 confidence limit.

(@) = data from Dogbey, 1995.

(!) = data from Agudogo, 1992.

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forms were significant repellents ($p<0.01$) the 3 and 7 days fermented forms were significant attractants at $p<0.001$ significant level.

Unlike the juveniles, no sharp difference in chemoresponse of the adult snails was observed for boiled and fermented forms of cassava tubers. Thus both forms of this material generated significant attractant indices for the adults at either $p<0.01$ or 0.05 confidence limits (Table 4.6). Fermenting cassava tubers beyond 1 day, however, tends to destroy its attractant effects on adult *B. truncatus* snails. In the case of cocoyam and sweet potato tubers, the boiled forms failed to elicit any significant attractant response from the adults whereas the 1 and 3 days fermented forms were significant attractants at $p<0.05$ confidence limit. Fermentation of these materials for up to 7 days, however, renders them incapable of stimulating the snails to any significant extent.

4.6.4 *Boiling and Oven-drying:*

It is apparent from the results shown in Table 4.6 that, compared to the oven-dried forms, boiled forms of bioactive natural products are not capable of eliciting any significant attractant response from the adult *B. truncatus* snails. Thus whereas oven-dried sweet potato tubers elicited statistically significant response index of 2.8 ($p<0.05$) from the adults the response index of the boiled form was insignificant. Both forms of this material were, however, insignificant as attractants to the juvenile conspecifics.

4.7 *Best processing technique :*

It appears that among the four processing techniques so far employed in the search for potent bioactive natural materials, fermentation seems to be the most promising method for enhancing

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the attractant and arrestant effects of natural materials. Oven-drying and boiling are more likely to render the materials as repellents. The extent of fermentation to be used, will, however, depend on the age group of the target snails. Thus whereas fermenting some materials (eg. cocoyam tubers) up to a period of 7 days improves their attractant effects on the juvenile snails the same technique is likely to repel the adult conspecifics.

4.8 *Suggestions for further work:*

Results from the present study suggest the need for further research in this direction to investigate the chemoresponses of *B. truncatus* and other planorbid snails towards the various forms of bioactive materials processed under these techniques. This would help to provide comprehensive information about the chemoreception niches of various *schistosome* host snails with respect to natural materials. The importance of this information is obvious. It would help to narrow down considerably, the search for natural bioactive products for use as attractants and arrestants in the manufacture of the envisaged controlled release formulations.

Other promising processing techniques that could be employed in further research include ;

1 Combination of two or more bioactive materials. It is possible that a higher response index may be produced when more than one known bioactive materials are combined in various proportions to form a single product.

2. Using two or more processing techniques to obtain a single material. For example, sun-drying followed by boiling ; oven-drying followed by fermentation, etc. It is likely that the repellent

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property of a material may be considerably reduced when subjected to more than one processing methods successively .

Sugarcane has been found to be a most promising potent bioactive material both in the present study (for juvenile *B. truncatus*) and in a previous work (for adult *B. truncatus*) reported by Dogbey (1995) . It would be of considerable interest to investigate the effects of the processed forms of this bioactive materials on planorbid snails using different processing techniques such as fermentation and boiling .

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CHAPTER 5.

EFFECTIVENESS OF ATTRACTANTS AND ARRESTANTS UNDER SIMULATED NATURAL ENVIRONMENTAL CONDITIONS.

5.1 INTRODUCTION

Most of the efforts at developing controlled release formulations for selective removal of *schistosome* host snails have been directed towards identification of bioactive materials using laboratory based bioassays (Thomas and Assefa,1979; Thomas et al.,1980, 1983,1985, 1986, 1989; Daldorph and Thomas,1988; Kpikpi,1990; Kpikpi and Thomas,1992,1993; Dogbey, 1995).

It is envisaged that known snail toxicants incorporated into such attractants and arrestants together with appropriate phagostimulants serving as feeding incitants could be used to selectively remove *schistosome* host snails and thereby reducing the adverse effects on non-target organisms associated with molluscicides in current use (Thomas and Assefa, 1979).

Having identified these materials it is necessary to devise a method of finding out how effective they can perform when applied in the field. In response to this need, Kpikpi (1990) and Dogbey (1995) individually tested some of the various bioactive materials they found in their bioassays under a simulated natural environmental condition (SNC). Each of these workers devised snail trapping units made of bamboo stems (*Bambusa vulgaris*), since these are readily available in the areas where schistosomiasis is endemic.

Beside finding out how long the material could remain effective when applied in the field, the test

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under SNC also acts as a further screening procedure to select only the most effective and appropriate materials for field testing and application and thereby reducing cost.

For these reasons, the significant attractants and arrestants identified in the bioassay tests as reported in chapters 2 and 3 were selected and screened further under SNC using trapping devices made of discarded plastic materials.

5.1.1 *CRITERIA FOR CHOICE OF MATERIALS TESTED UNDER SNC.*

Selection of bioactive materials for test under SNC was based on the criteria that :

1. They exhibit significantly high bioactivity, ie each material must generate statistically significant attractant or arrestant response index of not less than 4.0 from either adult or juvenile snails or both.

2. Relative cost of the material must be such that it can be affordable at all times by the local communities that might need it for the control purposes .

3. The material must be readily available and have only a few alternative uses in the areas where it occurs in abundance.

Based on the above criteria, the following materials shown in table 5.1 below were selected for further screening under the SNC.

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Table 5.1. **BIOACTIVE MATERIALS SELECTED FOR SNC TEST.**

FRUITS	*LEAVES*	*STEMS*	*ROOTS*
ripe mango	fresh cocoyam leaves	fresh sugarcane	1 day fermented cassava tuber
ripe cashew		fresh sweet potato tuber	7 days fermented cassava tuber.
ripe miraculin		1day fermented Sweet potato tuber	
water melon		3days fermented cocoyam tuber	
		7 days fermented cocoyam tuber	

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5.2. MATERIALS AND METHODS

5.2.1 *SNAIL BREEDING:*

About 1900 *Bulinus truncatus* snails were bred over a period of 2 months using the methods described in Chapter 2.2.1. The juvenile snails were transfered into a 66 x 38 x 21cm tank filled with 42.6 litres of tap water. The snails were routinely fed with fresh lettuce leaves every two days. The medium in which the snails were kept was changed once every week.

5.2.2 *SIMULATED NATURAL ENVIRONMENTAL CONDITION (SNC)*

A concrete tank of volume 1,108 litres was used for this purpose. The preparation was similar to that described by Dogbey (1995). The bottom of the tank was covered with sand, obtained from the bottom sediment of lake Weija, to a depth of 3cm and 60 litres of tap water added. Macrophytes such as *Ceratophyllum demersum*, *Pistia* sp, and *Nymphaea* sp. were added to cover nearly two-third of the surface of the water.

The water in the tank was kept at a temperature of 29 ± 1° C with a pH range of 5.8-6.7.

1200 young adult *B. truncatus* snails of at least 6 weeks old were introduced into the aquarium and allowed to acclimatise for 7 days. Two large leaves of fresh lettuce were added to the aquarium every other day during the period.

The snails were deprived of the lettuce food ration for a period of 24 hours prior to the experiment.

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5.2.3 *TRAP DESIGN*:

This is a modified version of a device used by Ayeh-Kumi (1996) as an experimental cage. It consists of transparent plastic spring water bottle ('ASTEK INSU' bottles). The top portion of the bottle was carefully cut off to obtain a cylindrical container of height 20cm and diameter 8.6cm. Two holes (7x8cm) were made at opposite sides of the container, one being 3cm above the base and the other 4cm from the cut top end of the container. By means of carpenter's glue, a nylon net (of mesh size 1.0mm^2) was used to cover the holes. Two other holes were made 0.5 cm from the top, one on opposite side of the container for suspending the traps in the aquarium by means of string (Plate 5.2.3, Appendix 7).

5.2.4. *TEST MATERIALS*:

The twelve bioactive materials tested nuder the SNC were those found to produce statistically significant response indices of at least 4.0 for either adult or juvenile *B. truncatus* in the bioassay tests. The procedure for fermentation of materials was the same as described in Chapter 3.2.11(3). Each material was cut into cylindrical forms with diameter similar to that of the lower portion of the trap. Test materials in the form of leaves were suspended inside the trap by means of a short stick through a small hole at the bottom of the trap. This was to prevent the material from moving out of the trap when placed in the water.

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5.2.5 *THE SNC EXPERIMENT:*

The inside of the traps was cleaned by washing thoroughly with tap water prior to and after testing each material. The traps were set by placing appropriate quantities of test material into the trap to make it 1/4 full (ie 5cm full). A small piece of stone (an inert material) was placed in each trap to act both as a sinker and a control material. The traps were set by suspending them vertically in the water by means of a piece of string hung over a wooden grid placed across the aquarium tank. The open end of the traps was 2cm below the water surface (Plate 5.2.4, Appendix 8). For each test material, ten test traps and ten control traps were set. Each test trap was alternated with a control trap. This is to ensure that each snail has equal chances of moving towards a test or control trap. The experiments were performed under a photoperiod of 12 hours of light and 12 hours of darkness.

The number of snails caught in each trap was counted and recorded after 24 hours for each test material. Snails found in and on any trap are deemed to have been caught by that trap. Student t-tests (Bailey,1981) were used to determine the levels of significance between numbers of snails caught in test and control traps. A trapping index (ie. total number of snails in test traps - total number of snails in control traps) was computed for each test material used. The data were used to evaluate the efficiency / effectiveness of the bioactive material in trapping *B. truncatus* snails under the SNC experiments.

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5.3 RESULTS :

5.3.1 NUMBER OF SNAILS CAUGHT:

The present results show that, apart from the ripe cashew test traps, all traps containing test materials caught significantly more snails than their corresponding controls ($p < 0.05$) (Table 5.3.1). The five materials that trapped significantly high numbers of snails under the present SNC experiments include fresh sugarcane > 7 days fermented cocoyam tuber > 1 day fermented cassava tuber > ripe mango > fresh cocoyam leaves (Fig. 5.3.1). Total number of snails caught by test traps was 990 whereas that for controls was 384 .

5.3.2 UNPROCESSED MATERIALS UNDER SNC TEST:

The results show that a total of 571 snails were caught by the seven unprocessed materials tested. This represents 57.7% of the total number of snails that entered the traps. The four most strongly bioactive unprocessed materials that caught significantly high numbers of snails are fresh sugarcane > fresh cocoyam leaves > ripe mango > fresh sweet potato. With a total of 150 snails in the test traps fresh sugarcane emerged as the strongest unprocessed bioactive material in the present SNC tests (Fig. 5.3.2).

5.3.3 PROCESSED MATERIALS:

All the five processed materials tested in the present experiments were in their fermented forms. The results show that of the total of 506 *B. truncatus* snails that entered the test traps containing

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Table 5.3.1

NUMBER OF *BULINUS TRUNCATUS* SNAILS ENTERING TRAPS WITH VARIOUS BIOACTIVE MATERIALS UNDER SNC TESTS.

TEST MATERIAL	TOTAL NUMBER OF SNAILS IN 10 TRAPS			
	TEST	CONTROL	TRAPPING INDEX	t-value ($p<0.05$)
UNPROCESSED				
FRUITS:				
Cashew	45	33	12	1.2451 (n.s)
Mango	93	28	65	3.6558**
Miraculin	42	19	23	2.4081*
Water melon	72	21	51	3.3966
LEAVES:				
Fresh cocoyam leaves	105	44	61	5.4487***
STEM:				
Fresh sugarcane	150	38	112	5.4679***
Sweet potato tuber	64	23	41	4.0354**
PROCESSED:				
1day fermented sweet potato	74	20	54	2.5861*
1 day ferm. cassava tuber	110	33	77	3.2154**
3 days ferm. cocoyam tuber	86	29	57	2.8108**
7 days ferm. cassava tuber	87	42	45	2.5399**
7 days ferm. cocoyam tuber	149	55	94	3.7737**

Note : ns = not significant.

*,**,*** = significant numbers of snails at 0.05, 0.01 and o.001 confidence limits respectively.

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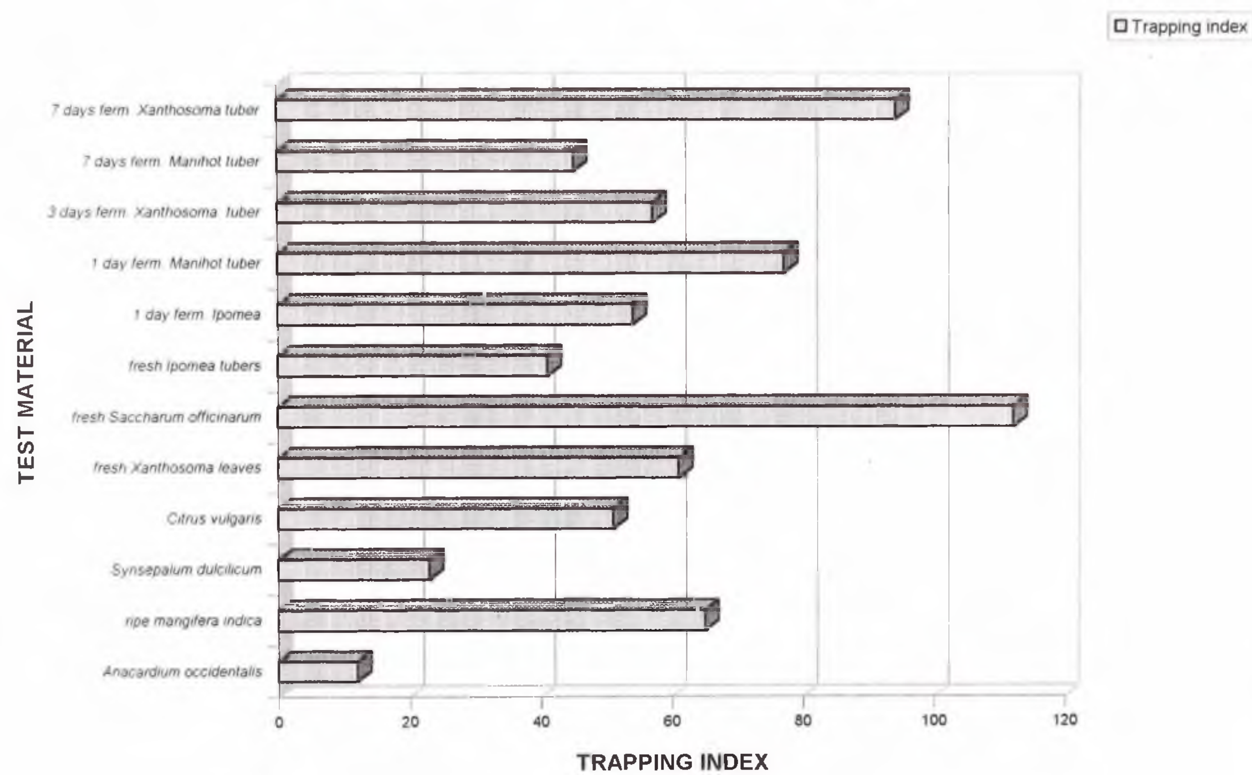
Fig. 5.3.1 TRAPPING EFFICIENCY OF SOME BIOACTIVE PLANT MATERIALS UNDER SIMULATED NATURAL CONDITIONS.



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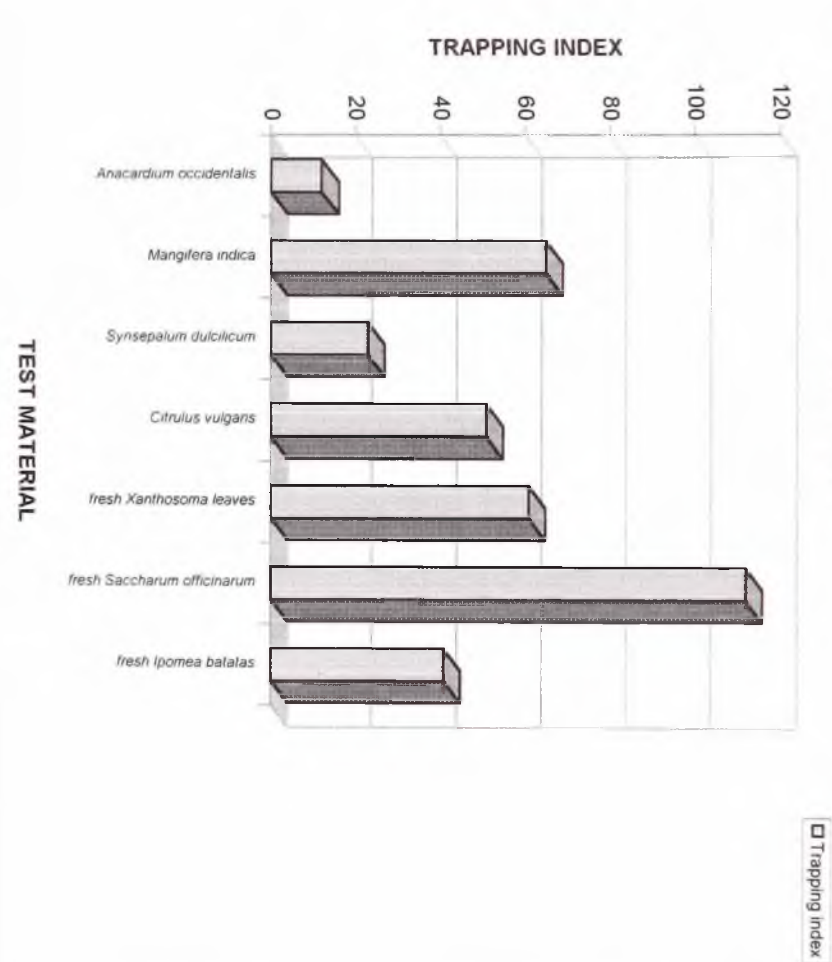

Fig.5.3.2 TRAPPING EFFICIENCY OF SOME UNPROCESSED BIOACTIVE PLANT MATERIALS UNDER SIMULATED NATURAL CONDITIONS.

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processed materials, 149 (ie.29.4%) were found in those traps containing 7 days fermented cocoyam tuber whereas those with 1 day fermented tuber caught 110 (21.7%) and those with 7 days fermented cassava tubers and 3 days fermented cocoyam tuber caught 87 and 86 snails respectively. Thus the three processed bioactive materials that caught the highest numbers of snails include 7 days fermented cocoyam tuber > 1 day fermented cassava tuber > 7 days fermented cassava tubers. The least number of snails was caught by 3 days fermented cocoyam tuber and 1 day fermented sweet potato test traps.

Thus the order of effectiveness of the processed bioactive materials from the present SNC tests is 7 days fermented cocoyam tuber > 1 day fermented cassava tuber > 3 days fermented cocoyam tuber > 1 day fermented sweet potato > 7 days fermented cassava tuber (Fig.5.3.3).

5.3.4 **BIOACTIVE FRUITS:**

It appears that fruits tested under the present SNC experiments caught much fewer snails than other bioactive materials. Thus, the total number of snails caught by test traps containing fruits were relatively lower. Among the four fruits tested, ripe mango emerged as the most potent with a total number of 93 snails caught by test traps containing this material. Other bioactive fruits that caught significantly high numbers of snails were water melon and ripe miraculin. The total number of snails caught by test traps containing ripe cashew was statistically insignificant ($p>0.05$) compared to those caught by the control traps. Thus ripe cashew is not a significantly strong bioactive material under the present SNC experiments (Fig. 5.3.4).

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Fig.5.3.3 TRAPPING EFFICIENCY OF SOME PROCESSED BIOACTIVE PLANT MATERIALS UNDER SIMULATED NATURAL CONDITIONS.

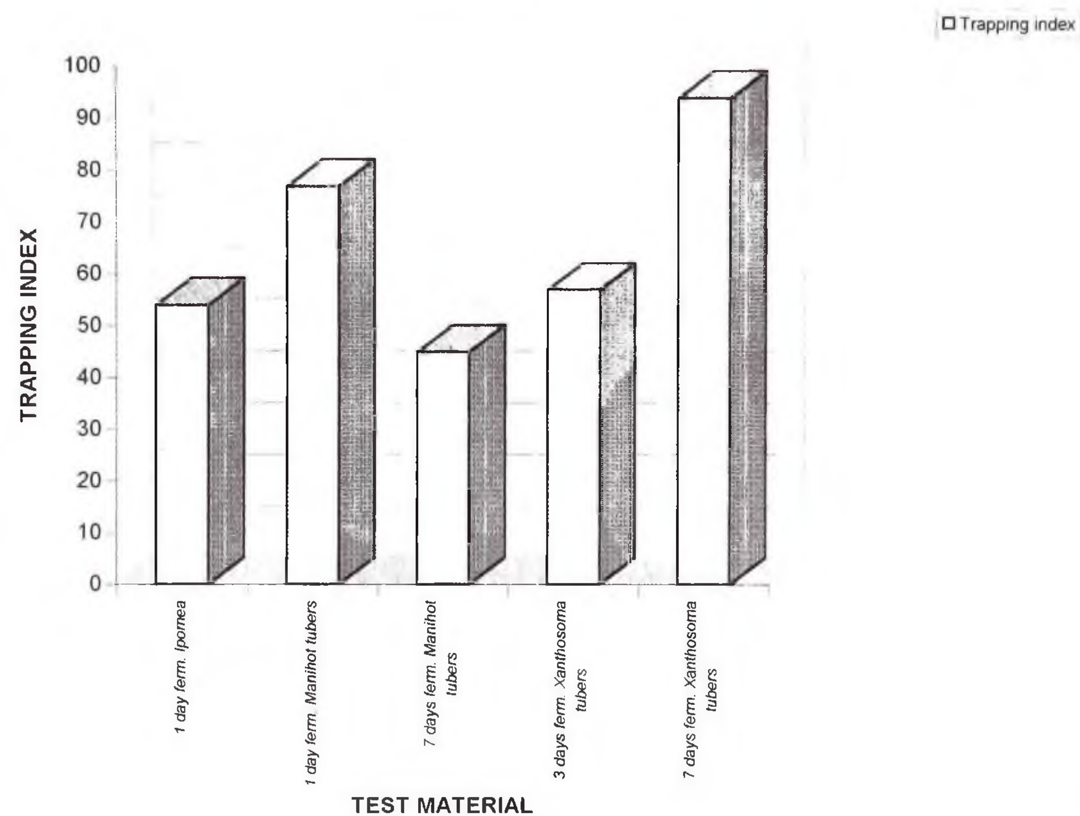

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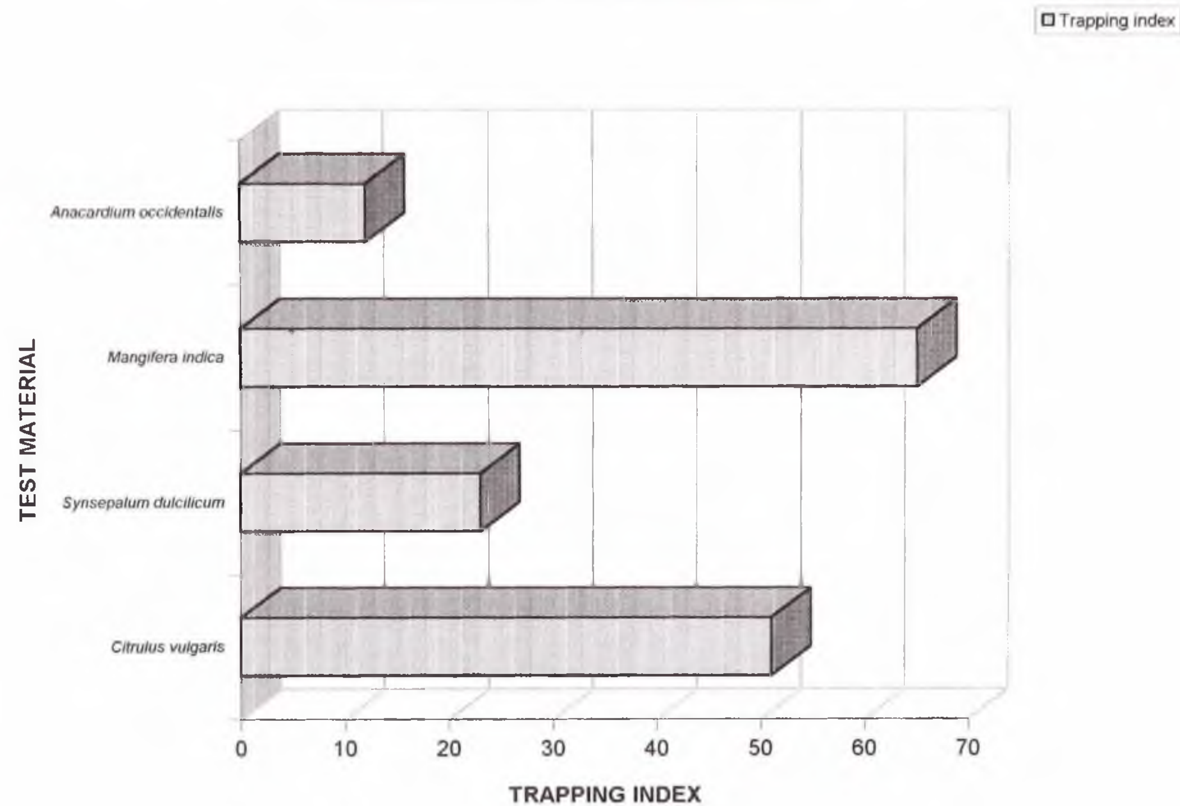
Fig. 5.3.4 TRAPPING EFFICIENCY OF SOME BIOACTIVE FRUITS UNDER SIMULATED NATURAL CONDITIONS.



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5.3.5 TRAPPING EFFICIENCY OF BIOACTIVE MATERIALS:

The trapping indices computed for each of the bioactive materials gives an indication of how effective a bioactive material is likely to function when deployed in trapping devices under field conditions.

The results of the trapping indices show that the five most efficient bioactive materials include fresh sugarcane > 7 days fermented cocoyam tuber > 1 day fermented cassava tuber > ripe mango > fresh cocoyam leaves (Fig.5.3.5). It is important to note that although sugarcane and 7 days fermented cocoyam tuber caught about equal numbers of snails, sugarcane proves to be much more efficient when used in trapping units (trapping index =112) than the fermented cocoyam (trapping index =94). Also fresh cocoyam leaves tend to catch more snails than ripe mango but the latter material has a much higher trapping index (65) than the former (61). Thus ripe mango is a more efficient bioactive material than fresh cocoyam leaves.

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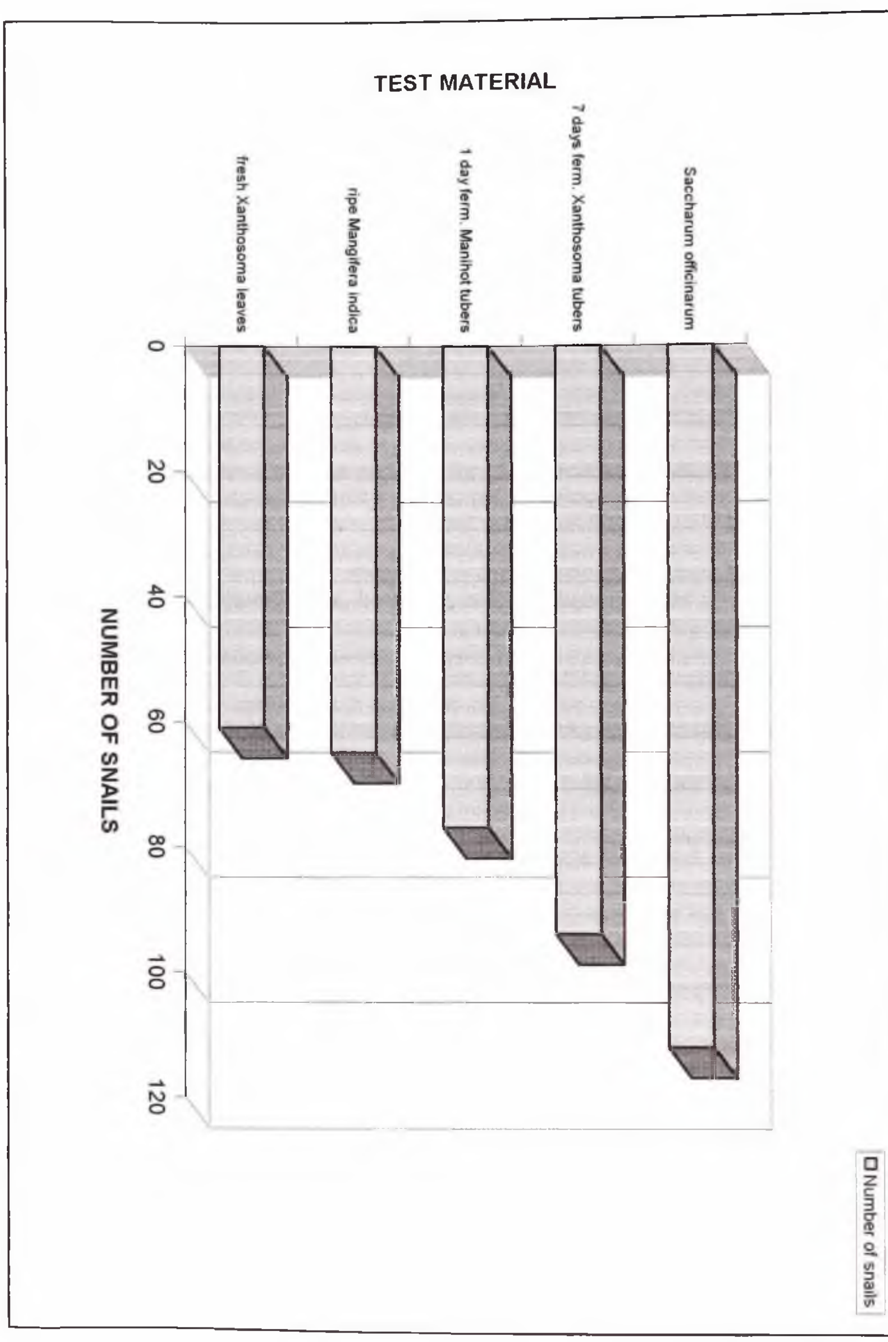
Fig. 5.3.5 TRAPPING EFFICIENCY OF SOME SIGNIFICANT BIOACTIVE PLANT MATERIALS UNDER SIMULATED NATURAL CONDITIONS.



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5.4 DISCUSSION.

5.4.1 *CONCORDANCE WITH BIOASSAY TESTS*:

The results of the present experiments under the SNC show some concordance with some of the results of the bioassay tests reported in chapters 2 and 3 of this study. Thus sugarcane and 7 days fermented cocoyam tuber which were found to be the two most potent processed and unprocessed bioactive materials respectively in the bioassay experiments also proved to be the materials that most strongly attracted *B. truncatus* in the test traps under the SNC experiments. This result supports similar SNC test results reported by Dogbey (1995) that sugarcane emerged as the strongest bioactive natural product for *B. truncatus*, although different trapping units were used.

One observation worthy of note, however, is that whereas in the olfactometer studies, sugar cane proved to be much more potent than 7 days fermented cocoyam tuber by virtue of its higher response indices, the results from the present SNC test indicate that there is no statistically significant difference between the total numbers of snails caught by test traps containing each of these two bioactive materials (150 and 149 snails for sugar cane and 7days fermented cocoyam tuber respectively (Table 5.3.1). A possible factor that may account for this difference in response indices during the bioassay test may be the forms in which these materials were used in the tests. As pointed out by Dogbey (1995), a directional diffusion gradient is more likely to be created from crude bioactive materials presented in their whole or solid forms, such as sugar cane, along which the snail could move into the test chamber of the olfactometer. On the other hand, diffusion

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gradient emanating from crushed or semi-solid bioactive materials, as in the case of 7 days fermented cocoyam tuber, would tend to be much more diffused and localised within the test chambers, making snails to move in all directions within the olfactometer chamber, hence the relatively lower response index generated by the fermented cocoyam tuber in the olfactometer experiments.

With regards to the efficiency, however, sugar cane emerged as the most efficient trapping material (Fig.5.3.1). Thus it is a better bioactive material than 7 days fermented cocoyam tuber.

5.4.2 *CONVERSION OF BIOACTIVE INGREDIENTS* :

Since about the same numbers of *B. truncatus* snails were caught by both sugar cane and 7 days fermented cocoyam tuber, it is reasonable to argue that both materials would contain quite similar active ingredients responsible for the observed chemoresponse movements of the snails.

Mature sugarcane stem is known to contain some 20% sucrose together with fructose and glucose as well as small amounts of maltose. Although attractant and arrestant responses of bulinid snails such as *B. globosus* and *B. rohlfsi* towards pure sucrose are not significantly high owing to the absence of ∝(1-4) glucosidic bonds in that molecule (Kpikpi,1993), it is likely that at favourably low ph levels, as found in the water medium, equilibrium may shift away from the sucrose molecules as a result of hydrolysis leading to formation of ∝(1-4) glucosidic bonds among the various glucose molecules (Stroev, 1986). It is also possible that some other molecules such as carboxylic acids may be involved in the chemoresponses of the snails towards the sugar cane.

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Root and stem tubers such as those of cocoyam and cassava contain large stores of polysaccharides in the form of starches and amylose. Fermentation processes lead to hydrolysis of these materials with formation of intermediate products such as maltose, glucose and some carboxylic acids (Ihenkoronye and Ngoddy, 1985) which may be largely responsible for the chemoresponses of the snails.

5.4.3 *FERMENTED MATERIALS AND FRUITS*:

Another point of interest in the present results is that traps containing fermented bioactive materials appear to catch significantly higher numbers of snails than those containing fruits, except in the case of ripe mango. A possible reason accounting for this observation may be due to the relative calcium levels in these materials. Apart from the large amounts of carbohydrates which may be utilised by the snails for energy generation (de Zwaan et al., 1976) as well as for location of target organisms in their environment (Thomas, 1982; Croll, 1983; Audesirk and Audesirk, 1985), calcium and iron are two elements present in stem and root tubers (Ihenkoronye and Ngoddy,1985) that are of physiological importance to aquatic molluscs including *B. truncatus*. A lot of experimental evidence indicates that there is a positive correlation between concentration of dissolved calcium and distribution and abundance of freshwater snails (Boycott, 1936, Macan, 1950; Russel-Hunter, 1964) and that the large amounts of calcium needs of these snails are obtained directly from their foods (Greenaway,1971a; Jodrey, 1953, Kado,1960; van der Borght,1963; Wilbur,1972; Thomas et al., 1974).

Iron, an important element for the synthesis of respiratory pigments or enzymes (Taylor,1969; Southward and Southward, 1972; Jørgenson, 1976; Ferguson, 1982) is also present in

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greater quantities in these tubers than in ripe fruits (Ihenkoronye,1985). Thus the higher levels of these elements in tubers than in ripe fruits may be responsible for the observed difference in snail numbers caught by the respective traps.

It is possible that some other molecules which are presently unknown may also be acting as stimulants in addition to the calcium, iron and the carbohydrates. Further work needs to be done to find out the other active ingredients in these bioactive materials that are involved in the chemoreception profile observed in this study.

One fruit which caught the least number of snails and also proved to be the most ineffective bioactive material was ripe cashew. This was not unexpected as it has been shown that cashew , although contains some sugars, has significant level of chemicals with molluscicidal properties (Ayeh-Kumi, 1996; Webbe and Lambert, 1983). The active ingredients, however, are yet to be identified .

Fresh sugar cane, 7 days fermented cocoyam tubers and 1 day fermented as well as fresh cocoyam leaves and ripe mango can therefore be recommended for field testing .

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CHAPTER SIX

GENERAL DISCUSSION

The present chemoreception study of *B. truncatus* has revealed some interesting aspects of the responses of both adult and juvenile *B. truncatus* to some unprocessed and processed forms of naturally occurring bioactive materials from common plants. It has also given the efficacy of some of these materials under simulated natural environmental conditions.

This chapter attempts to summarize these findings, pointing out the implications of some of these findings to the control of the snail hosts of schistosomiasis. Possible areas of future research will also be pointed out.

6.1 CHOICE OF MATERIALS FOR BIOASSAY TESTS:

The purpose of these tests was to identify materials from naturally occurring common products that can strongly attract or arrest *B. truncatus* snails (or both). Known snail toxicants could be incorporated into these bioactive materials for the manufacture of controlled release formulations that can be used to selectively destroy the schistosome host snails.

Previous work done in this field (Dogbey,1995) influenced to a large extent the selection of materials for the present work. Where one form of the material was previously tested, a different form of it was chosen for the present study. For example Dogbey (1995) tested the boiled and raw forms of sweet potato stem tubers. In the present work the raw, fermented and oven-dried

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forms of sweet potato were tested. This is to provide the opportunity to determine which form of a bioactive material would be most stimulating to the *schistosome* host snails. Sugar cane was, however, tested in the present work although it has already been tested previously. The emphasis has been on the availability and cost of the test material. Thus all the materials selected are from common plants found in areas where schistosomiasis is endemic. For example leaves of neem plant (*Azadirachta indica*), fruits of mango, cashew, water melon, cassava and sweet potato are common materials found in these areas and some are even used as food although not staples. The expectation is that where a potent bioactive material identified happens to be used as food, the active ingredients responsible could be investigated and closely related analogues could be used as alternatives.

6.2 BIOASSAY EXPERIMENTS:

6.2.1 *UNPROCESSED MATERIALS :*

Adult *B. truncatus* snails exhibited a much wider chemoreception niche towards unprocessed materials than their juveniles. Thus they were significantly attracted, arrested or both towards 15(ie 42.86%) of the unprocessed materials tested ($p<0.05$, 0.01 or 0.001). The five most significant attractants found for adult *B. truncatus* from this study were as follows:

ripe mango > water melon > sugar cane > date palm > fresh pawpaw leaves (Appendices 1-3).

There were some overlaps between the chemoreception niches of adult and those of the juveniles towards unprocessed materials. Thus all the six unprocessed materials that elicited significant

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attractant responses from the juvenile snails were also attractants to the adults at either $p < 0.05$, 0.01 or 0.001 confidence limits. These include sugar cane > fresh sweet potato tuber > locust beans > ripe cashew > fresh lettuce leaves > showers of gold. The extent of overlap, however, varied with each material.

Some overlaps also emerged between the chemoreception niches of adult and juvenile snails in terms of arrestant responses. Thus five of the unprocessed materials were observed to act as significant arrestants for both adult and juvenile *B. truncatus* snails, although the extent of these arrestant effects varied. These include sugar cane > fresh lettuce leaves > showers of gold > sweet potato > ripe cashew.

These results lend credence to the findings of other workers that there were intra and inter- specific overlaps in the chemoreception niches of pulmonate snails in their response towards various naturally occurring materials (Agudogo, 1992; Ansa, 1994; Dogbey, 1995; Kpikpi, 1990).

6.2.2 *PROCESSED MATERIALS* :

The purpose of processing materials for the bioassay experiments was to alter the form and texture of the materials with a view to enhancing their bioactive properties.

Results from this investigation indicate that juvenile *B. truncatus* respond to a much wider range of processed naturally occurring materials than the adults with some important overlaps. Thus it was found that four of the 23 processed materials significantly attracted both adult and juvenile *B. truncatus* snails at either $p < 0.05$, 0.01 or 0.001 confidence limits. These include 1 day fermented sweet potato tuber > 3 days fermented sweet potato tuber > 1 day fermented cassava root tuber > 3 days fermented cocoyam tuber (Appendices 4-6). No overlaps were observed between the

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chemoreception niches of adults and juveniles of these bulinid snails in terms of their arrestant responses to processed materials. In fact the adults were significantly arrested by only 3 processed materials : oven-dried sweet potato leaves > sun-dried cocoyam leaves > 1 day fermented cocoyam tuber.

The juvenile snails were significantly attracted and arrested by 9 of the processed materials all of which happened to be fermented to various extents. These include 7days fermented cocoyam tuber > 7 days fermented cassava root tuber > 3 days fermented cocoyam and cassava tubers > 7 days fermented sweet potato > 3 days fermented cassava leaves > 1 day fermented sweet potato> 3 days fermented cocoyam tuber.

It appears that juvenile *B. truncatus* snails in the present experiment were more responsive to fermented materials than the adults. It is possible that the fermentation process significantly altered the texture of these materials, and more importantly, shifted the hydrolysis equilibrium reactions from polysaccharides such as starches towards oligosaccharides like maltose, maltotriose and the formation of some carboxylic acids. Planorbid snails have been shown to be significantly responsive to these molecules (Thomas, 1986; Kpikpi, 1990; Thomas and Assefa, 1978 ; etc.).

As pointed out by Thomas and Assefa (1979), intraspecific differences in chemoreception niches have been evolved to reduce the intensity of intraspecific competition for food and other target molecules among these snails . Thus in the natural environment food preferences of the juveniles *B. truncatus* could be expected to vary from those of the adults to some extent. Although considerable work has been done on the search for significant specific bioactive natural materials for use as attractants, arrestants and phagostimulants, there is the need to investigate the

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active ingredients responsible for the bioactive effects so far identified. It is also important to study the phagostimulation properties of these materials. The value of identifying the functional molecules in these materials lies in the fact that it would help to narrow down the search to a few related natural materials that are likely to contain them. Also if a material is identified as a phagostimulant for the snail, it is also likely to be an attractant and arrestant.

6.3. EVALUATION OF EFFICACY OF BIOACTIVE MATERIAL UNDER SIMULATED NATURAL ENVIRONMENTAL CONDITIONS.

As pointed out in chapter 5, this experiment was carried out with the aim of screening further the bioactive materials identified from the bioassay tests. There were two important choices involved in this evaluation. These were the choice of bioactive materials and the method to be used.

6.3.1 *CHOICE OF BIOACTIVE MATERIALS:*

The characterisation of the chemoreception profile of *B. truncatus* led to the identification of some natural plant materials as attractants and arrestants for these snails. Ideally, the most potent of these factors should be selected for the SNC tests. A desirable feature of such materials should be that of di-bioactivity. Thus materials that acted both as attractants and arrestants should be preferred to those which had only a single significant effect on the snails. However, two other requirements in addition to the potency of the material influenced the final choice of materials selected for further evaluation. These were cost and availability of the test material in the area where the disease occurs.

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6.3.2 *CHOICE OF TRAPPING DEVICE:*

Two main requirements need to be met in the design of the trapping units. The need for the system to be able to hold the test material and release them at a slow rate such that some of the active factors could still remain in the traps at the end of the test period. Secondly, there was the need for a rigid framework in which the snails could be trapped (Kpikpi, 1990).

Alternatively, the cost of the materials also needs consideration. Thus discarded plastic containers were used for the trapping units. Being non-biodegradable, traps made of this material are more likely to be used over a longer period than those from plant materials such as bamboo.

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CONCLUSION:

From the bioassay studies using diffusion olfactometers, the conclusions can be drawn that

Adult *B. truncatus* snails exhibit wider chemoreception niche towards unprocessed naturally occurring plant materials than the juveniles .

Juvenile *B. truncatus* snails exhibit wider chemoreception niche towards processed forms of these materials than the adults.

Response of juvenile snails towards fermented materials is more profound than those of the adults .

There were some overlaps in responses of adult and juvenile snails towards both processed and unprocessed forms of some bioactive materials except in their arrestant responses towards processed materials.

Sugar cane emerged as the most potent, all round bioactive natural material for both adult and juvenile *B. truncatus*. Ripe mango and 7 days fermented cocoyam tuber were the strongest bioactive natural products for adult and juvenile conspecifics respectively.

Processed bioactive materials seem to offer more promising prospects for use as attractants and arrestants than unprocessed forms.

In the SNC experiments to test the efficacy of the 12 selected bioactive materials using trapping units made of plastic containers, sugarcane and 7 days fermented cocoyam tuber caught the highest number of snails. Sugarcane, however, generated a much higher trapping index value and therefore emerged as the most efficient bioactive material.

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Thus sugar cane followed by 7 days fermented cocoyam tubers are most likely to be efficient when used as baits in the field.

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Appendix 1 UNPROCESSED BIOACTIVE MATERIALS WITH SIGNIFICANT CHEMORECEPTION EFFECTS ON *BULINUS TRUNCATUS*.

TEST MATERIAL	MEAN RESPONSE INDICES			
	ADULTS		*JUVENILES*	
	Attract. ind.	Arrest. ind.	Attract. ind.	Arrest. ind.
FRUITS:				
Anacardium occidentalis	5.2***	2.4**	3.6***	1.9**
Ripe *Mangifera indica*	8.8***	7.3***	-1.6	0.9
Citrulus vulgaris	8.7***	6.8***	0.7	2.0
Citrus aurantifolia	-3.9@	-0.5	-7.9@@@	-2.8@@
Synsepalum dulcilicum	4.8***	1.6	3.0	3.6*
Phoenix dactylifera	7.7***	5.0***	1.9	1.8
Theobroma cacao : pod	-	0.2	-	-4.9@@@
Beans	5.0***	3.2**	1.5	0.6
Duranta plumieri	3.8*	3.8***	4.0***	3.2***
Musa paradisiaca	3.0*	0.6	0.6	0.2
Calotropis procera	3.9*	3.6*	-2.6@	-2.3@
Azadirachta indica	-4.9@	-3.2@@@	-5.0@	-3.6@@
Parlaa clappertoniana	6.2***	0.2	6.0***	2.2
Chrysophyllum albicum	-5.2@@	0.1	-8.2@@@	-1.0
LEAVES:				
Xanthosoma maffafa	5.6***	4.5***	1.4	1.1
Latuca sativa	5.2**	4.6***	4.6**	2.4**
Carica papaya	6.7***	5.2***	3.5	1.7

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TEST MATERIAL	MEAN RESPONSE INDICES			
	ADULTS		JUVENILES	
	Attract. ind.	Arrest. ind.	Attract. ind.	Arrest. ind.
STEMS:				
Zingiber officinale	-7.2@@@	-4.7@@@	-9.3@@@	2.2@@
Saccharum officinarum	8.5***	5.0***	10.0***	6.7***
Ipomea batatas	5.0***	2.9**	6.3***	2.7**
ROOTS:				
Manihot esculentus	5.2***	3.2***	-2.9	0.1

1. *,**,*** = statistically significant response indices at 0.05, 0.01 and 0.001 probabilities respectively.
2. @, @@, @@@ = statistically significant repellent indices at 0.05, 0.01 and 0.001 probabilities respectively.

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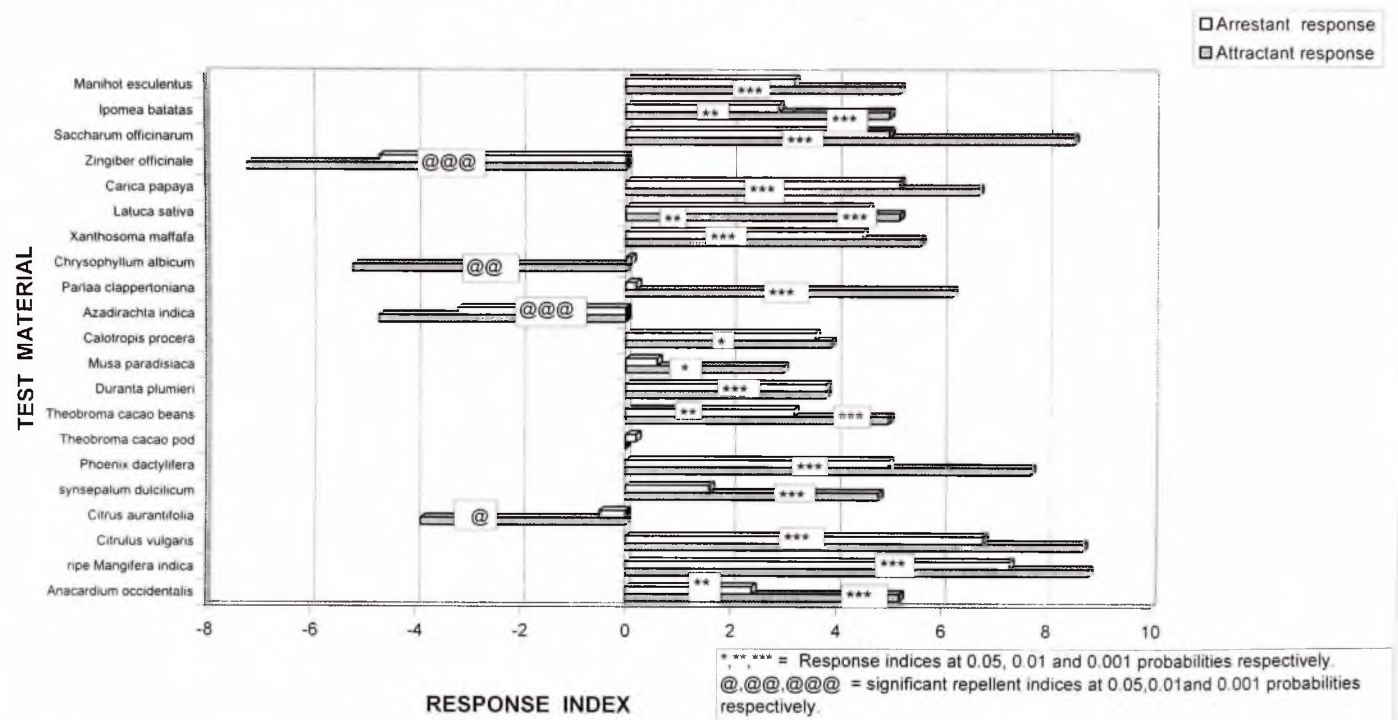
Appendix 2 SOME UNPROCESSED BIOACTIVE MATERIALS WITH SIGNIFICANT CHEMORECEPTION EFFECTS ON ADULT *BULINUS TRUNCATUS*.



*,**,*** = Response indices at 0.05, 0.01 and 0.001 probabilities respectively.
@,@@,@@@ = significant repellent indices at 0.05,0.01and 0.001 probabilities respectively.

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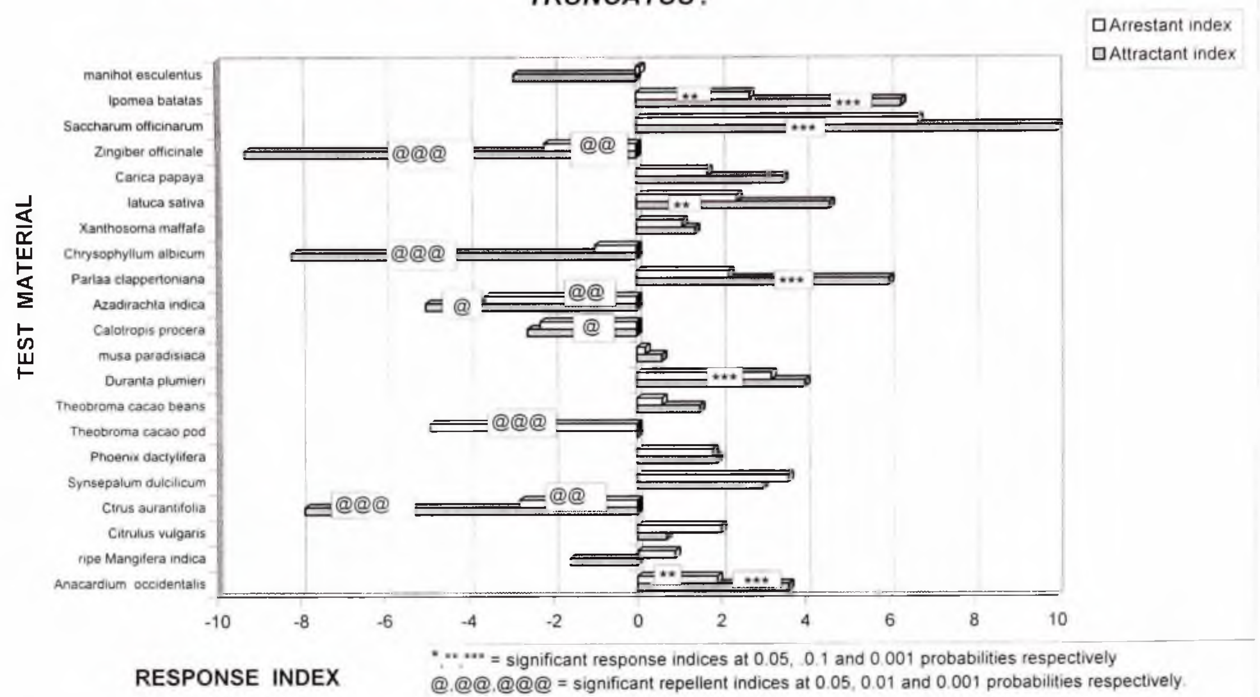
Appendix 3 SOME UNPROCESSED BIOACTIVE MATERIALS WITH SIGNIFICANT CHEMORECEPTION EFFECTS ON JUVENILE *BULINUS TRUNCATUS*.



*,**,*** = significant response indices at 0.05, .01 and 0.001 probabilities respectively
@,@@,@@@ = significant repellent indices at 0.05, 0.01 and 0.001 probabilities respectively.

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Appendix 4 **PROCESSED BIOACTIVE MATERIALS WITH SIGNIFICANT CHEMORECEPTION EFFECTS ON *BULINUS TRUNCATUS*.**

TEST MATERIALS	MEAN RESPONSE INDICES			
	ADULTS		*JUVENILES*	
	Attract. ind.	Arrest. ind.	Attract. ind.	Arrest.. ind.
FRUITS:				
Sun-dried *Thevetia nerifolia*	3.1*	-	2.3	-
LEAVES:				
Oven-dried *Azadirachta indica*	-3.2@	-3.3@@	-2.0	-0.1
3 days ferm. *Carica papaya*	3.1*	2.6*	2.1	1.0
Sun-dried *Xanthosoma maffafa*	3.8**	4.0***	0.1	1.1
3days ferm.*Xanthosoma maffafa*	1.6	1.1	3.6**	2.3**
Oven-dried *Manihot esculentus*	-2.4@	-2.2	0.0	0.0
STEMS:				
Oven-dried *Zingiber officinale*	-4.2@@	-2.6@@	-2.1	-3.7@@@
Oven-dried *Ipomea batatas*	2.8**	2.1*	1.3	0.6
1 day ferm. *Ipomea batatas*	5.0**	1.8	6.5**	2.4**
3 days ferm. *Ipomea batatas*	3.5**	0.8	5.1**	1.9*
7 days ferm. *Ipomea batatas*	2.4	-0.4	5.7***	4.0***
1 day ferm. *Xanthosoma maffafa*	3.8*	3.6**	2.8	0.3
3days ferm.*Xanthosoma maffafa*	3.4*	-0.5	8.2***	4.3***
7days ferm.*Xanthosoma maffafa*	2.0	-0.3	9.7 ***	9.5***
ROOTS:				
1 day ferm. *Manihot esculentus*	4.6**	1.0	4.1**	0.4
3 day ferm. *Manihot esculentus*	-1.8	-1.8	5.3**	4.3**
7 days ferm. *Manihot esculentus*	0.7	-0.6	8.5***	6.4***

*,**,*** = statistcally significant response indices at 0.05, 0.01 and 0.001 probabilities respectively.

@, @@, @@@ = significant repellents at 0.05, 0.01 and 0.001 probabilities respectively.

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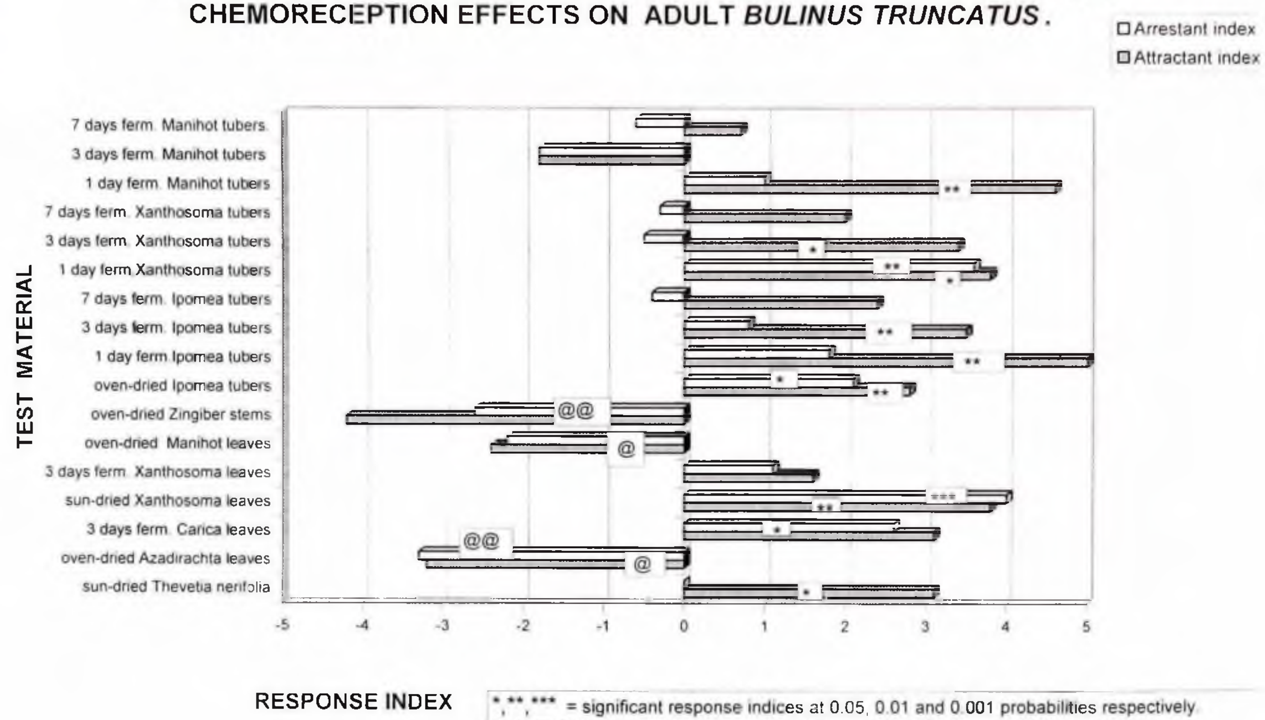
Appendix 5 SOME PROCESSED BIOACTIVE MATERIALS WITH SIGNIFICANT CHEMORECEPTION EFFECTS ON ADULT *BULINUS TRUNCATUS*.



*, **, *** = significant response indices at 0.05, 0.01 and 0.001 probabilities respectively.
@,@@,@@@ = significant repellent indices at 0.05, 0.01 and 0.001 probabilities respectively.

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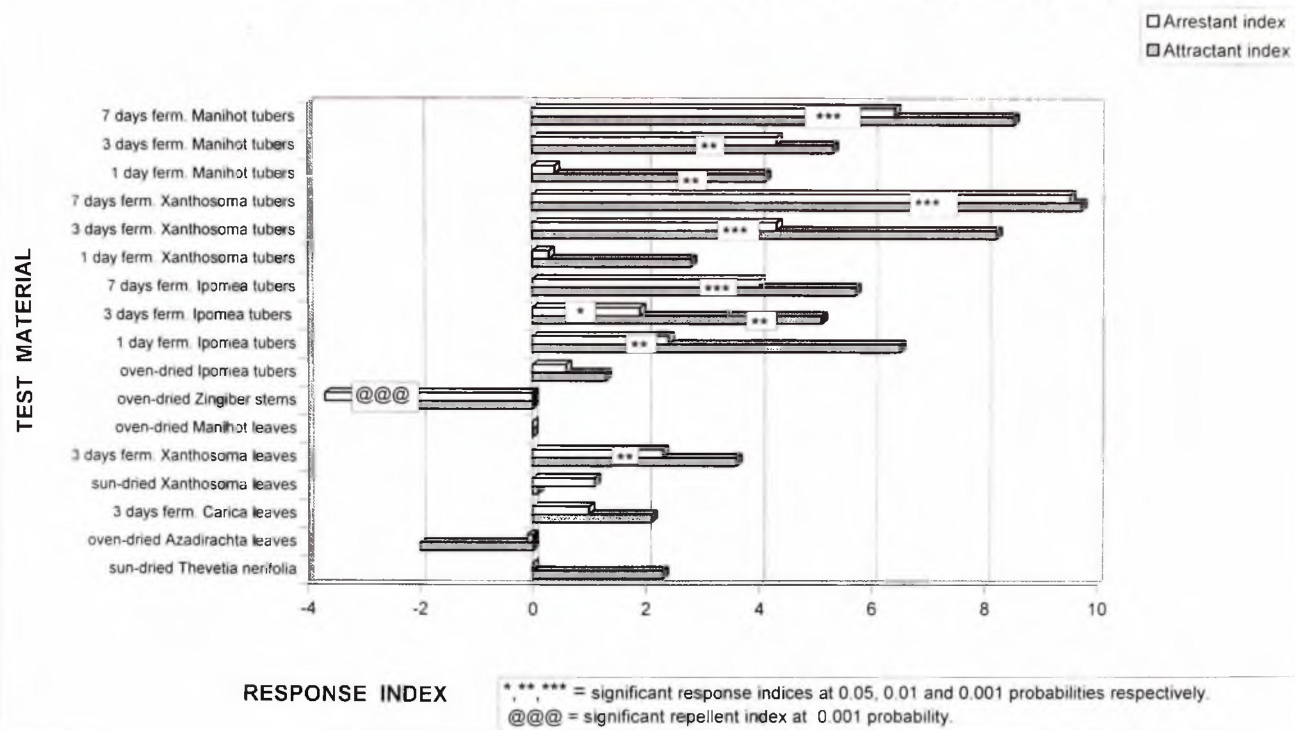
Appendix 6 SOME PROCESSED BIOACTIVE MATERIALS WITH SIGNIFICANT CHEMORECEPTION EFFECTS ON JUVENILE *BULINUS TRUNCATUS* .



*,**,*** = significant response indices at 0.05, 0.01 and 0.001 probabilities respectively.
@@@ = significant repellent index at 0.001 probability.

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Appendix 7:

PLATE 5.2.3.

TRAPPING DEVICE USED FOR SNC EXPERIMENT

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Appendix 8.

PLATE 5.2.4

EXPERIMENT SETUP FOR SNC TESTS. THE TRAPPING DEVICES ARE SHOWN IN THE WATER.

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