Identification and field evaluation of bioactive natural products for use in schistosome host snail traps

A Thesis presented by

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DECLARATION

I hereby declare that except for the references to the literature and to the works of other researchers which have been duly cited herein, this work is the result of my own original research and that this thesis has not been presented elsewhere for another degree.

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DEDICATION

I dedicate this work to God Almighty for His protection and guidance.

ABSTRACT

The behavioural responses of two schistosome host snails (*Bulinus truncatus* and *Biomphalaria pfeifferi*) to twenty-six naturally occurring (crude and semi-processed) products were measured in diffusion olfactometers. 15 out of these 26 materials (i.e. 57%) were found to act as statistically significant attractants for adult *B. pfeifferi* while only 5 (19%) of the test materials elicited statistically significant responses from the adults of *B. truncatus*. The juveniles on the whole were less responsive to the test materials compared with their adult conspecifics; thus the juveniles of *B. truncatus* responded to only 3 (i.e. 11.5%) of the 26 natural products tested while those of *B. pfeifferi* responded to 34% of the natural products. 13 out of the 15 significant attractants for adult *B. pfeifferi* were found to be statistically significant arrestants while only 2 out of the 5 significant attractants for adults of *B. truncatus* elicited statistically significant arrestant responses. None of 26 test materials acted as statistically significant arrestant for juveniles of *B. truncatus*. Of the 9 test materials which acted as significant attractants for juveniles of *B. pfeifferi* 5 were found to act as statistically significant arrestants.

Preliminary investigations conducted under simulated natural conditions (SNC) revealed that sugarcane (*Saccharum officinarum*) presented as chunks (whole) in bamboo cylinders (trapping units) attracted more snails (both *Bulinus truncatus* and *Biomphalaria pfeifferi*) than controls. This particular trapping unit was also found to be more effective than comparable units which used pawpaw (*Carica papaya*), sweet potato (*Ipomoea batatas*) and cassava (*Manihot esculenta*). The efficacy of the sugarcane-bamboo cylinder combination was again demonstrated under field conditions in Weija lake where in three

separate cases a significantly higher number of schistosome host snails were caught in the test traps compared with controls. The implications of this study for the development of controlled released formulations for the selective control of schistosome host snails are discussed.

CHAPTER ONE

1. GENERAL INTRODUCTION

Schistosomiasis commonly known as Bilharziasis is a parasitic disease. The causative parasites were discovered by a German pathologist Theodore Bilharz in 1852 during an autopsy in Cairo, Egypt. However, it was not until 1915 that the life cycles of *Schistosoma haematobium* and *Schistosoma mansoni* were unravelled by Leiper (1915), revealing the link between the disease, freshwater bodies, and the planorbid snails which act as intermediate hosts for the *schistosoma* parasites. Since that time, in the light of this new knowledge about the etiology of the disease, several recommendations, and to a lesser extent practical attempts have been made to eradicate or control the disease. These efforts have favoured a range of different approaches including chemotherapy, environmental improvement/manipulation, the control of the molluscan host of the parasites and more recently, vaccination. Despite all these attempts, however, the world prevalence rates of the disease have been increasing. The Japonica (Eastern) schistosomiasis is endemic in Japan, much of China, the Philippines and Celebes. Manson's schistosomiasis (intestinal bilharziasis) occurs in northern Egypt and many other parts of Africa, Yemen and other places in Arabia, Puerto Rico and several islands of the lesser Antilles, Venezuela, Dutch Guiana, and extensive areas in Brazil. Urinary Schistosomiasis (bladder bilharziasis) exists throughout practically all of Africa, the southern tip of Portugal, Cyprus, and the Near East.

The disease is mostly found in agricultural and fishing communities sited near streams, rivers, lakes, dams and ponds. The disease is prevalent in most developing countries and it is currently estimated that about 600 million people are exposed to it and 250 million people actually infected by the parasite (WHO, 1987). The high prevalence is attributed to developmental projects undertaken by governments (such as damming of rivers for hydroelectric power, water supply and canalization for irrigation purposes) to improve the living conditions of the people. However, such projects tend to create favourable sites for the growth and development of the fresh water snails which act as the intermediate host of *schistosoma* parasites, therefore increasing the transmission rate of schistosomiasis.

1.1 The parasites and their transmission

The adult flukes, which are dioecious , live in the human body in the blood vessels of the hepatic and mesenteric systems. The eggs which are laid by the females find their way out of the human body by breaking out of the finer blood vessels which supply the bladder and the large intestines, and eventually gain entry into the lumen of the bladder and the rectum. From these locations, the eggs are excreted in urine in the case of urinary schistosomiasis (due to *Schistosoma haematobium*) or in faeces in the case of intestinal schistosomiasis (due to *Schistosoma mansoni*, *S. intercalatum* and *S. japonicum*) (McCullough, 1965).

The eggs will normally not hatch in the concentrated media of urine or faeces but as soon as the excreta enters water and becomes dilute the egg which already contains a developed first stage larva hatches. This larva, termed miracidium ,is a ciliated, motile, non-feeding

organism which must find and enter (by penetration) an appropriate snail usually within 12 to 18 hours in our ambient field temperatures or perish.

The appropriate intermediate hosts are specific species of fresh water pulmonate snails, *Bulinus* and *Biomphalaria* species. These snails usually inhabit stagnant or sluggish waters and are often encountered in ponds, pools, marshes, reservoirs and lakes. They are found also in weedy areas of streams and rivers where the flow of water is slow. In the various freshwater bodies they tend to be associated with certain aquatic weeds, but they may be found in water devoid of weeds where they subsist on encrusted algae. Inside the body of the snails (the intermediate hosts) the miracidium loses its ciliated epithelium and develops into a sac-like larva termed a sporocyst. This mother sporocyst is capable of multiplying asexually, to produce daughter sporocysts. These daughter sporocysts eventually produce, also asexually, the third stage larvae, the cercariae. They are motile fork-tailed organisms and are discharged in large numbers from the snail into the water medium in which the snails live. It is reckoned that offspring of a single sporocyst derived from a miracidium will produce cercariae all of one sex. This intra-molluscan development in the snail takes up to 4 weeks at a temperatures of 29 ± 1°C . An infected snail continues to shed cercariae over several weeks.

The cercariae are infective to man who is the definitive host. The cercariae which are non-feeding must find and enter the definitive host within 48 hours or perish. They infect man usually by directly penetrating the naked skin. But it is also possible to acquire the infection through drinking contaminated water containing cercariae. The tail of the cercariae is shed

as it penetrates the host. The rest of the body develops into a young schistosoma (schistosomulum) which enters the blood system where it grows into adult.

When mature, the adult males fertilize the adult females, the latter have to be in target sites and then lay their eggs into venules of the bladder and mensentry. The eggs which are armed with characteristic spines, also contain developing miracidia. By means of histolytic enzymes produced by the developing miracidia, the eggs digest their way through the wall of the blood vessels and of the bladder or intestine and finally gain entry into the lumens of these organs. From here they are discharged with the excreta or urine to complete the life cycle. The cycle in man takes about 2 to 3 months. But the worms can live and reproduce in man for years. As a result of the damage done to the blood vessels , blood usually accompanies the discharge of the excreta in the early and acute phases of schistosomiasis infection. It is observed that the pathological effects of schistosomiasis arise mainly from the presence and activities of the eggs in the human body and tissues, as the body reacts against them as foreign bodies. In chronic *S. haematobium* infection pathological changes may develop in the urinogenital tract and the kidneys, urethra and bladder. Malignant tumours of the bladder may also develop. Intestinal schistosomiasis infection causes fever, general weakness, loss of appetite, hepatomegaly and splenomegaly in severe cases.

1.2 Schistosomiasis in Ghana

Both forms of schistosomiasis i.e. urinary and intestinal, are found in Ghana (McCullough, 1956, 1962, 1965). In the savanna areas of the north, schistosomiasis is associated with temporary streams, pools and dams. In the forest areas of Central, South, East and Western Ghana, it is associated with slow flowing rivers and streams and dams. In the savanna areas

of the South-east, it is associated with the larger freshwater "lagoon" in the Volta flood plains where a lot of fishing is done. Along the 4,500 miles shore-line of the Volta lake are a number of fishing and farming communities which are affected to various degrees.

In the fresh water lagoons and in the Volta lake area, the disease mostly affects the fisherfolk, males and females, who are in contact with infected water. In certain large water reservoirs, it is found among the labour gangs who use and maintain the reservoirs. Children are generally affected through repeated contact with infected water used for recreational purposes, bathing and washing. In places religious practices which demand ablution and therefore frequent contact with water, produce a higher rate of infection among those adherents who use infected water than those who use pipe-borne water.

Water conservation programmes and the proximity and free entry of people into impounded water bodies have heightened transmission in many areas. Certain irrigation fields are becoming transmission sites due to improper drainage and management. As already indicated, impounded water bodies which serve as reservoirs for water supply for man and livestock or for hydroelectric power generation and where indiscriminate human water contact is allowed to take place have led to increased transmission. In most of these areas proper sanitary facilities for the communities are either non-existent or inadequate. Close proximity of settlements to the shores of these impounded water bodies have encouraged frequent contact with the water and therefore, transmission.

In addition to fishing in the main inland waters, the Volta lake, the lagoons, rivers and streams, fish-farming is being encouraged in private ponds and small dams and is on the increase. Since most of these water bodies already harbour the snail hosts of schistosomiasis, there is a real danger of increased transmission from these agricultural practices.

The construction of the Akosombo dam in 1964 and later the Kpong dam in 1981 across the river Volta in Ghana has created ecological conditions downstream and in the Volta estuary which have enhanced the invasion and proliferation of submerged, emergent and floating aquatic weeds and the snail intermediate hosts of schistosomiasis, *Bulinus*, *Biomphalaria* and also *Lymnaea* (Odei, 1983). The intensive water contact activities of the riparian communities who are mainly fishermen and clam-diggers have resulted in the establishment and transmission of urinary and intestinal schistosomiasis due to *S. haematobium* and *S. mansoni* respectively in the river Volta itself and in the Volta estuary almost at the sea coast. The Weija lake was created by the construction of a dam on Densu River in 1977. A preliminary investigation of the lake 15 years after impoundment by the Institute of Aquatic Biology (I. A. B.) reveals a high prevalence rates of *S. haematobium* in communities close to the lake.

Schistosomiasis has been endemic in Ghana for a long time. The government's awareness of its public health importance has led to organised studies of the problem by various health and research agencies. Work done so far has shown that before 1964, when the Volta lake was formed both *S. haematobium* and *S. mansoni* were endemic in Ghana (Odei, 1975).

The former which caused urinary schistosomiasis was widespread and was characterized by its consistently high prevalence in certain areas of the country. The snail hosts of *S. haematobium* are *B. globosus and Bulinus truncatus truncatus*. The former was widely distributed in both forest and savanna areas of the country. The latter, *B. truncatus truncatus*, was rather restricted to the savanna areas, the north and the south-east. *Schistosoma mansoni* which caused intestinal schistosomiasis was rather less prevalent than *S. haematobium*. The snail host was *Biomphalaria pfeifferi* which was also not as widely distributed as the bulinid snails.

The known distribution of the two forms of the disease matched the distribution of their snails hosts. But it was apparent that there were areas where the snails existed without the disease, suggesting that the disease could spread to wider areas. The area of the middle Volta basin was rather sparsely populated by the host snails. This was thought to be due to the scarcity of permanent surface water in the area (McCullough, 1965).

After the Volta lake was formed the Volta basin became flooded and provided conditions which encouraged host snail proliferation. The post-lake findings show that all sections of the Volta lake complex except the Daka branch, are infested with *B. truncatus truncatus* and *B. forskalii*. *B. globosus* does not seem to have succeeded in invading the lake though it has been recorded in Obosum basin since 1967 (Paperna, 1969). Biomphalaria is also absent from the lake though it has been found in the riverine section of the Dayi branch of the lake in 1972 (V.L.R.P., 1972). *S. haematobium* is therefore the only form of the infection so far encountered in the lake and it is transmitted by *B. truncatus truncatus*.

The snails in the lake tend to be associated with certain aquatic and semi-aquatic weeds, *Ceratophyllum*, *Spirodela*, *Pistia*, *Vossia*, *Alternanthera* and *Ludwigia* which provide a favourable micro-habitat in the lake for their proliferation and dissemination. Where *Ceratophyllum* grows submerged in deep water, it serves as reservoir from which snails colonize the shallower in-shore areas, where there human contact is frequent (Odei, 1965). Where marginal weeds, e.g. *Alternanthera* , *Polygonum* and *Vossia* grow, they create favourable conditions in otherwise inhospitable areas and so encourage the establishment of host snails in new areas.

The lake level fluctuates through a vertical height of 3.05m during the yearly cycle of flooding and drawn-down. These changes in lake level affect the ecology of the snails and influence the snail population density. The effect is rather severe in areas where marginal weeds provide the main weed-substrate for the snails when the lake margins become flooded. In these areas when the water recedes the snails in the weeds are stranded and some aestivate and manyof them may die. Elsewhere outside the lake, it is known that the main factor which affects the snail populations is the amount of rainfall. The population falls during the early rainy seasons but it is highest at the end of the rainy season and early dry season(Odei, 1965). In the lake, the population declines during the flooding from July to October and it is highest at the early draw-down period when the lake level recedes.

Studies in the Volta and elsewhere in Ghana in the numerous impounded bodies of water show that the host snails are spreading to new areas (Odei, 1975). The danger therefore

exists that the spread of the snails will be followed by a spread of the disease also to the new areas. Studies are therefore being carried out to provide data for the control of Schistosomiasis in Ghana.

Though mortality rate of Schistosomiasis is under 1%, the disease is a public health menace in that the infection rate is high (may reach 60% in some endemic areas with even higher rates particularly among children) and this tends to have negative socio-economic effects on countries in which the disease occurs. For example, the Volta River Authority (VRA), an autonomous body which is responsible for the generation and transmission of hydroelectric power at the Akosombo and Kpong dams and the management of the Volta lake basin, spends millions of cedis annually on the control of the disease. In 1990, the VRA spent about ₵2,500,000 to treat 2,683 infected people living around the Volta lake with praziquantel, a drug given orally according to the weight of the victim. Man hour loss to the country as a whole may be substantial (VRA annual report, 1990). This is also a drain on the foreign exchange resource of the country because money which should have gone into more productive development projects is thus diverted in the purchase of drugs and control of the disease.

1.3 Schistosomiasis control.

Concerted and directed efforts to control schistosomiasis in Ghana commenced in the late 1950s. It was then becoming evident that the disease was fairly widespread in the country. It is important to mention also that during that period the disease had begun to receive

attention by World Health Organisation (WHO) in its reports from other parts of the world. Work done before and during this period has been documented by Odei (1965) and McCullough (1965). The major commitment to control the disease was made by the establishment of a Snail Control Unit within the medical field units of the Ministry of Health in the late 1950s. This effort was later strengthened by assistance offered by WHO in the form of material and expert advisers. The task of the unit was to undertake what was termed at the time, a snail eradication programme aimed at controlling Schistosomiasis. The unit was directed up to undertake a country-wide search for, identify and record the snail intermediate hosts, to study their biology and ecology, and to devise and apply measures for their "eradication" (later modified as control) (Odei, 1983).

The strategy adopted was to attach trained snail collectors to schistosomiasis survey teams so that information collected on the existence and prevalence of the disease was simultaneously related to the findings on the snails in the localities covered. Most of these findings have been duly documented by McCullough and Ali (1965) and Odei (1974). It became evident that both *Schistosoma haematobium* and *S. mansoni* presenting as urinary and intestinal Schistosomiasis respectively were endemic in Ghana. The former was more prevalent (17 - 20%) than the latter (0 - 3%) as at 1965. The classical case of the outbreak of urinary schistosomiasis in the Volta lake created in 1964 has been reported by Paperna (1969) and Odei (1974). Subsequent water conservation projects undertaken in the 1970s have invariably been followed by increased schistosomiasis transmission (Odei, 1983). The life cycle of schistosomiasis and the transmission of schistosomiasis require four basic components - (i) the definite host (man, in most cases), (ii) the intermediate hosts (fresh-water snails), (iii) the parasites (*schistosoma* species), (iv) the transmission medium

(freshwater) for active transmission to be possible in a locality. In terms of control, therefore, the elimination of any of the above 4 components from the chain should break the cycle and thus eventually result in actual eradication of the disease which the parasite causes.

Elsewhere in the Gezira irrigation system of the Sudan a new type of palm leaf trap was designed for the control of the molluscan vectors of schistosomiasis (Markowski, 1955).

1.3.1 Chemotherapy

Measures to control the disease have been traditionally based on chemotherapy and been hospital based. This continues to be principally the unproclaimed policy, with metrifonate and praziquantel being the current drugs of choice. In the late 1970s a host of factors forced a rethink of molluscicide strategies. Probably the most important of these factors was the increasing cost of Bayluscide which was linked to the price of oil. Another major factor was the appearance in 1975 of Praziquantel - the first safe, effective, single dose, oral drug capable of reducing morbidity in population-based treatment campaigns. As a result, anti-schistosomiasis programmes aimed more and more at morbidity control than transmission control (Appleton, in press). Treatment is now based in decentralized local Health Centres and clinics in the Primary Health Care systems under the general supervision of the Schistosomiasis Control Division of the Ministry of Health.

1.3.2 Schistosome host snail control

Snail control measures have been undertaken over the years at various times by the snail control unit, the Volta Lake Research Project and the Institute of Aquatic Biology. Initially

molluscicides were used either alone or in conjunction with appropriate herbicides or the mechanical clearance of aquatic weeds. These measures have proven to be successful under certain limited conditions, mainly in small impoundments and when applied focally. The chemicals used were mainly sodium pentachlorophenate (NaPCP), Bayluscide, Frescon as molluscicides and Paraquat and Diquat as herbicides.

Current research effort in snail control by the Institute of Aquatic Biology is in the search for biological control agents, mainly microbial pathogens and competitor snails. The Institute is also searching for and screening plants with molluscicidal properties.

1.3.2.1 Competitor snails

Melanoides tuberculata is being tested as a possible competitor snail under natural field conditions (McCullough, 1981). The results show that this snail because of its high fecundity and being also parthogenetic has competitive advantage over the host snails and is thus able to "swamp" the shared habitat shared with the host snails. But after 3 years of study it has not completely excluded the host snails. The host snails which initially constituted about 90% of the snail population were reduced to only 1 - 5% at the study area (Odei, 1983). It is very interesting to note that a few host snails do become infected and therefore are able to transmit the infection. This indicates that *M. tuberculata* does not act as decoy of schistosome miracidia in the medium.

1.3.2.2 Plant molluscicides

The potential of plant molluscicides was first recognised in 1933 with Archibald's observation on the toxicity to *Biomphalaria sudanica* of the berries of the tree *Balanites aegyptiaca* in Sudan. This failed however to elicit more than token interest and it was only 50 years later, in the late 1980s that the number of studies on plant molluscicides exceeded those on synthetic molluscicides (Appleton, in press). The first paper on *Phytolacca dodecandra* was published by Lemma (1965). This enthusiasm for plant molluscicides lasted only a decade or so and in the early 1990s interest reverted to synthetic compounds, though at a low level. It has been argued by some, e.g. Gryseels, *et al.* (1992), that snail control has not been shown to be effective and should not be considered as a control measure.

Plant molluscicides are likely to reduce the high cost of Bayluscide if they can be brought 'on stream'! In an ongoing research programme at the University of Natal a combination of WHO guidelines (Kloos & McCullough, 1982 as cited in Appleton (In press) and the ideas of local scientist have been followed in drawing up a short-list of three molluscicidally-active plant species that are also useful in traditional medicine. These three species, all indigenous to the schistosomiasis-endemic area, were thoroughly assayed against the target snails as well as various non-target organisms, both animal and plant (Clark, 1994 as cited in Appleton (In press). Ranking them afterwards, aqueous suspensions of the leaves of the tree *Gardenia thunbergia* (White Gardenia) and *Apodytes dimidiata* (White pear) seemed to have the greatest potential. Both species were molluscicidally active (Anon, 1983).

The Institute of Aquatic Biology in Ghana is making strenuous efforts on plant molluscicides in Ghana, and particularly on *Phytolacca dodecandra*. A number of plants indigenous to Ghana are being screened including those containing saponins and those which have been reported in the literature as having molluscicidal properties. These include *Tephrosia vogelii, Balanites aegyptiaca, Luffa sp., Jatropha sp., Blighia sapinda, Canna indica* and *Ricinus communis*. Among these plants *Tephrosia* and *Balanites* are the only ones which have shown molluscicidal activity using water extracts. Unfortunately both of them have been temporarily suspended. Irvin ,1961(as cited in Odei, 1983) recorded *Phytolacca dodecandra* at Larteh in Ghana where its local name is 'Ahoro'. *P. dodecandra* has actually been located by Dr. A. A. Entwi (Odei, 1983).

1.3.2.3 Inorganic molluscicides

The potential value of inorganic molluscicides in controlling African schistosomiasis was noted as far back as 1915 when Leiper suggested that snail control in irrigation canals in Egypt could be improved by the use of ammonium sulphate in pools remaining after the canals had been emptied. In 1942 the Egyptian Ministry of Health established a Snail Destruction Section in Fayoum (Ministry of Health, 1945). Copper sulphate was the molluscicide of choice and *Bulinus truncatus* the target snail. Serious attempts to evaluate candidate molluscicidal compounds did not begin until the late 1940s and 1950s when about 7000 were screened (Ritchie, 1973). Interest in molluscicides rose to a peak in the 1960s but declined during the 1970s due partly to the very high costs of developing new compounds (Appleton, in press). During the 1960s and 1970s however, the intensive spraying of water bodies with molluscicides was the mainstay of schistosomiasis control

programmes and the eradication of snail populations was seen as both feasible and desirable. Of the thousands of compounds tested in the laboratory, only a few were subjected to field trials. Principal amongst these were NaPCP and $CuSO_4$ which were widely used, NaPCP particularly in Brazil and Japan and $CuSO_4$ particularly in Africa (Appleton, in press).

For various reasons, these chemicals were found to be unsuitable for field work. Instability in the presence of sunlight and health hazards to spray men restricted the usefulness of NaPCP (Meyling *et al.*, 1959, Blair, 1961) and the effectiveness of $CuSO_4$ required the clearance of vegetation and frequent applications (Pitchford *et al.*, 1960). Other disadvantages were non-toxicity to snail eggs (Annecke & Peacock, 1951), adsorption onto organic matter and non-target toxicity (Shiff & Garnett, 1961).

In 1960 the first specially formulated molluscicide, Bayluscide (Bayer 73), was produced (Foster *et al.*, 1960). This has been the most successful molluscicide ever produced and has been used in many control programmes. It is currently the only synthetic molluscicide being marketed for use in fresh water. Bayluscide is the ethanolamine salt of niclosamide and its active ingredient, clonitralide, is toxic not only to aquatic snails, but also their eggs and miracidia and cercariae of schistosome and fasciolid flukes.

The disease can be controlled in many ways. The parasites have an obligate relationship with their snail intermediate hosts. Consequently, the depression of snail populations should be an essential element in control programmes aimed at this disease. Snail control methods involve manipulation of the biological, physical and chemical environment to render it unsuitable for snail multiplication and survival. Of these methods, chemical

manipulation using molluscicides is the most efficient for reducing snail numbers. The method of molluscicide control measures is effected in two ways. (i) Dripfeed and (ii) Aerial application.

The dripfeed method is carried out in flowing water by applying the molluscicide at the water edges and allowing it to be carried away by the flow. In aerial application, the molluscicide is sprayed from the air by means of an aircraft. In these methods, precautions are taken with regards to the concentration of the molluscicide. If the concentration is low, very little or no effect occurs. On the other hand, more harm than good is caused if the concentration is too high. The general approach in molluscicide application is laborious and expensive. Often snails escape molluscicides by burrowing into the mud hence leading to a high failure rate. Also because of non-target tixicity and the biodegrading nature of these chemicals, non-target species get killed and chemical residues left in water bioaccumulate in edible fauna rendering them unwholesome for human consumption. Water for domestic purposes also become contaminated. The use of broad spectrum molluscicides such as Bayluscide in the fight against schistosome host snails has generated certain problems such as high financial cost, destruction of non-target organisms and chemical pollution of water bodies (McCullough *et al.*, 1980). The fact that Bayluscide is piscicidal at molluscicidal concentrations can be a problem. Experience in South Africa showed that dead fish continue to float on the surface for about three days after spraying (Appleton, 1985). This means that, particularly in stock farming areas, repeat visits must be made to each treated site to collect dead and rotting fish. No farmer is going to want his stock to drink from water sources polluted by dead fish.

These problems have prompted the Scientific Working Group of the WHO (1977) to recommend that the prime aims of research concerned with control should be the development of cheaper methods of control with increasing target specificity. In the same year, Caraderelli *et al* (1977) (as cited by J.D. Thomas *et al* (1980) had investigated the possibilities of using controlled release technology to increase the efficiency of molluscicide applications. More recently Thomas *et al* (1986) has suggested that the efficiency of a controlled release formulation (CRF) could be enhanced by combining attractants and phagostimulants that would allow target snails to be removed selectively, with minimal adverse effects on the environment.

1.3.2.4 Bioactive natural materials

Recent research has been directed towards identifying artificially formulated food materials, Michelson (1960) and naturally occurring crude Etges (1963), and pure chemical factors (Thomas, 1980 as cited by Kpikpi ,1990) that could be used as baits in the proposed controlled release formulations. This concept of identifying pure chemical factors for CRF, first formulated by Thomas *et al* (1979), has led to the search for attractants, arrestants and phagostimulant substances. To date, pure carbohydrate compounds (Thomas, 1986; Kpikpi, 1990; Kpikpi and Thomas, 1992) and amino acids and carboxylic acids (Thomas *et al*, 1986; 1989) have been explored for bioactivity.

However, most of the experimental work in this field has been focused on *Biomphalaria glabrata* , the snail host of human intestinal schistosomiasis in South America. As a result, the chemoreception niche of this species has been well characterised in terms of its response

to crude extracts and to pure compounds (Thomas *et al*, 1980a & 1980b). The main objective of the investigations carried out by Thomas and his colleagues was to identify chemicals which would serve as potent specific attractants, arrestants and phagostimulants for incorporation into ingestible controlled release formulations (CFSs) designed to remove *B. glabrata* selectively in cost effective ecologically acceptable manner. Of the many chemicals that were found to be effective attractants and arrestants to *B. glabrata* were short chain carboxylic acid (C3 - C5) (Thomas *et al*, 1983) while maltose was found to be the strongest phagostimulant.

Kpikpi (1990) developed and used a combination of bamboo cylinders and foam materials soaked in test chemicals. In addition to demonstrating the effectiveness of the two snail trapping units, he also pointed to the possible modifications that would transform these units into tools for snail control. Although the fact that schistosome host snails respond to attractants, arrestants and phagostimulants (Kairomones or bioactive factors) is now well documented (Thomas et al 1989; Thomas *et al* 1990; Kpikpi 1990, 1992; Kpikpi and Thomas, 1992), the application of this knowledge towards the selective removal of schistosome host snail has only just begun (Kpikpi, 1990). In his study, Kpikpi (1992) describes the detailed measurement of components of sugar chemoreception niches of *Bulinus globosus* and *Bulinus truncatus*. He found maltose, maltriose and xylose to be attractants, arrestants and phagostimulants for *B. globosus*. Pure amylose is also a potent phagostimulant for both *B. truncatus* and *B. globosus*. He concluded that these findings can be utilized for selective removal of schistosoma host snails. Kpikpi (1992) describes the study in which the efficacies of maltose, hydrolysed starch, amylopectin carbohydrates (M

A. H.) were identified as kairomones under laboratory conditions. These were then tested under field conditions in the Kpong lake in Ghana, the result of which revealed that only maltose (at 4%) and M. A. H. produced significant effects on *Bulinus globosus*, and hence the assessment of the implications of the findings on slow release technology in schistosomiasis control.

1.4 The present study

The present study intends to investigate the suitability of natural products which may be cheaper than pure chemicals and more easily available in the areas where schistosomiasis is endemic. The work involved the identification of natural products which act as attractants to snail hosts of schistosomiasis. The efficacy of these bioactive factors was tested using modifications of already developed bamboo traps (Kpikpi, 1990) under simulated natural conditions (SNC). The SNC tests were designed to identify the ideal combination of bioactive factor and bamboo cylinder which would produce the most efficient trapping units. The efficacy of the ideal trapping units were evaluated under field conditions in a natural water body, the Weija Lake. Consequently the work was divided into three phases. The first two phases were laboratory based while the third phase was undertaken in the field.

One stem, four roots and five fruits yielding a total of twenty-six naturally occurring materials under different treatment regimes, were tested on juveniles and adults of the schistosome host snails of *Bulinus truncatus (B. truncatus)* and *Biomphalaria pfeifferi* to

identify natural products which act as attractants to these snails. They were tested further using bamboo traps under SNC to identify the ideal combination of bioactive factor and bamboo cylinder which would produce the most efficient trapping units.

CHAPTER TWO

BIOASSAY STUDIES USING DIFFUSION OLFACTOMETERS

2.1 INTRODUCTION

The parasites which cause schistosomiasis in man have an obligate relationship with their snail intermediate hosts. Consequently, the suppression of snail populations should be an essential element in control programme aimed at this disease. Snail control methods involve manipulation of the biological, physical and chemical environment to render it unsuitable for snail multiplication and survival. Of these methods, chemical manipulation using molluscicides is at present the most efficient for reducing snail numbers. The molluscicides currently available however, have some major disadvantages; these include high financial cost and adverse effects on non-target organisms. These disadvantages can be mitigated to a large extent by using species - specific molluscicides. This may be done by the use of snail attractant substances combined with toxicants. The idea is that the snails will be selectively drawn towards the toxins and hence selectively eliminated. This concept, first formulated by Thomas & Assefa, (1979), has led to the search for attractant arrestants and phagostimulatory substances. To date, pure carbohydrate compounds (Thomas, 1986; Kpikpi, 1990; Kpikpi and Thomas, 1992) and amino and carboxylic acids (Thomas et al, 1986; Thomas 1989) have been explored for bioactivity. In the study reported in this chapter, the responses of *Bulinus truncatus* and *Biomphalaria pfeifferi* to whole plant materials are investigated. In all twenty-six plant materials were tested.

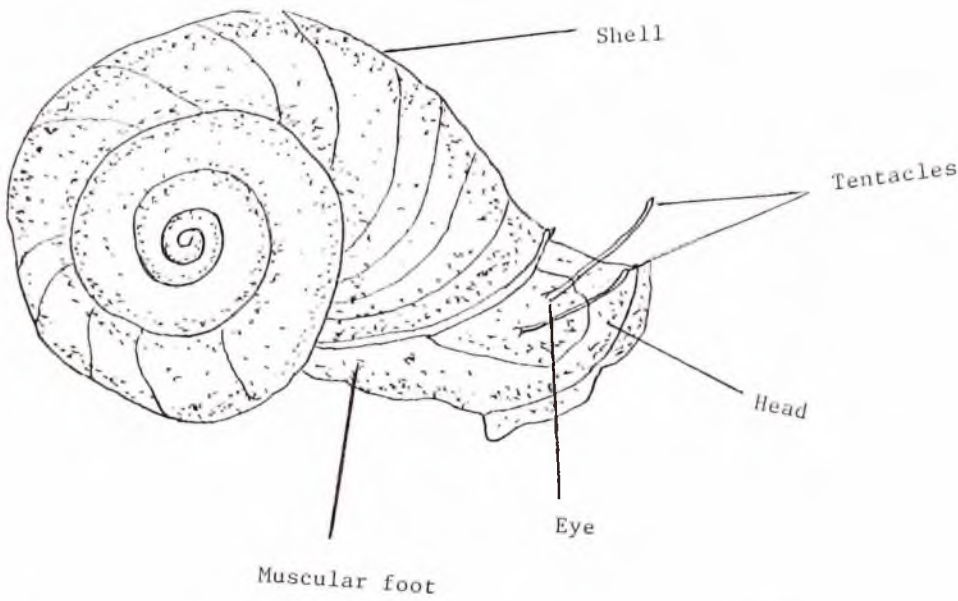
2.2 MATERIALS AND METHODS

2.2.1 Snail Breeding

Adult snails of *Bulinus truncatus* and *Biomphalaria pfeifferi* (Figs. 1A & 1C), were collected from the Weija Lake and kept in the laboratory at a temperature of 26 ± 1 °C under natural light regime (approximately 12 hours of light and 12 hours of dark). Seven adult snails of each species of snails were kept in each of twelve 3000ml plastic bowls filled with 2000ml tap water (Plate 1). The snails were routinely fed every other day with three fresh leaves of wild lettuce *(Lactuca taraxactifolia)*. The water in which the snails were kept was changed twice a week. Eggs were laid within three days and five days respectively by *B. truncatus* and *B. pfeifferi*. The eggs of both species of adult snails were hatched within 7-9 days. The juveniles of both *B. truncatus* and *B. pfeifferi* of 17-24 days old were used in the first phase of the experiment. The average weight of these juvenile snails was 0.01 g (n=50).

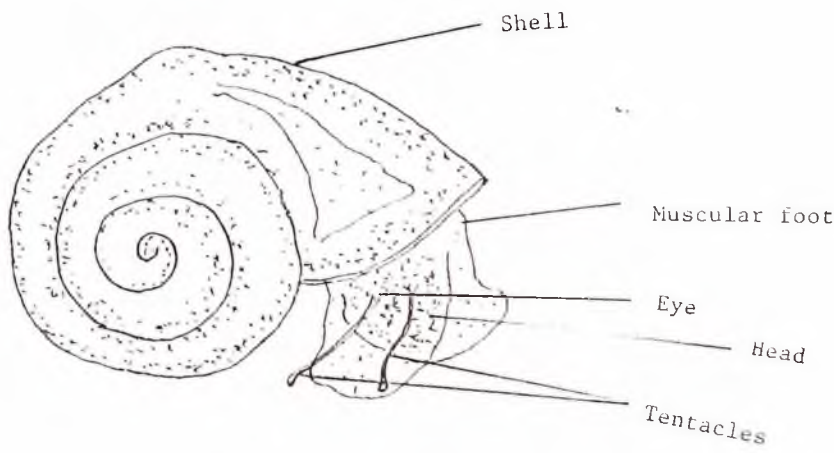
2.2 . 2 Pre-treatment of snails

Prior to the experimentation, 50 active juvenile snails of each of the two species were selected and placed in a 250ml beaker containing 160ml tap water and deprived of food for a period of 20-24 hours. They were thenfed with two discs of wild lettuce of 1cm diameter. The above procedure was followed for the next set of experiments involving adult snails of *B. truncatus* and *B. pfeifferi*. These adults, however were fed with two discs of wild lettuce of 2cm diameter after each experiment. The adults of the two species weighed 0.1 ± 0.01g on average (n=50)



X 10

FIGURE 1A: ADULT BIOMPHALARIA PFEIFFERI



X 12.5

FIGURE 1B: JUVENILE BIOMPHALARIA PFEIFFERI

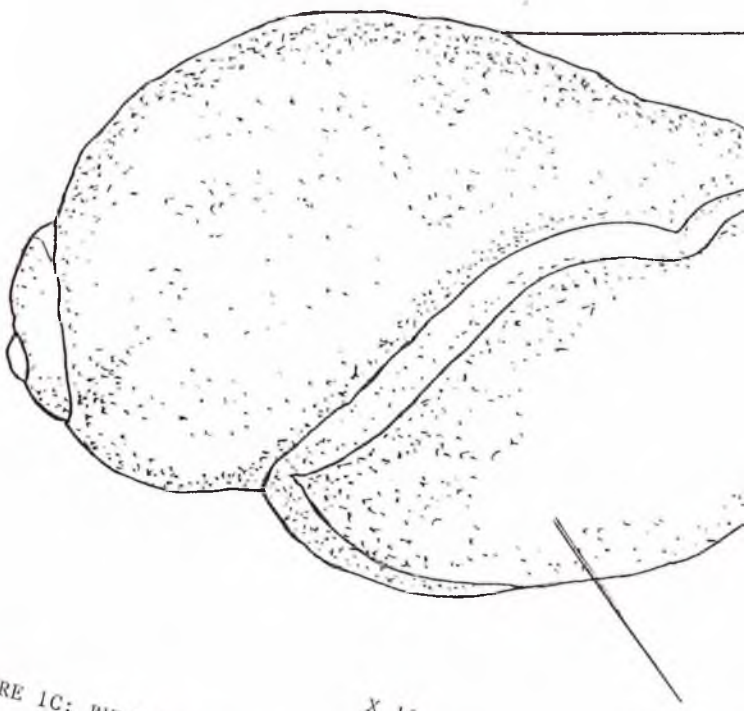


FIGURE 1C: BULINUS TRUNCATUS X 10

PLATE 1: Breeding of snails of Biomphalaria pfeifferi and Bulinus truncatus in plastic bowls in the laboratory for bioassay study. The snails were fed on wild lettuce.

2.2.3 Preparation of Test Materials

Ten natural plant materials known to contain some sugars were chosen and tested as raw (R) or boiled (B) for 15 minutes and in addition, for the fruits, in a unripe green condition (G) or ripe mature condition (M). Combinations of the different states of the plant materials gave a total of 26 test substances (Table 1a). Polystyrene, an inert material, was used as control material. The food (bioactive) materials were cut into circular disc forms of diameter 1.0cm and thickness 1.2cm for the juvenile snails and 2.0cm diameter and 1.5cm thickness for the adult snails, with cylindrical cutters of appropriate dimensions. Some of the food materials were boiled for fifteen minutes on a hot metal plate before cutting into circular forms. The polystyrene materials were of similar dimensions.

Table 1a: Test materials and the various conditions in which they were used

	PLANTS	R	B	G	M	B&G	B&M
ROOT							
1.	*Manihot esculenta* (Cassava)	*	*				
STEMS							
1.	*Saccharum officinarum* (Sugar cane)						
2.	*Dioscorea rotundata* (Yam)	*	*				
3.	*Ipomoea batatas* (Sweet potato)	*	*				
4.	*Xanthosoma muffafa* (Cocoyam)	*	*				
FRUITS							
1.	*Musa paradisiaca* (short Banana)			*	*	*	*
2.	*M. sapientum var paradisiaca* (long Banana)			*	*	*	*
3.	*Musa sapientum* (Plantain)			*	*	*	*
4.	*Carica papaya* (Pawpaw)			*	*	*	*
5.	*Ananas comosus* (Pineapple)				*		

Key: **R**=Raw, **B**=Boiled, **G**=Unripe green, **M**=Ripened Mature

2.2.4 Boiling Procedure

The boiled materials were prepared by dropping the raw materials into a 1000ml beaker containing 600ml boiling water on a hot plate. It was then allowed to boil for 15 minutes. The boiled test materials were then cut into discs.

2.2.5 Diffusion or gradient olfactometers

The olfactometers used in the experiments were similar to those used by Thomas *et al* (1979). Those for juvenile snails consisted of a rectangular central chamber measuring 3.8 by 1.0 by 2.0cm, joined at each end to a cylindrical end chamber measuring 1.2cm in diameter and 2.0cm in depth (fig. 2.1). The olfactometers used for adult snails were larger, with a central chamber measuring 7.8 by 1.9 by 2.0cm, joined at each end to a cylindrical chamber measuring 2.4cm in diameter and 2.0 in depth (Fig. 2.1). The olfactometers used for the experiments were each washed thoroughly with hot water and a detergent and rinsed several times with tap water prior to and after each experiment. By using a perspex block of 20 olfactometers it was possible to replicate each test 20 times. Snails are known to respond to gravity (Lever & Geuze, 1965), therefore care was taken to ensure that the olfactometers were level. Also, to counteract any directional bias, due to light gradients, the locations of the test food and control materials were alternated in successive olfactometers.

2.2.6 THE BIOASSAY EXPERIMENT

Each chamber was filled with 30ml of tap water and a snail placed in the middle of the central chamber at the start of the experiment (Plate 2). The position of each assay snail was noted at 2.5 minute intervals. They were scored positive if they were on the test side +, or test

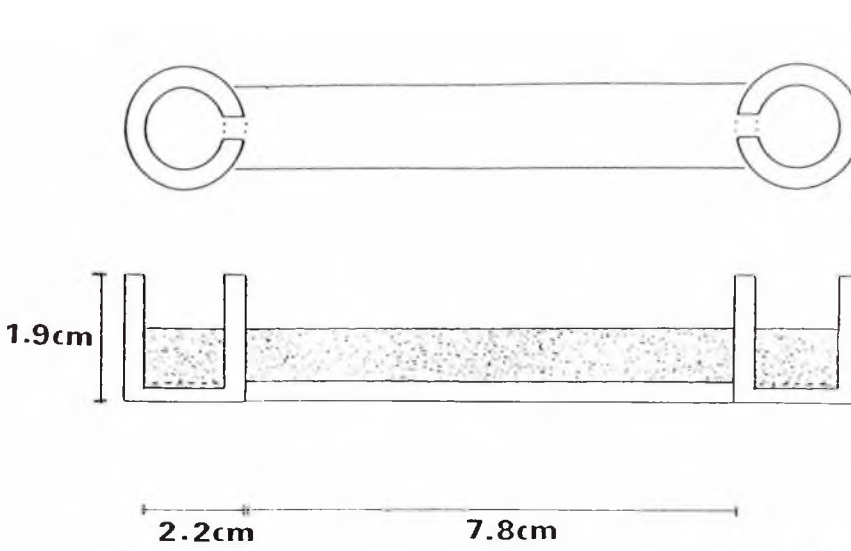


Fig. 1D: Diagrammatic representation of both surface and side views of the diffusion olfactometer.

PLATE 2: Student performing bioassay tests using olfactometers

disc (+), and negative if on the control side -, or control disc(-). The mean number of time units spent by the snail in each location was then calculated. Paired t-test (Bailey 1981) were used to determine the levels of significance. The mean values of the t-tests are referred to as attractant and arrestant indices.

Plant materials which cause the snails to spend significantly more time on the test side or test disc, compared with controls, are deemed to have attractant or arrestant effects respectively.

2.3 RESULTS.

2.3.1 Attractants and Arrestants.

Test materials which cause snails to spend significantly more time on the test disc compared with controls are classified as having arrestant effect. Those which make the snails spend significantly more time on the test side compared with controls are deemed to have an attractant effect. Test materials which caused the snails to spend more time on the control discs/sides were classified as repellants.

In all twenty-six plant materials were tested. Of these five were statistically significant attractant or arrestant for adult *B. truncatus*, while three were attractant for juvenile *B. truncatus*. Adult snails of *B. pfeifferi* showed an attractant or arrestant response to fifteen materials, while nine were effective attractants or arrestants for juveniles of *B. pfeifferi*. Four of the plant materials acted as significant repellants for juveniles of *Bulinus truncatus* - ripe fruits of boiled plantain; unripe fruits of raw plantain and boiled long banana, and boiled cocoyam. Unripe fruits of raw plantain also acted as repellant for *B. pfeifferi* juveniles. The strongest attractant and arrestant responses for both adults and juveniles of *B. truncatus* and *B. pfeifferi* were elicited by sugar cane.

juveniles responded significantly ($p < 0.05$) to only 9 out of the 26 materials tested (Table 1). These were raw cassava, boiled unripe plantain, sugar cane, boiled cassava, boiled ripe pawpaw, boiled cocoyam, raw unripe long banana, raw ripe pawpaw and raw yam (Table 4). Of the products which were found to be effective attractants and arrestants for *B. pfeifferi*, sugar cane, raw ripe long banana, raw unripe pawpaw, raw cassava and boiled unripe plantain had the greatest effect on the snails ($p < 0.005$) with sugar cane producing the highest response index of 8.1 for the juveniles (Table 4), and raw ripe long banana elicited the highest response index of 7.8 for the adults (Table 7). Only one out of the 26 natural products tested on *B. pfeifferi* juveniles acted as a statistically significant repellent ($p < 0.05$). This was raw unripe plantain. None of the 26 materials tested on *B. pfeifferi* adults acted as statistically significant repellent.

2. 3.2 Response of snails to types of test materials.

2.3.2.1 Response to raw and boiled natural products.

B. truncatus

Adults of *B. truncatus* responded to 3 (21.4%) out of the 14 raw products tested, and responded to 2 (16.8%) out of the 12 boiled materials tested on them (Table 2) The juveniles responded to 2 (14.3%) out of 14 raw products, and to only one (8.3%) of the 12 boiled materials tested on them (Table 2).

B. Pfeifferi

Out of the 14 raw products tested on the adults 9 (64.4%) acted as attractants/arrestants. The adult snails responded to 5 (41.7%) out of 12 boiled products tested on them (Table 2).

The juveniles responded to 5 (35.7%) out of the 14 raw products and to 4 (33.3%) out of the 12 boiled products tested on them (Table 2)

2.3.2.2 Response to stems, roots and fruits materials.

B. truncatus

Adults responded (100%) to the only stem (i.e sugar cane) tested on them. They responded to 2 (25%) out of the 8 root products, and to 2 (11.8%) out of the 17 fruit materials tested on them. The juveniles responded (100%) to the only stem used, to none (0%) of the root, and to 2 (11.8%) out of the 17 fruit materials tested on them (Table 3).

B. Pfeifferi

Out of the 1 stem, 8 roots and 17 fruit products tested on the adults, they responded to 1 stem (100%), 5 roots (62.5%), 9 fruits (52.9%). The juveniles responded (100%) to the stem, to 4 (50%) of the 8 roots and to 4 (23.5%) of the 17 fruits materials tested on them (Table 3).

TABLE 1b: Proportions of test factors acting as significant attractants for adults and juveniles of two snail species.

	TOTAL NO. OF PRODUCTS TESTED	NO. OF ATTRACTANTS	% ATTRACTANTS
BULINUS TRUNCATUS (Juveniles)	26	3	11.5
B. TRUNCATUS (Adults)	26	5	19.2
BIOMPHALARIA PFEIFFERI (Juveniles)	26	9	34.6
B. PFEIFFERI (Adults)	26	15	57.7

TABLE 2

Proportions of raw and boiled test factors acting as significant attractants for adults and juveniles of two snail species.

SPECIES	TOTAL NO. OF RAW PROD. TESTED	NO. OF RAW ATTR.	% OF RAW ATTR.	TOTAL NO. OF BOILED PROD. TESTED	NO. OF BOILED ATTR.	% OF BOILED ATTR.
B.TRUN. (Juv.)	14	2	14.3	12	1	8.3
B.TRUN. (Adults)	14	3	21.4	12	2	16.7
B.PFEIF. (Juv.)	14	5	35.7	12	4	33.3
B.PFEIF. (Adults)	14	9	64.3	12	5	41.7

ATTR. – *ATTRACTANTS* ; *B.TRUN.* – *BULINUS TRUNCATUS*; *Juv.* *Juveniles*

B.PFEIF. – *BIOMPHALARIA PFEIFFERI.* *PROD.* *PRODUCTS*

TABLE 3: Proportions of stems, roots, and fruits test factors acting as significant attractants for adults and juveniles of two snail species.

SP	TOT. NO. OF STEM TEST.	NO. OF STEM ATTR	% OF STEM TEST	TOT. NO. OF ROO. TEST.	NO. OF ROO. ATTR	% OF ROO. TEST	TOT. NO. OF FRUI. TEST.	NO. OF FRUI ATTR	% OF FRUI TEST
B.RO (Juv)	1	1	100	8	0	0	17	2	11.8
B.RO (Ad.)	1	1	100	8	2	25	17	2	11.8
B.PF (Juv)	1	1	100	8	4	50	17	4	23.5
B.PF (Ad.)	1	1	100	8	5	62.5	17	9	52.9

B.RO = BULINUS TRUNCATUS; B.PF= BIOMPHALARIA PFEIFFERI; SP = SPECIES; TEST. = TESTED; ATTR.. = ATTRACTANTS; FRUI = FRUITS; Juv = Juveniles; Ad. = Adults. ROO=ROOTS, TEST=TESTED

TABLE 4: BIOACTIVE MATERIALS WHICH PRODUCED A SIGNIFICANT EFFECT ON JUVENILES OF *BIOMPHALARIA PFEIFFERI*

TEST MATERIAL /NATURAL PRODUCT	MEAN ATTR INDEX	LEVEL OF SIGN.	MEAN ARREST INDEX	LEVEL OF SIGN	MEAN REPELL INDEX	LEVEL OF SIGN.
CASSAVA (RAW)	7.400	* * *	4.850	* * *	-	-
UNRIPE PLANTAIN (BOILED)	6.700	* * *	5.200	* * *	-	-
SUGAR CANE	8.100	* * *	5.100	* * *	-	-
CASSAVA (BOILED)	5.000	* *	3.650	* *	-	-
RIPE PAWPAW (BOILED)	5.600	* *	-	-	-	-
COCOYAM (BOILED)	3.900	*	4.250	* * *	-	-
UNRIPE LONG BANANA (RAW)	4.700	*	-	-	-	-
RIPE PAWPAW (RAW)	4.700	*	-	-	-	-
YAM (RAW)	3.300	*	-	-	-	-
UNRIPE PLANTAIN (RAW)	-	-	-	-	-4.200	*

*ATTR. = ATTRACTANT; SIGN. = SIGNIFICANCE; ARREST. = ARRESTANT; REPELL. = REPELLENT; * = P = 0.05; ** = P = 0.01; *** = P = 0.001.*

TABLE 5 BIOACTIVE MATERIALS WHICH PRODUCED A SIGNIFICANT EFFECT ON ADULTS OF *BULINUS TRUNCATUS*

TEST MATERIAL/ NATURAL PRODUCT	MEAN ATTR. INDEX	LEVEL OF SIGN.	MEAN ARREST. INDEX	LEVEL OF SIGN.	MEAN REPELL. INDEX	LEVEL OF SIGN.
SUGAR CANE	8.500	* * *	4.050	* *	-	-
POTATO (RAW)	7.400	* * *	3.250	*	-	-
UNRIPE PAWPAW (RAW)	5.600	*	-	-	-	-
RIPE PAWPAW (BOILED)	4.600	*	-	-	-	-
CASSAVA (BOILED)	4.600	*	-	-	-	-

ATTRACT. = ATTR.; SIGN = SIGNIFICANCE; REPELL. = REPELLENT

*ARREST. = ARRESTANT; * = P < 0.05; ** = P < 0.01; *** = P < 0.001.*

TABLE 6: BIOACTIVE MATERIALS WHICH PRODUCED A SIGNIFICANT EFFECT ON JUVENILES OF *BULINUS TRUNCATUS*.

TEST MATERIAL /NATURAL PRODUCT	MEAN ATTR. INDEX	LEVEL OF SIGN.	MEAN ARREST. INDEX	LEVEL OF SIGN.	MEAN REPELL. INDEX	LEVEL OF SIGN.
RIPE PAWPAW (BOILED)	8.500	* * *	-	-	-	-
RIPE PAWPAW (RAW)	5.100	* *	-	-	-	-
SUGAR CANE	4.000	*	-	-	-	-
COCOYAM (BOILED)	-	-	-	-	-7.400	* *
RIPE PLANTAIN (BOILED)	-	-	-	-	-5.0000	*
UNRIPE LONG BANANA (BOILED)	-	-	-	-	-4.900	*
UNRIPE PLANTAIN (RAW)	-	-	-	-	-4.800	*

*ATTR. = ATTRACTANT; SIGN. = SIGNIFICANCE; REPELL. = REPELLENT; ARREST. = ARRESTANT; * = P < 0.05; ** = P < 0.01; *** = P < 0.001.*

Table7: BIOACTIVE MATERIALS WHICH PRODUCED A SIGNIFICANT EFFECT ON ADULTS OF BIOMPHALARIA PFEIFFERI

TEST MATERIAL/NATURAL PRODUCT	MEAN ATTR. INDEX	LEVEL OF SIGN.	MEAN ARREST. INDEX	LEVEL OF SIGN.	MEAN REPELL. INDEX	LEVEL OF SIGN.
RIPE LONG BANANA (RAW)	7.800	* * *	3.850	* *	-	-
YAM (BOILED)	6.200	* * *	3.250	* *	-	-
UNRIPE PLANTAIN (BOILED)	4.300	* * *	3.700	* *	-	-
SUGAR CANE	6.400	* *	5.400	* * *	-	-
UNRIPE PAWPAW (RAW)	5.800	* *	3.600	* *	-	-
CASSAVA (RAW)	6.700	* *	-	-	-	-
RIPE SHORT BANANA (RAW)	5.200	* *	2.450	*	-	-
COCOYAM (BOILED)	4.700	* *	5.000	* * *	-	-
COCOYAM (RAW)	4.000	*	4.550	* * *	-	-
RIPE SHORT BANANA (BOILED)	4.800	*	3.000	*	-	-
RIPE PLANTAIN (BOILED)	4.600	*	2.500	*	-	-
UNRIPE SHORT BANANA (RAW)	4.600	*	-	-	-	-
POTATO (RAW)	3.900	*	2.800	*	-	-
PINEAPP.	4.400	*	2.500	*	-	-
RIPE PLANTAIN (RAW)	4.300	*	3.350	* *	-	-

*ATTR. = ATTRACTANTS; SIGN. = SIGNIFICANCE; ARREST. = ARRESTANT; REPELL. = REPELLENT; PINEAPP. = PINEAPPLE. * = P< 0.05; ** = P< 0.01; *** = P< 0.001*

Figures 1A(a) & 1A(b): Attractant and arrestant responses of adults of *Bulinus truncatus* to selected plant materials.

R = Raw
GR = Green / Unripe raw
GB = Green / Unripe boiled
MR = Mature / Ripe raw
MB = Mature / Ripe boiled

* = $p<0.05$
** = $p<0.01$
*** = $p<0.001$

ATTRACTANT(A) = Attractant responses of adults.
ARRESTANT(A) = Arreatant responses of adults.

Figure 1A(a)

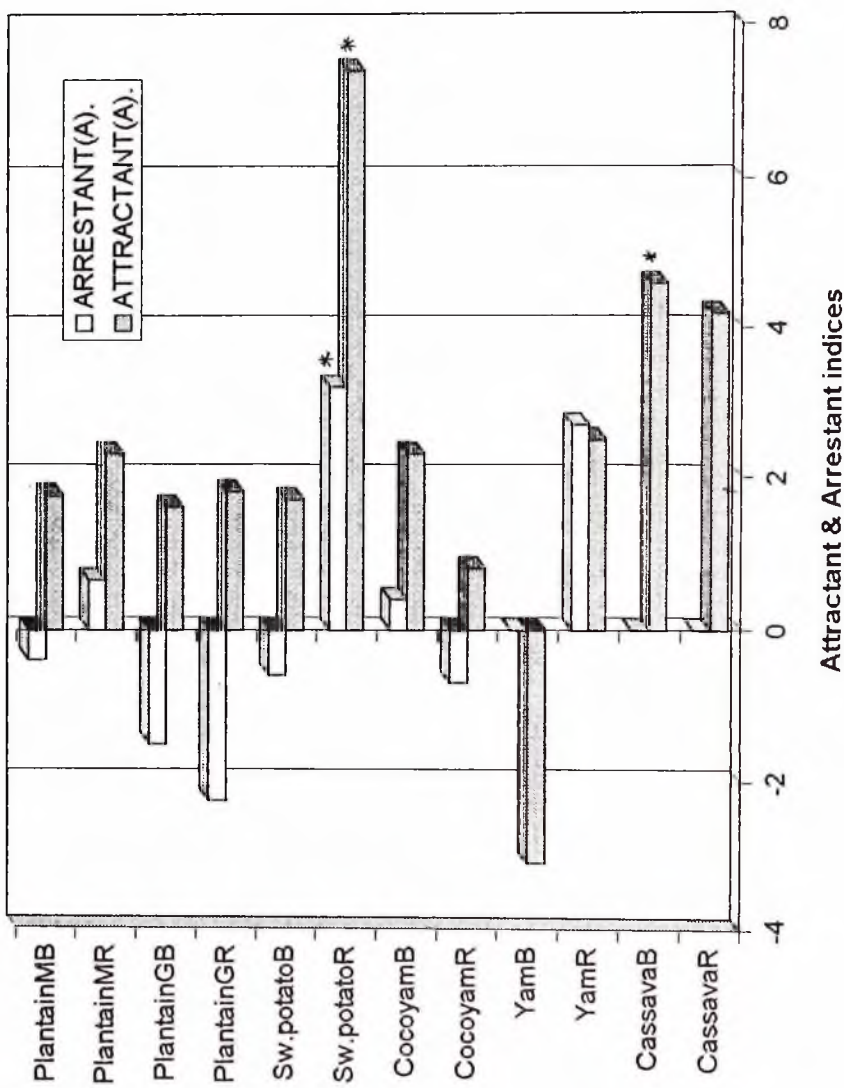
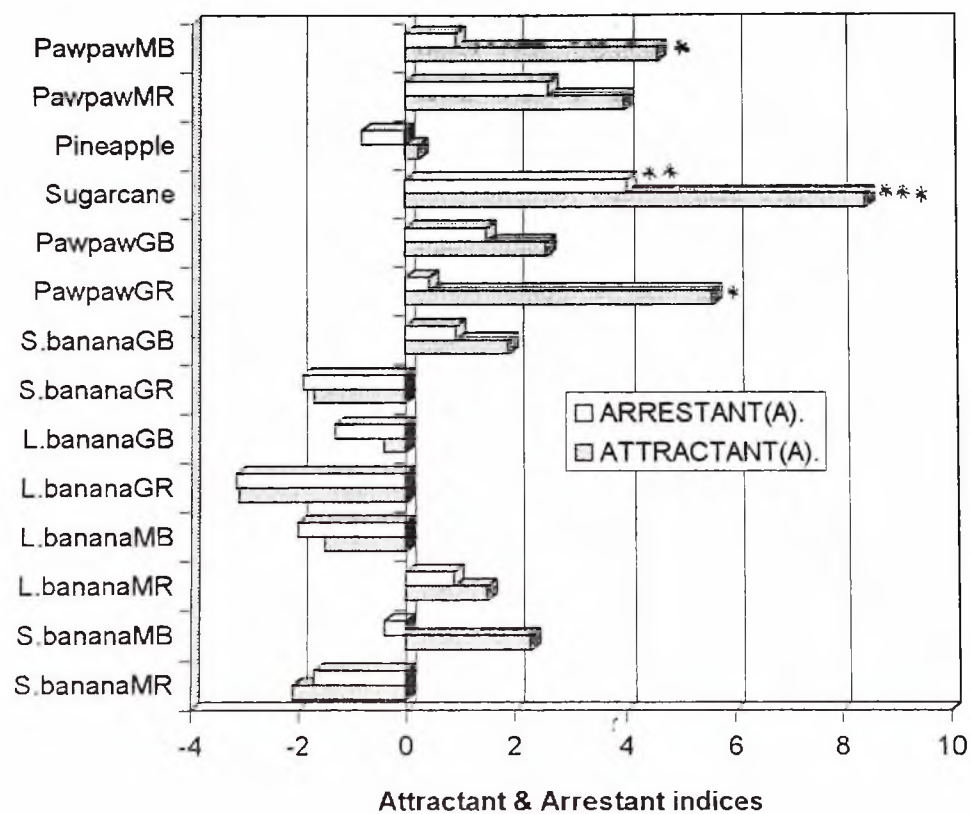


Figure 1A(b)



Figures 1B(a) & 1B(b): Comparison of effects of test materials on adults and juveniles of *B. truncatus* using attractant index.

R = Raw
GR = Green / Unripe raw
GB = Green / Unripe boiled
MR = Mature / Ripe raw
MB = Mature / Ripe boiled

* = $p<0.05$
** = $p<0.01$
*** = $p<0.001$

ATT.BRJ = Attractant responses of *Bulinus truncatus* juveniles.
ATT.BRA= Attractant responses of *Bulinus truncatus* adults.

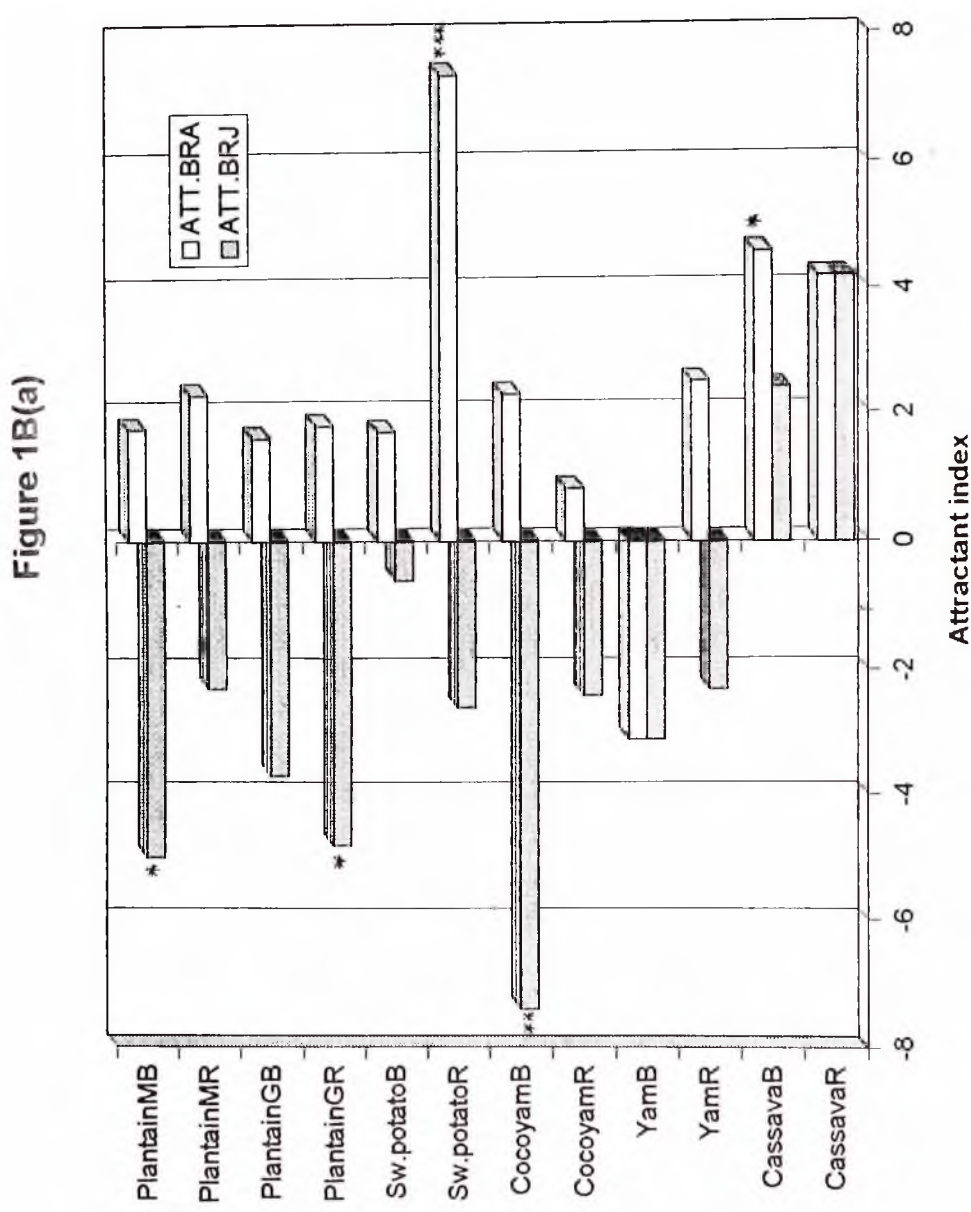


Figure 1B(a)

Figures 1B(c) & 1B(d): Comparison of effects of test materials on adults and juveniles of *B. truncatus* using arrestant index.

R = Raw
GR = Green / Unripe raw
GB = Green / Unripe boiled
MR = Mature / Ripe raw
MB = Mature / Ripe boiled

* = $p<0.05$
** = $p<0.01$
*** = $p<0.001$

ARR.BRJ = Arrestant responses of *Bulinus truncatus* juveniles.
ARR.BRA= Arrestant responses of *Bulinus truncatus* adults.

Figure 1B(c)

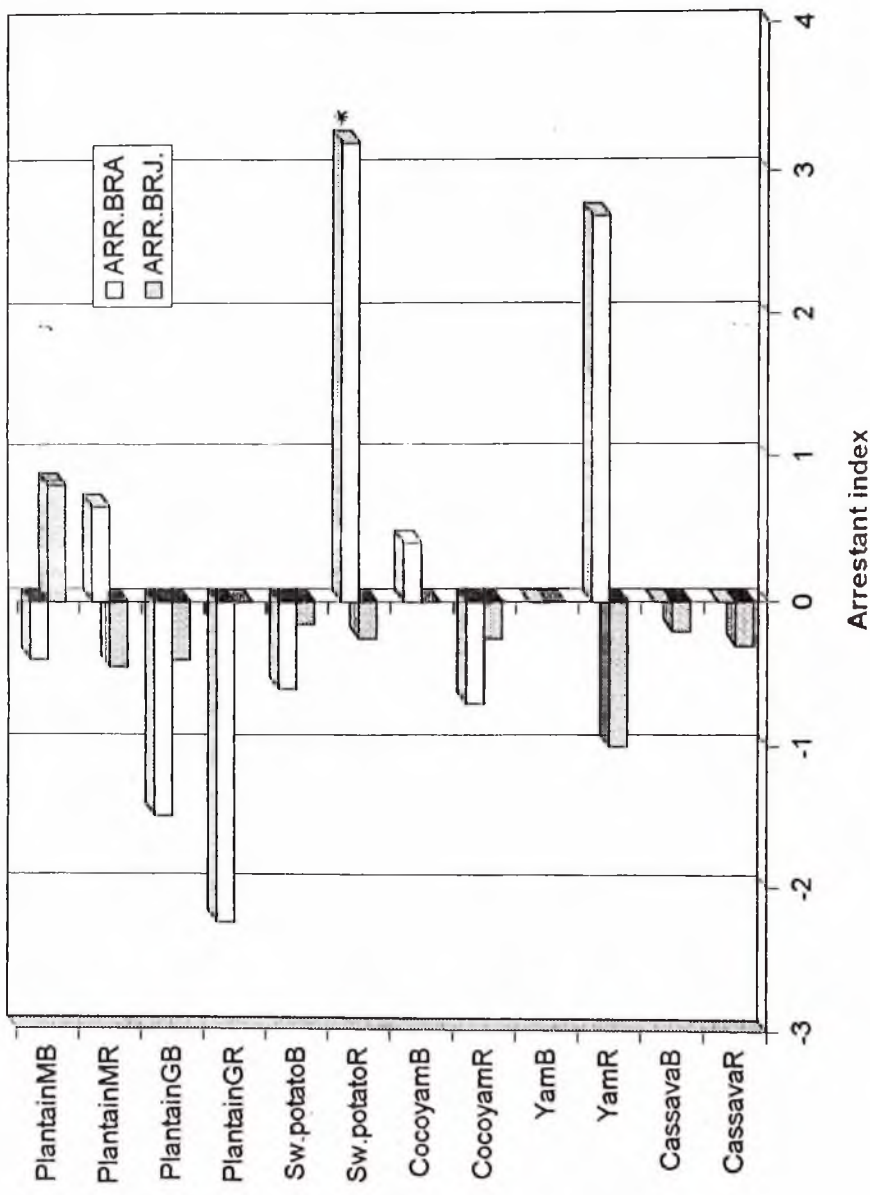
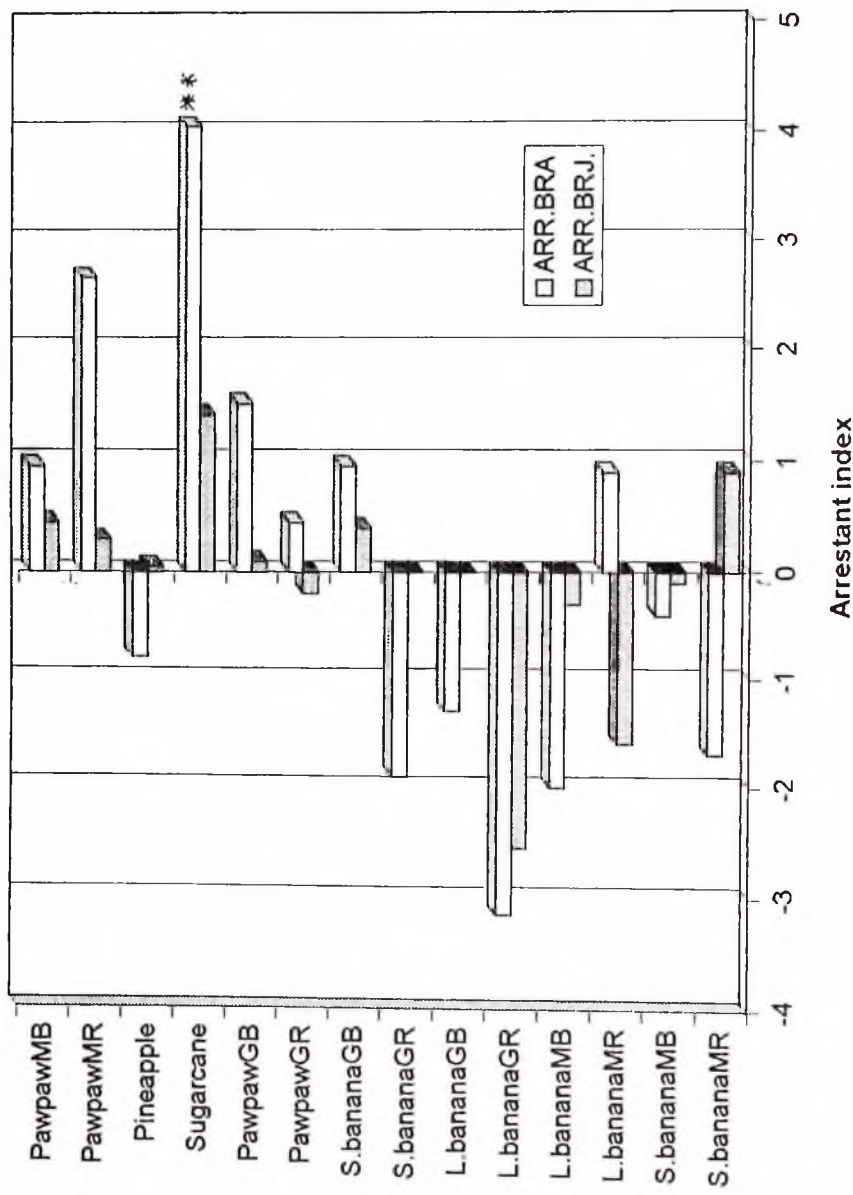


Figure 1B(d)



Figures 1C(a) & 1C(b): Comparison of attractant and arrestant responses of *B. truncatus* juveniles to selected plant materials.

R = Raw
GR = Green / Unripe raw
GB = Green / Unripe boiled
MR = Mature / Ripe raw
MB = Mature / Ripe boiled

* = $p<0.05$
** = $p<0.01$
*** = $p<0.001$

ATTRACTANT(J) = Attractant responses of juveniles.
ARRESTANT(J) = Arrestant responses of juveniles.

Figure 1C(a)

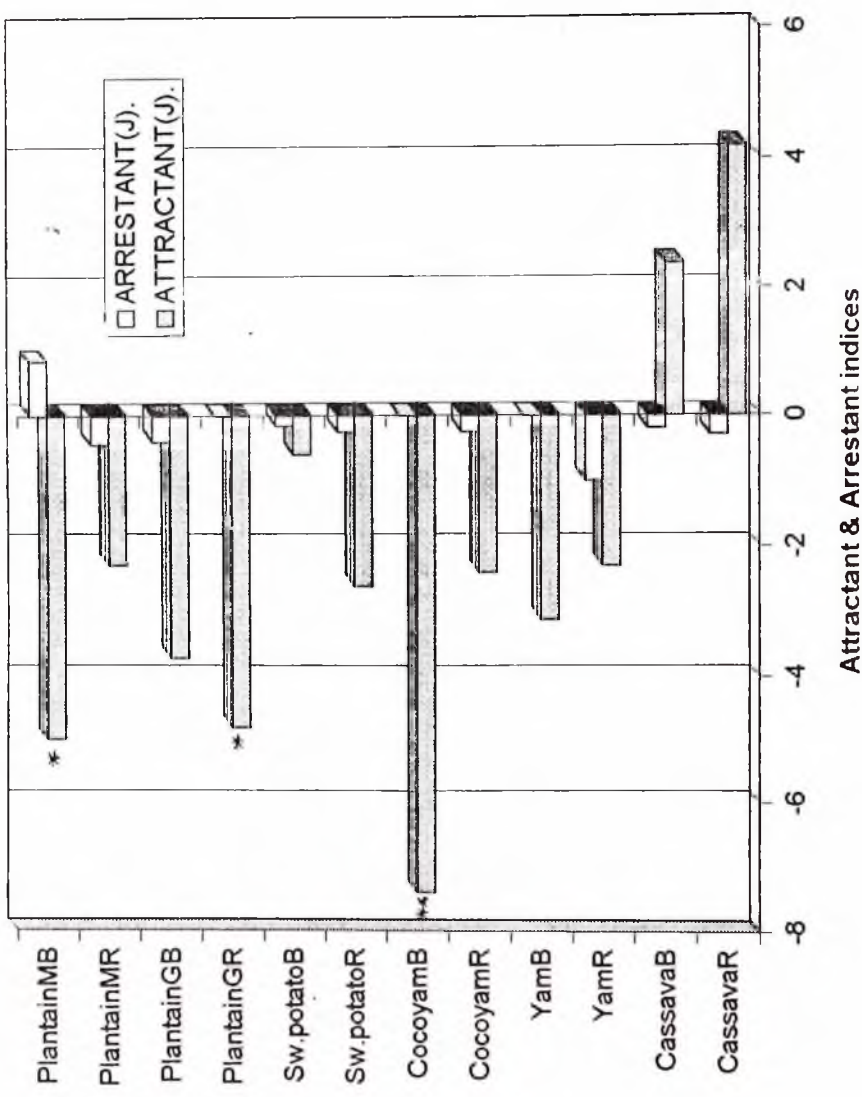
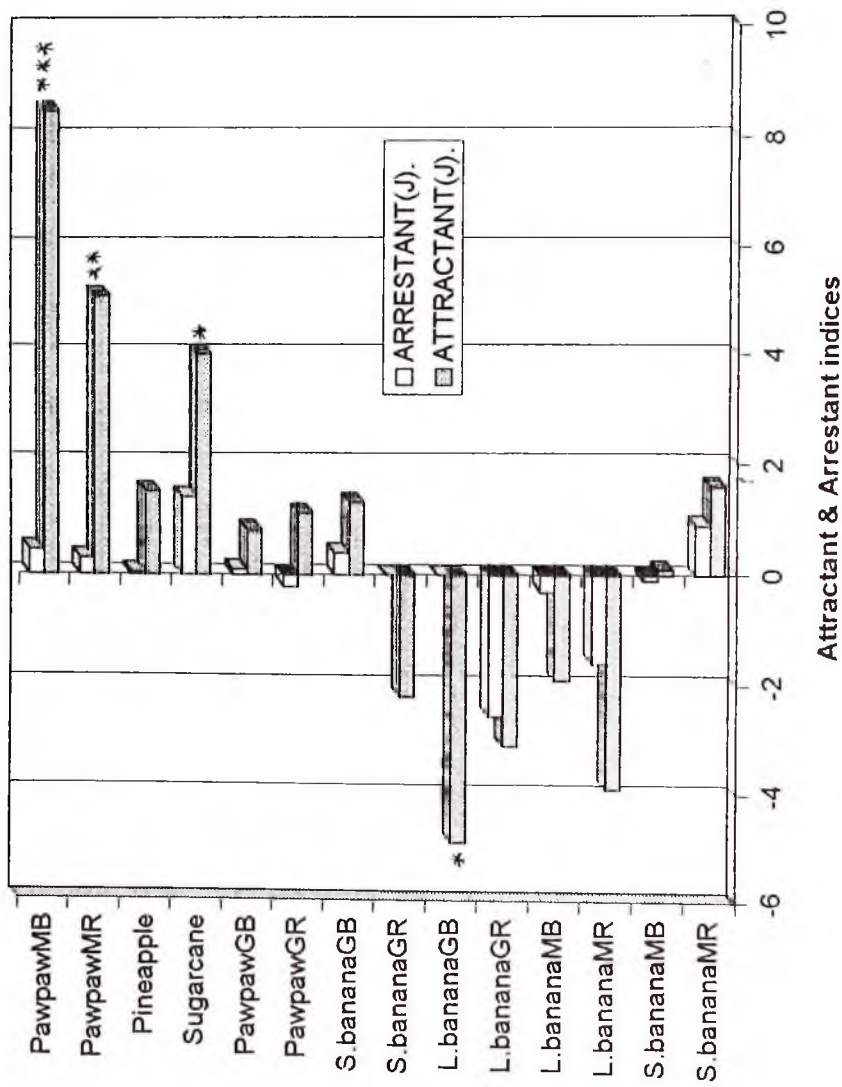


Figure 1C(b)



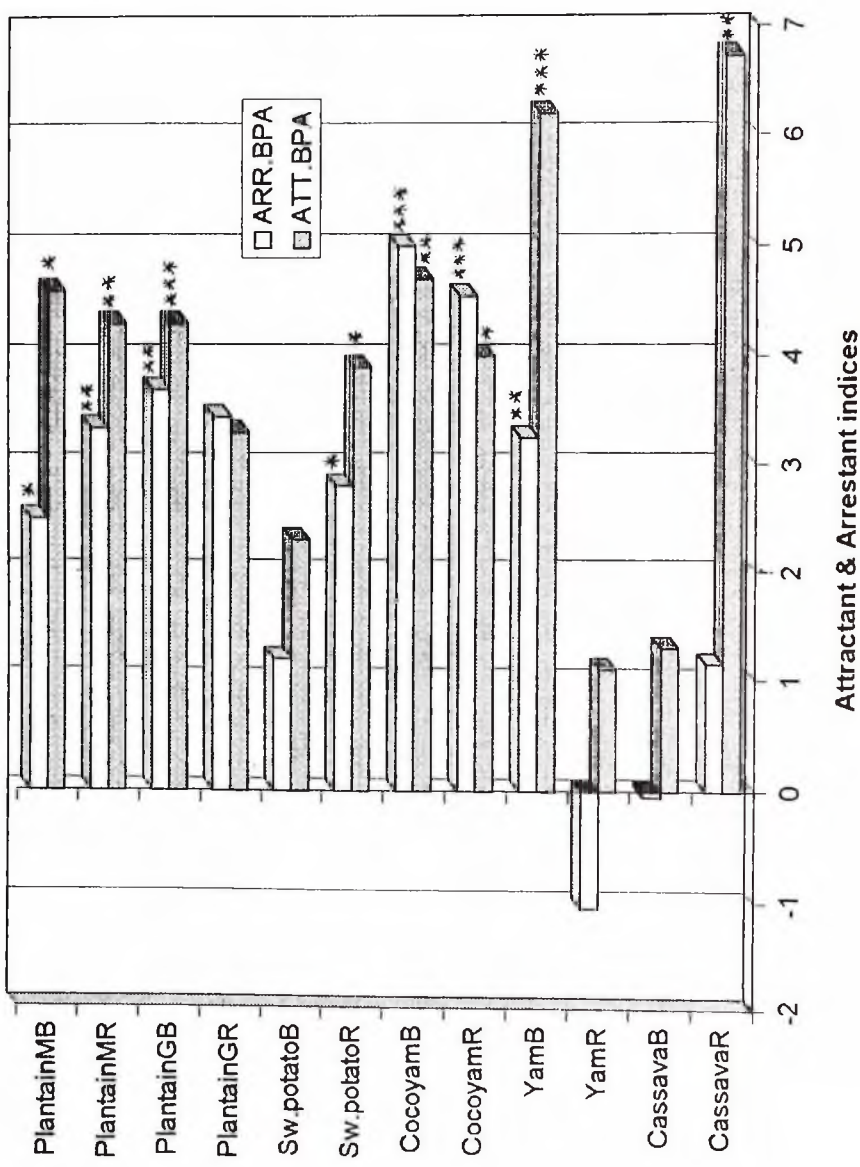
Figures 2C(a) & 2C(b): Attractant and arrestant responses of *B. pfeifferi* juveniles to selected plant materials.

R = Raw
GR = Green / Unripe raw
GB = Green / Unripe boiled
MR = Mature / Ripe raw
MB = Mature / Ripe boiled

* = $p<0.05$
** = $p<0.01$
*** = $p<0.001$

ATT.BPJ = Attractant responses of *Biomphalaria pfeifferi* juveniles.
ARR.BPJ = Arrestant responses of *Biomphalaria pfeifferi* juveniles.

Figure 2A(a)



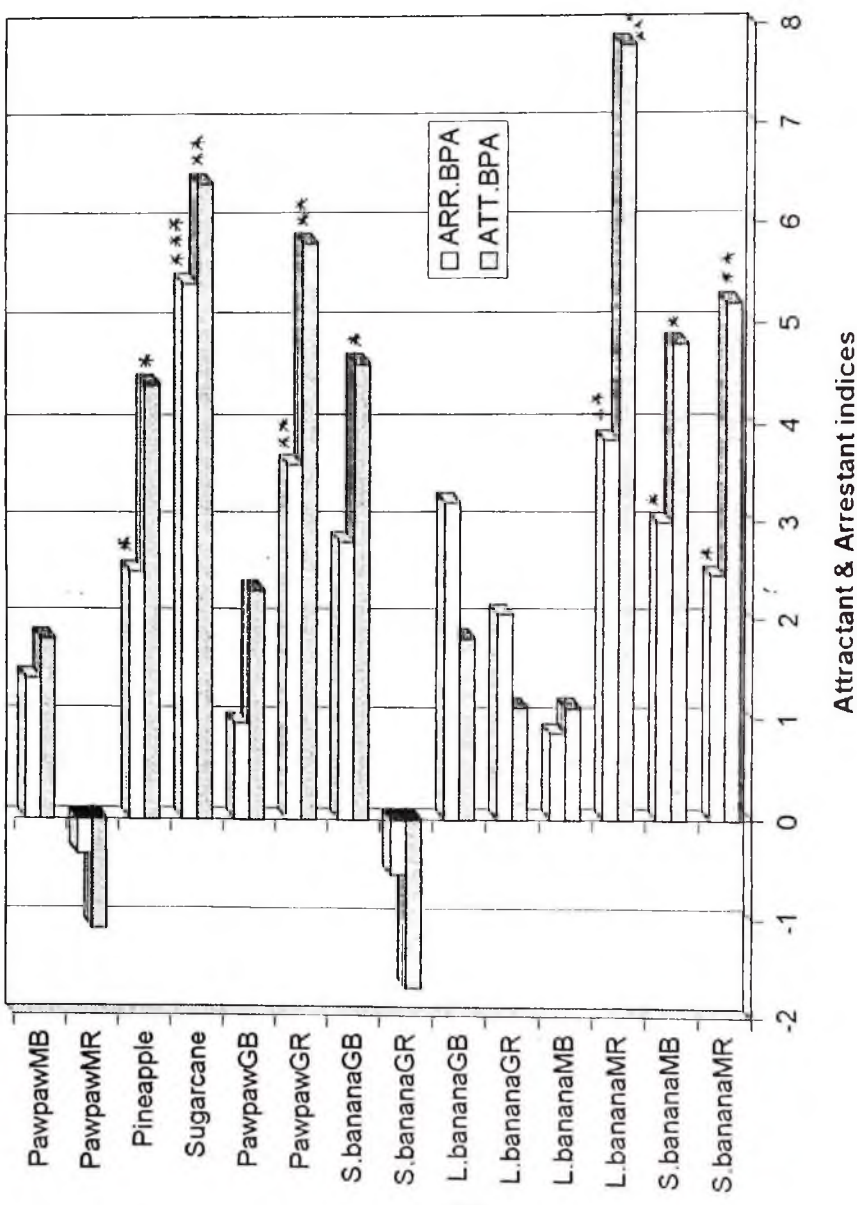


Figure 2A(b)

Figures 2B(a) & 2B(b): Comparison of effects of test materials on adults and juveniles of *B. pfeifferi* using attractant index.

R = Raw
GR = Green / Unripe raw
GB = Green / Unripe boiled
MR = Mature / Ripe raw
MB = Mature / Ripe boiled

* = $p<0.05$
** = $p<0.01$
*** = $p<0.001$

ATT.BPJ = Attractant responses of *Biomphalaria pfeifferi* juveniles.
ATT.BPA = Attractant responses of *Biomphalaria pfeifferi* adults.

Figure 2B(a)

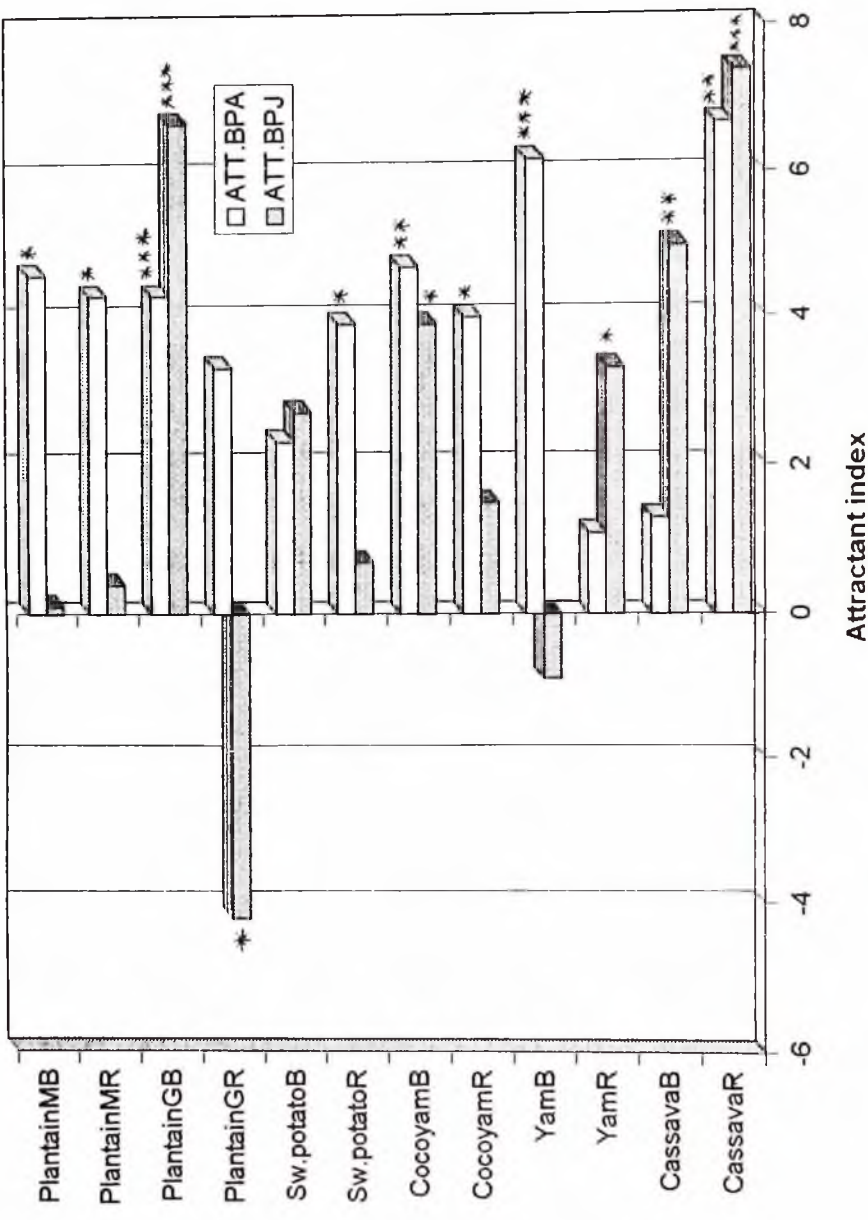
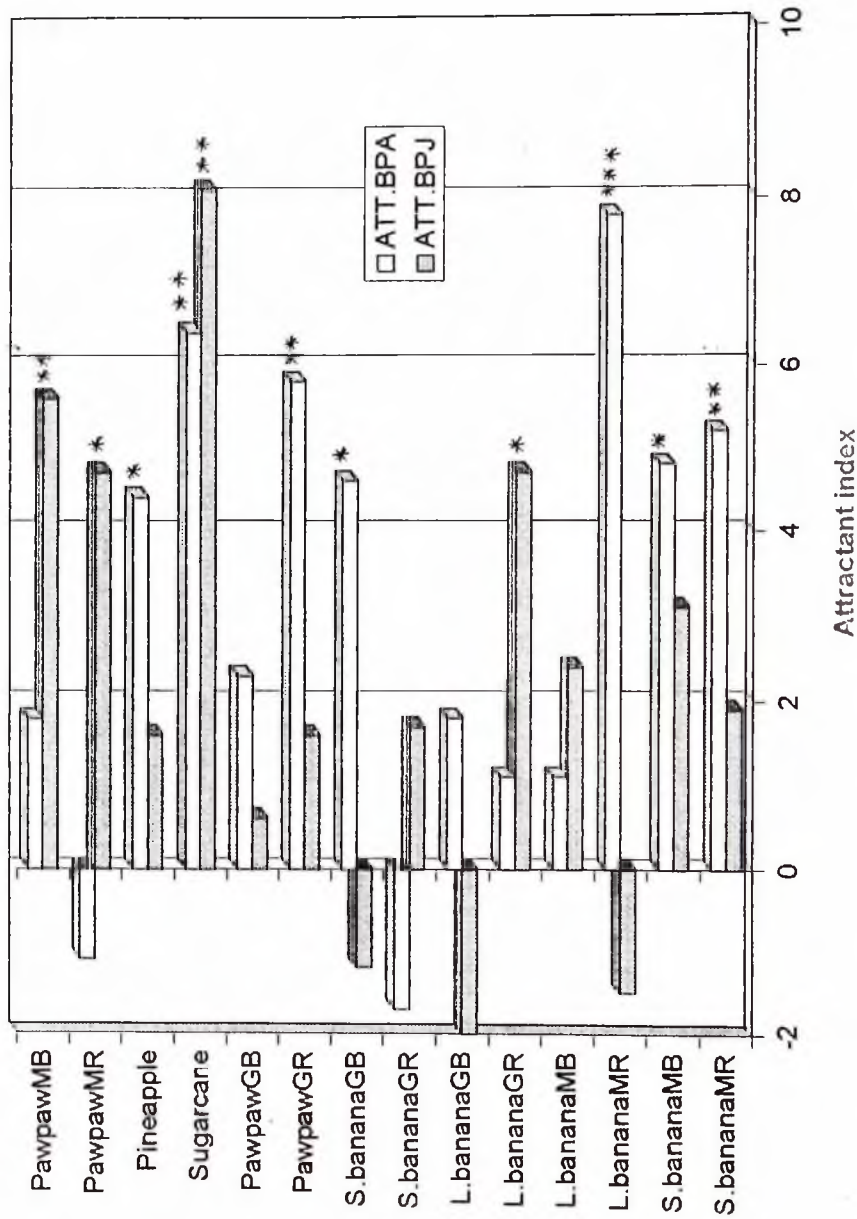


Figure 2B(b)



Figures 2B(c) & 2B(d): Comparison of effects of test materials on adults and juveniles of *B. pfeifferi* using arrestant index.

R = Raw
GR = Green / Unripe raw
GB = Green / Unripe boiled
MR = Mature / Ripe raw
MB = Mature / Ripe boiled

* = $p<0.05$
** = $p<0.01$
*** = $p< 0.001$

ARR.BPJ = Arrestant responses of *Biomphalaria pfeifferi* juveniles.
ARR.BPA =Arrestant responses of *Biomphalaria pfeifferi* adults.

Figure 2B(c)

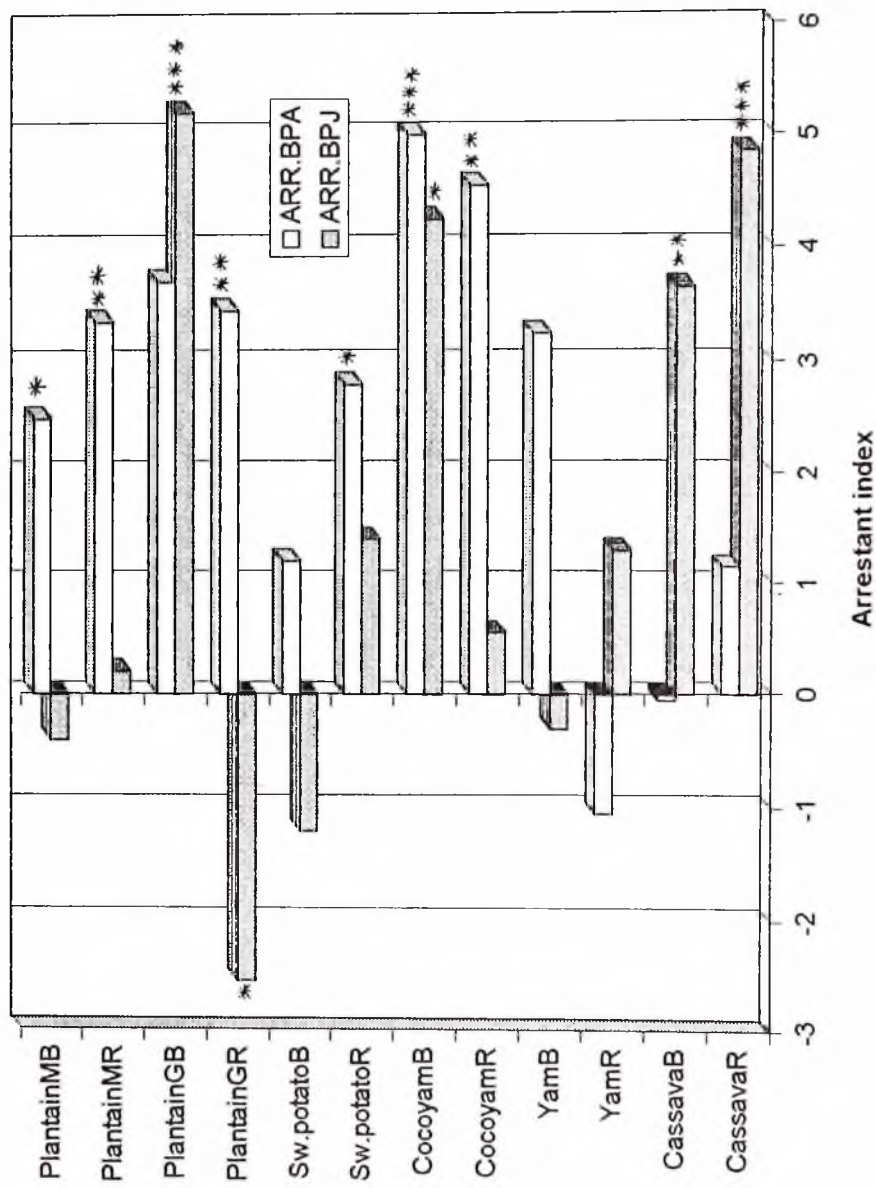
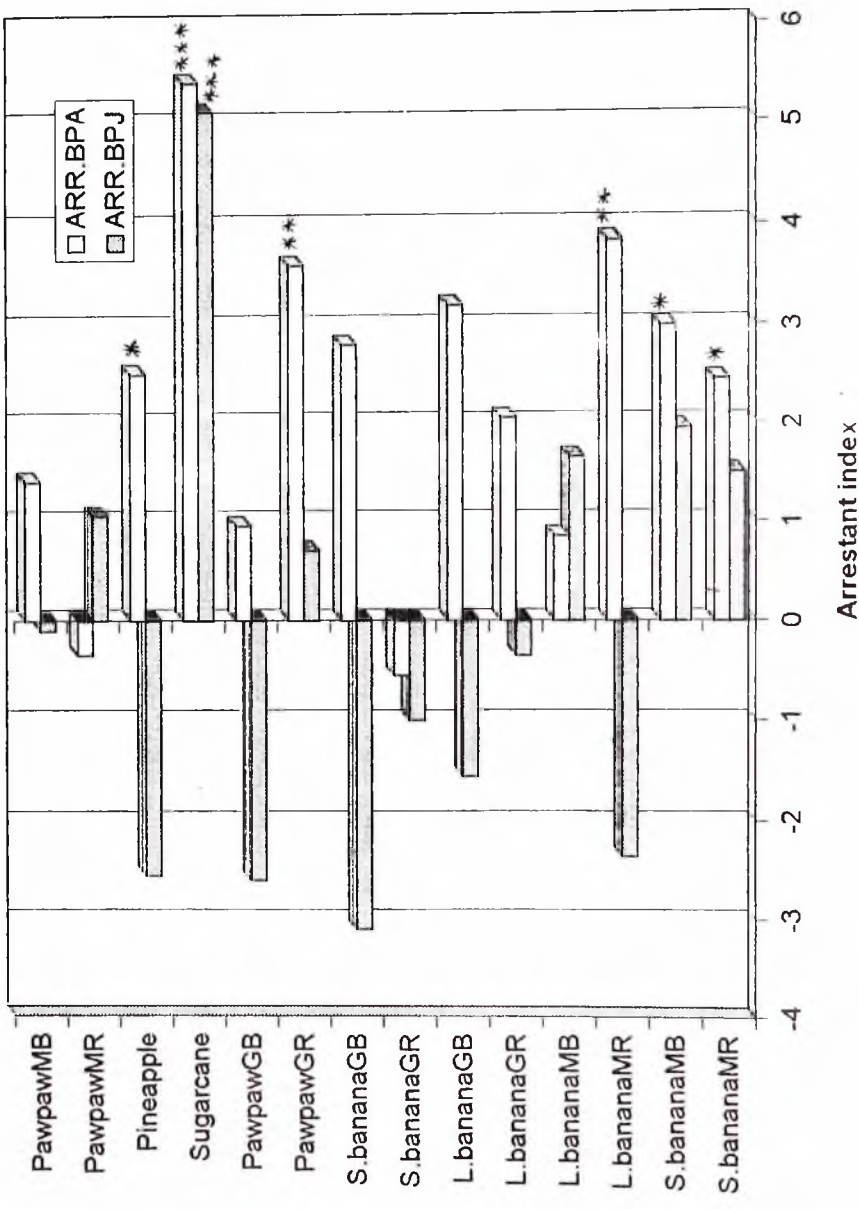


Figure 2B(d)



Figures 2C(a) & 2C(b): Attractant and arrestant responses of *B. pfeifferi* juveniles to selected plant materials.

R = Raw
GR = Green / Unripe raw
GB = Green / Unripe boiled
MR = Mature / Ripe raw
MB = Mature / Ripe boiled

* = $p<0.05$
** = $p<0.01$
*** = $p<0.001$

ATT.BPJ = Attractant responses of *Biomphalaria pfeifferi* juveniles.
ARR.BPJ = Arrestant responses of *Biomphalaria pfeifferi* juveniles.

Figure 2C(a)

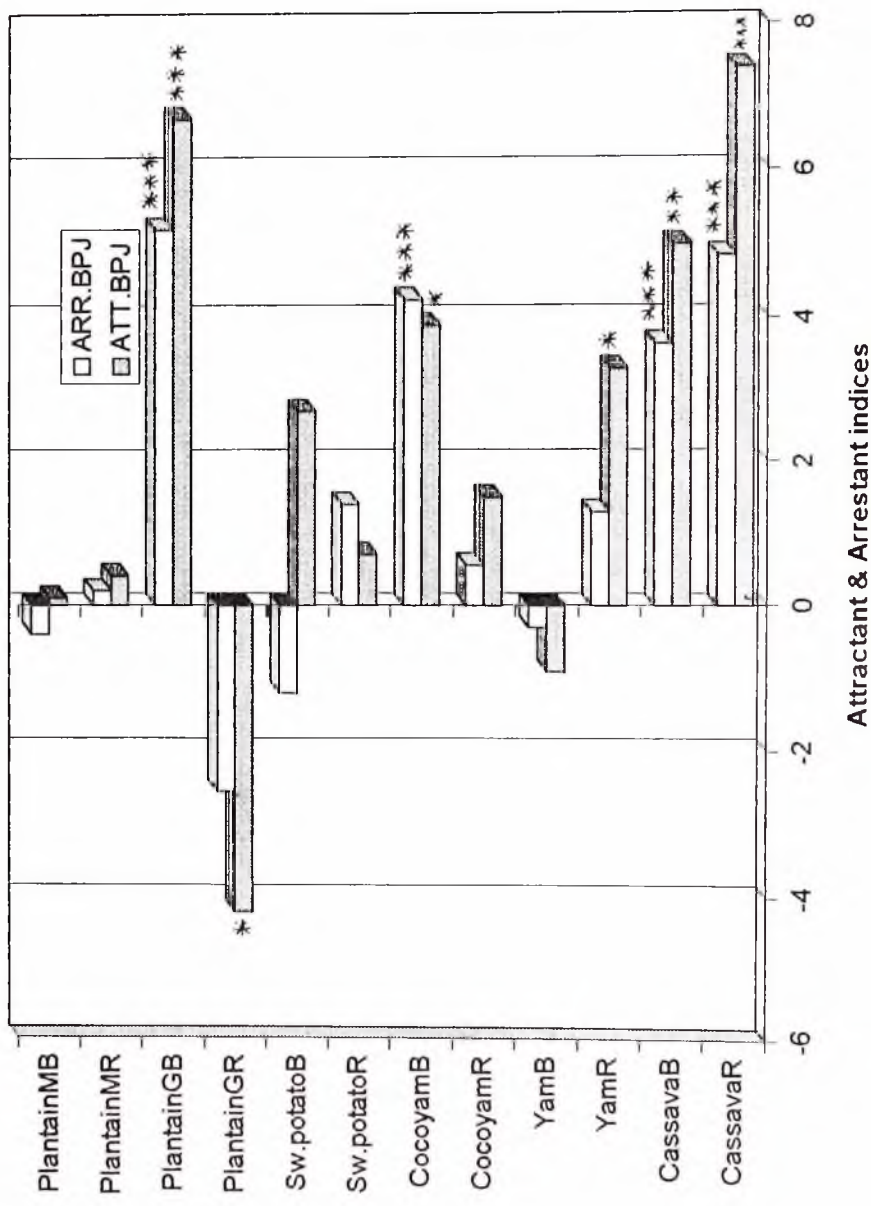
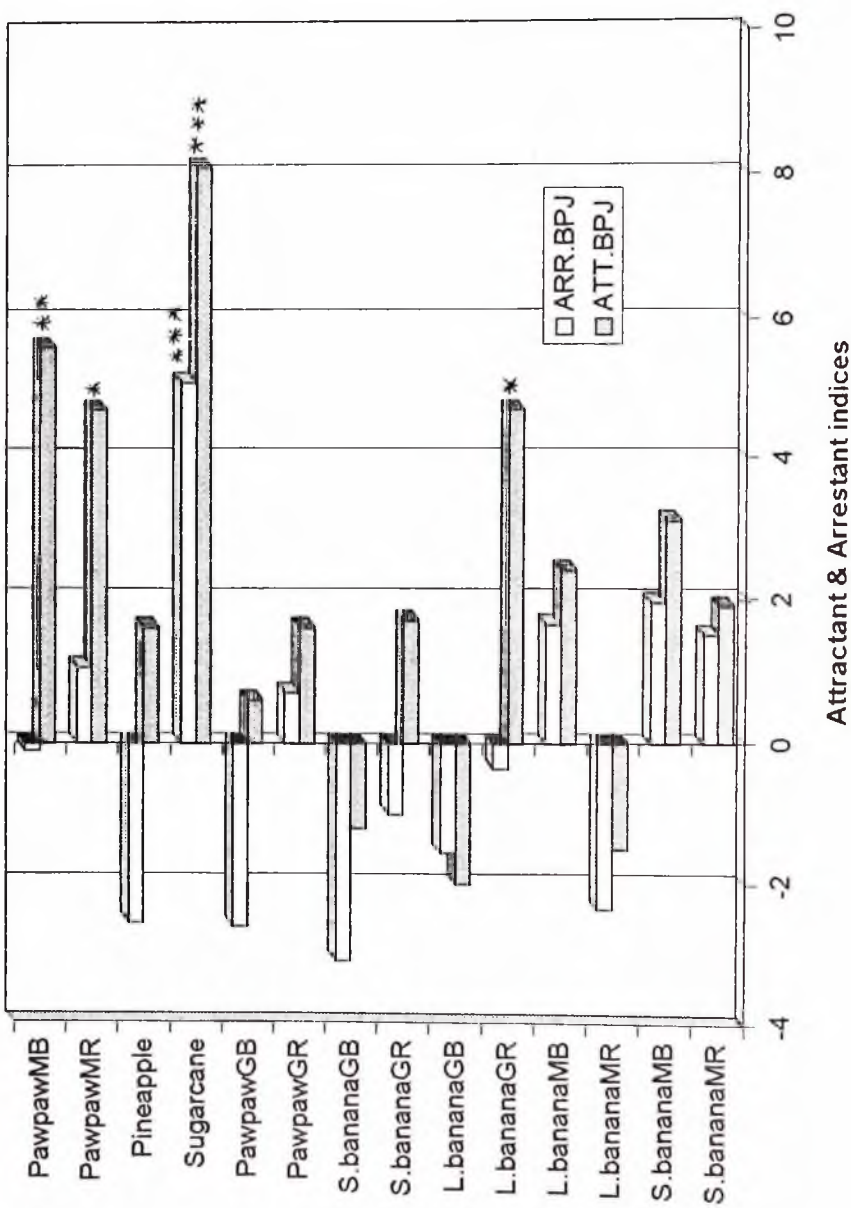


Figure 2C(b)



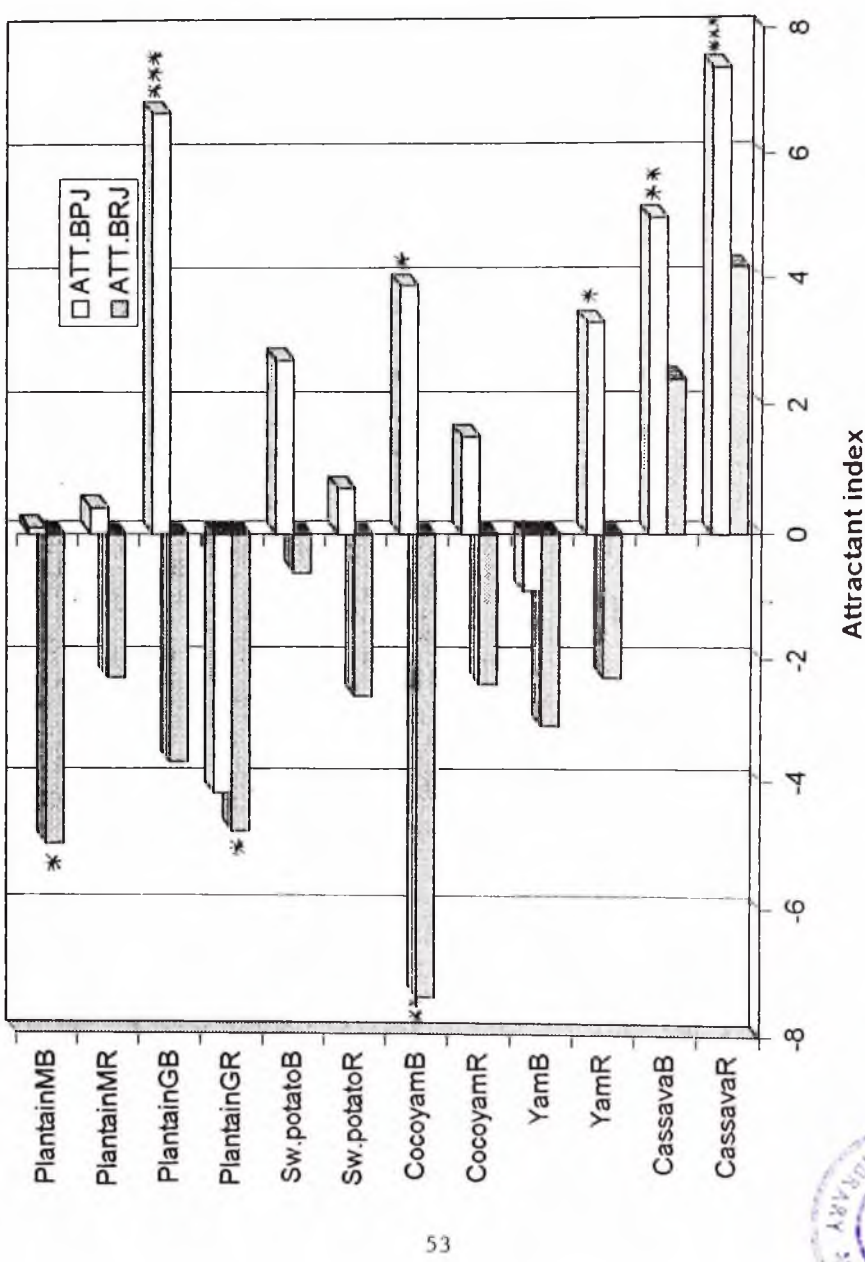
Figures 3A(a) & 3A(b): Comparison of effects of test materials on juveniles of *B. pfeifferi* using attractant index.

R = Raw
GR = Green / Unripe raw
GB = Green / Unripe boiled
MR = Mature / Ripe raw
MB = Mature / Ripe boiled

* = $p<0.05$
** = $p<0.01$
*** = $p<0.001$

ATT.BRJ = Attractant responses of *Bulinus truncatus* juveniles.
ATT.BPJ = Attractant responses of *Biomphalaria pfeifferi* juveniles.

Figure 3A(a)



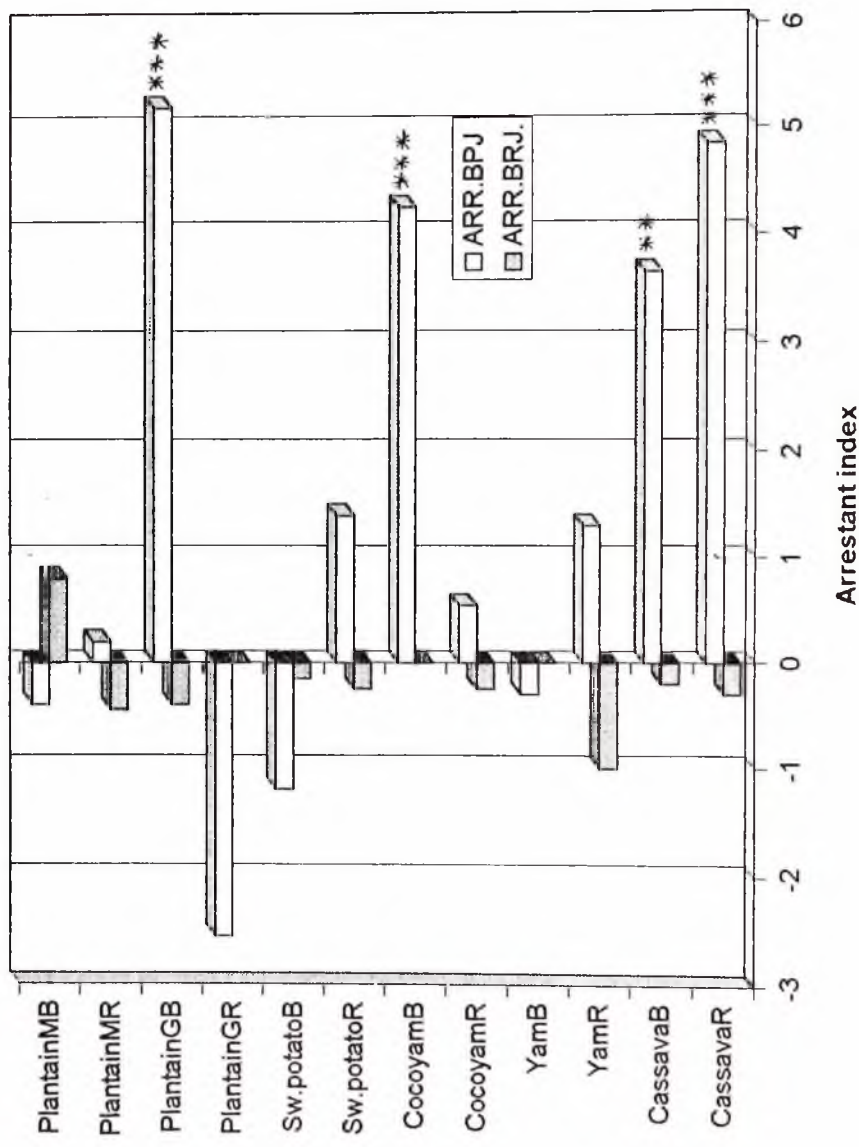
Figures 3A(c) & 3A(d): Comparison of effects of test materials on juveniles of *B. truncatus* and *B. pfeifferi* using arrestant index.

R = Raw
GR = Green / Unripe raw
GB = Green / Unripe boiled
MR = Mature / Ripe raw
MB = Mature / Ripe boiled

* = $p<0.05$
** = $p<0.01$
*** = $p<0.001$

ARR.BRJ = Arrestant responses of *Bulinus truncatus* juveniles.
ARR.BPJ = Arrestant responses of *Biomphalaria pfeifferi* juveniles.

Figure 3A(c)



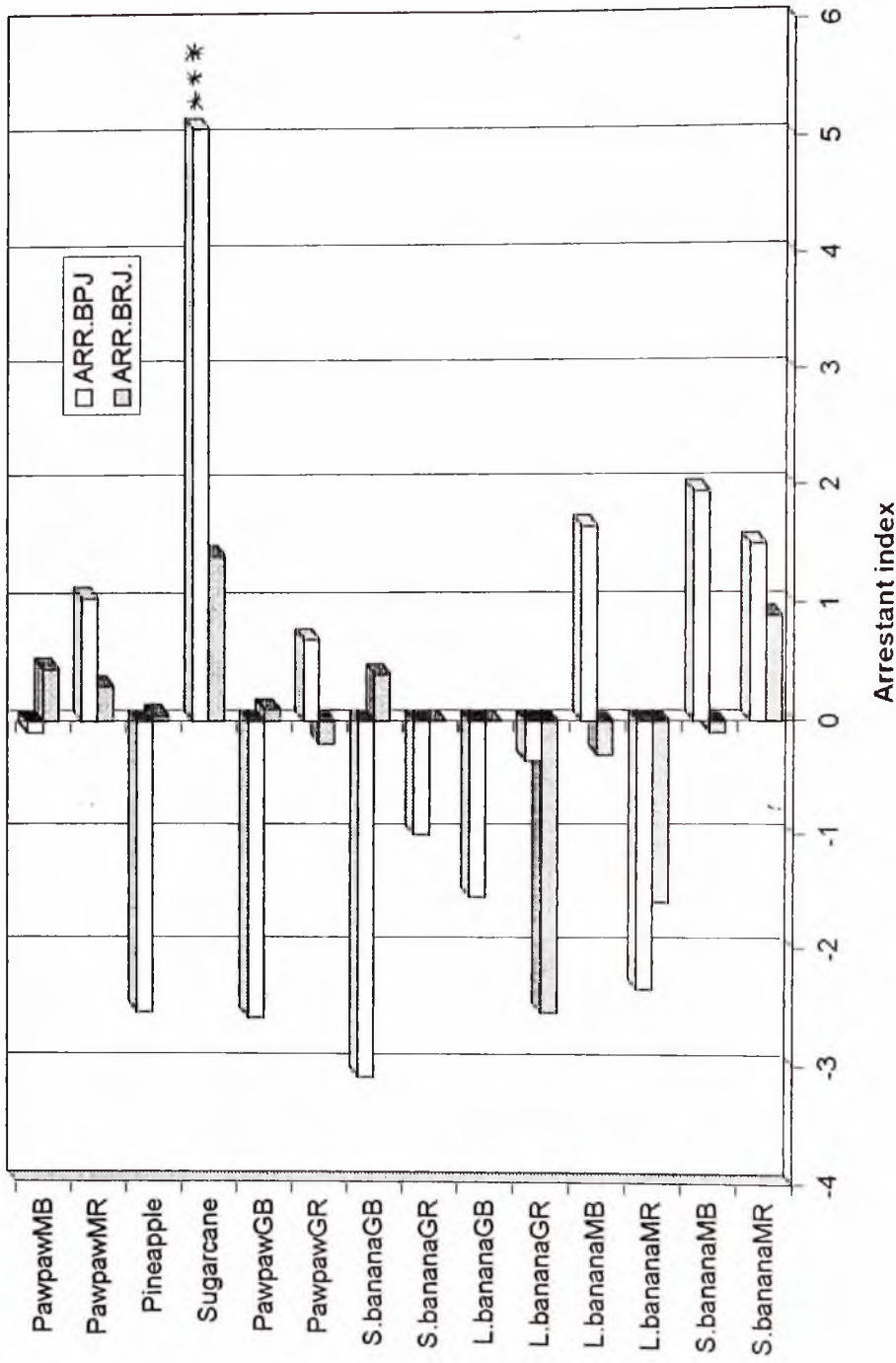


Figure 3A(d)

Figures 3B(a) & 3B(b): Comparison of effects of test materials on adults of *B. truncatus* and *B. pfeifferi* using attractant index.

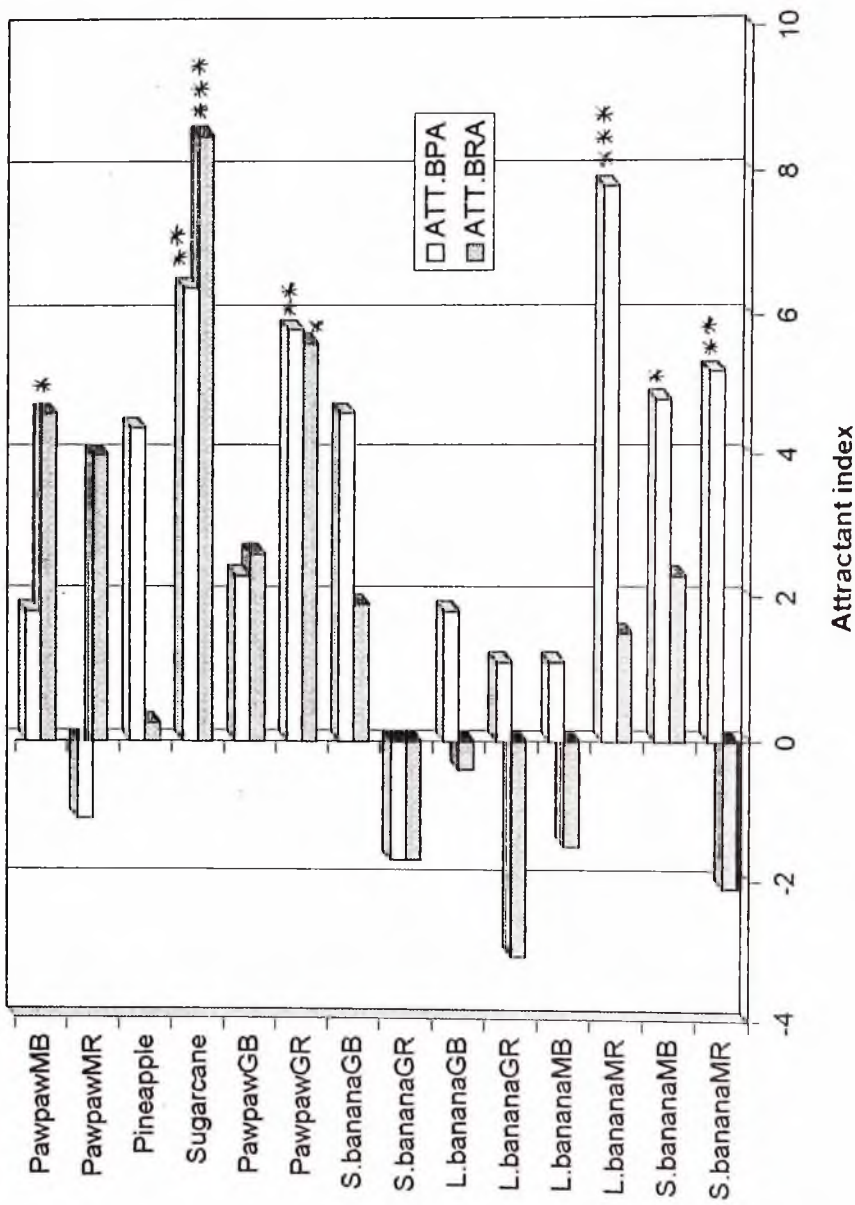
R = Raw
GR = Green / Unripe raw
GB = Green / Unripe boiled
MR = Mature / Ripe raw
MB = Mature / Ripe boiled

* = $p<0.05$
** = $p<0.01$
*** = $p<0.001$

ATT.BRA = Attractant responses of *Bulinus truncatus* adults.
ATT.BPA = Attractant responses of *Biomphalaria pfeifferi* adults.

Figure 3B(a)

Figure 3B(b)



Figures 3C(a) & 3C(b): Comparison of effects of test materials on adults of *B. truncatus* and *B. pfeifferi* using arrestant index.

R = Raw
GR = Green / Unripe raw
GB = Green / Unripe boiled
MR = Mature / Ripe raw
MB = Mature / Ripe boiled

* = $p<0.05$
** = $p<0.01$
*** = $p<0.001$

ARR.BRA = Arreatant responses of *Bulinus truncatus* adults.
ARR.BPA = Arrestant responses of *Biomphalaria pfeifferi* adults.

Figure 3C(a)

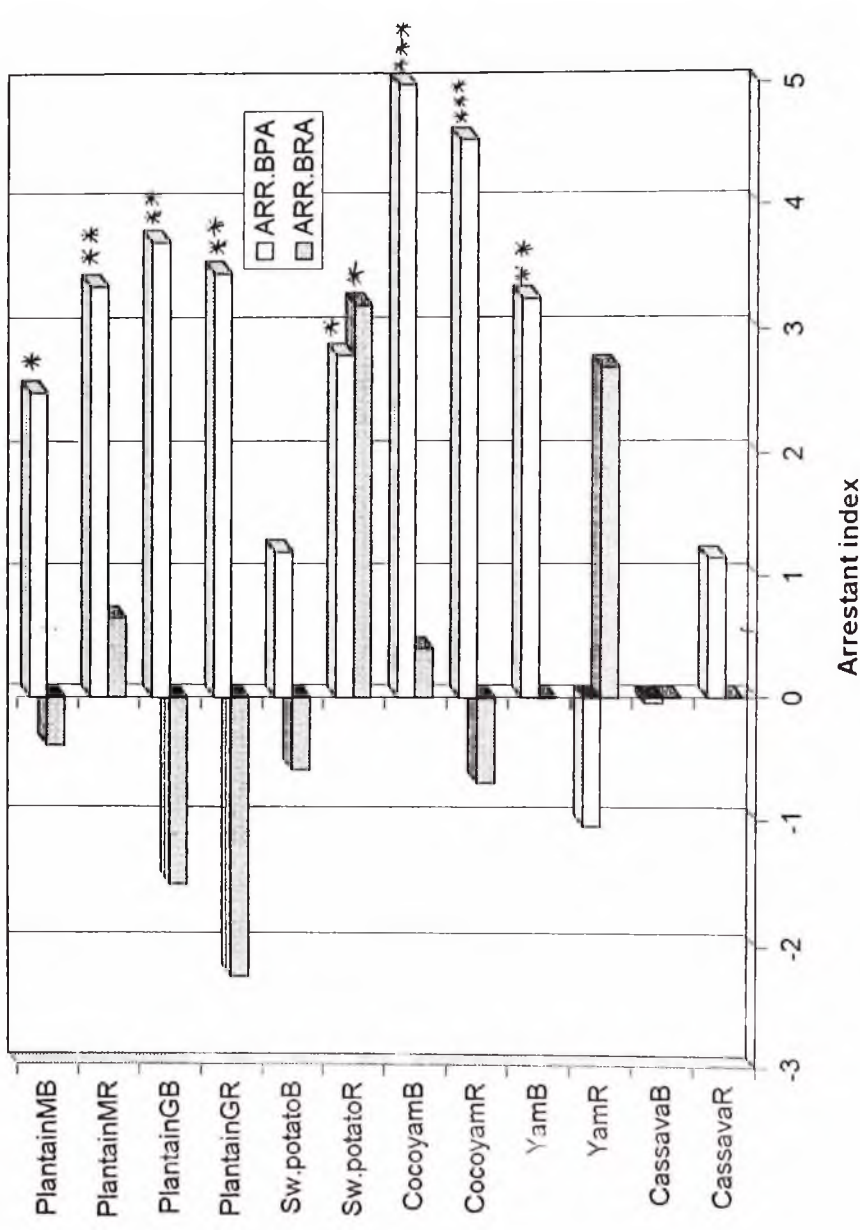
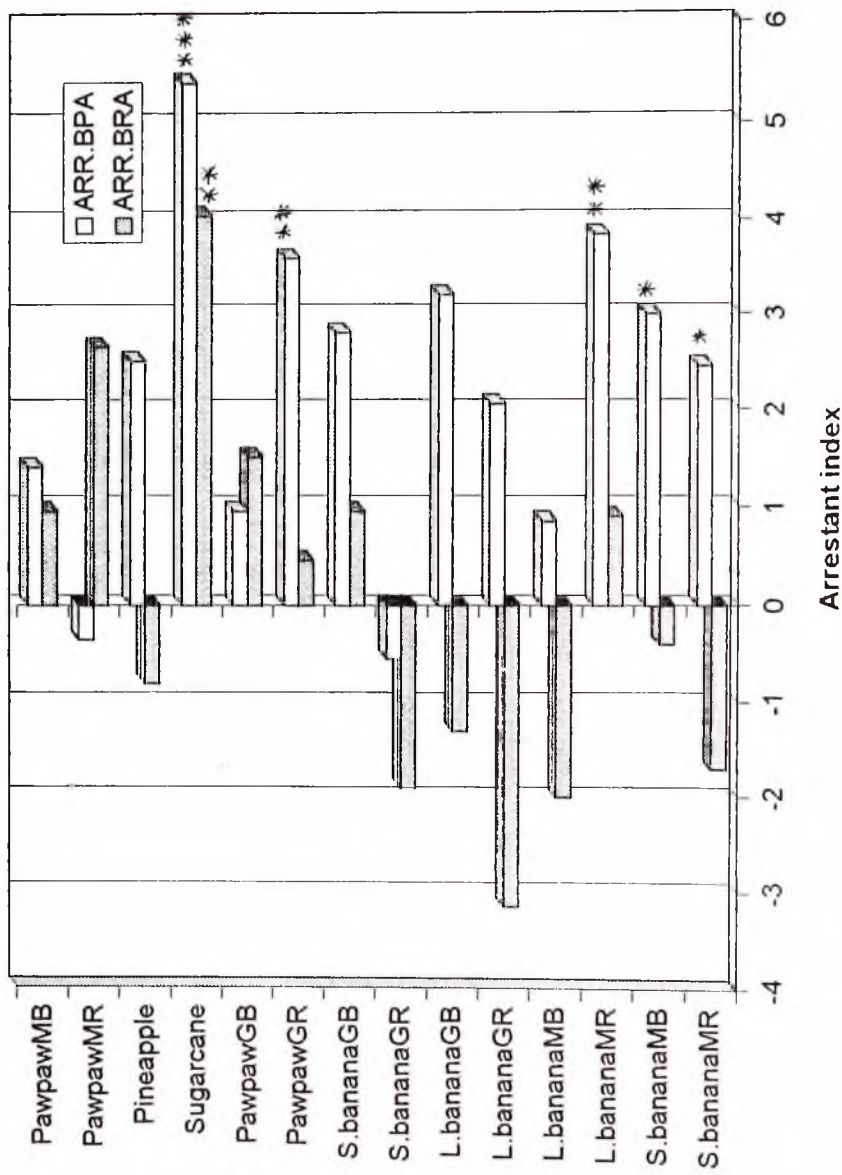


Figure 3C(b)



2. 4 DISCUSSION

The present results show that both *Bulinus truncatus* and *Biomphalaria pfeifferi* discriminate between the materials tested. Adults and juveniles of *B. truncatus* responded to a statistically significant extent to only 19.2% and 11.5% of the materials tested respectively. Corresponding figures for *B. pfeifferi* were 34.6% and 57.7% for juveniles and adults respectively (Table1). This phenomenon of selective responses to chemical factors is similar to those observed in other freshwater planorbid snails. Thomas et al (1979) have shown that the South American schistosome host snail *Biomphalaria glabrata* responds to a statistically significant extent to only a few species of amino acids. Similar observations have since then been made by Thomas et al, (1980b and 1985) on *B. glabrata* and *Bulinus rohlfsi* respectively in their responses to carboxylic and amino acids. Furthermore *Biomphalaria glabrata* has been shown to exhibit similar discriminative behaviour patterns when tested with sugars (Thomas 1986 a;). Adult *B. glabrata* were found to respond significantly to only 39.1% of 23 sugars tested, while adults of *Bulinus truncatus* were found to respond significantly to only 43.7% and 23.1% of carboxylic and amino acids respectively. Daldorph and Thomas (1988) have recorded comparable behaviour patterns in all six British freshwater gastropods tested. There are both differences and points of resemblance between the chemoreception profiles of the two species of snails used in this study. Interspecific comparisons show that *B. truncatus* was, on the whole, less responsive to the test materials than *B. pfeifferi*. The differences in chemoreception niches of the two species may reflect differences in the biochemistry of their chemoreceptors and metabolism (Kpikpi, 1990). Intraspecific comparisons reveal that adults of both snail species have a wider

chemoreception profile than the juveniles although there is some degree of overlap. The same pattern was observed for both adults and juveniles of *Biomphalaria pfeifferi*. This observation is at variance with those of Thomas and Assefa, 1979 and Thomas et al, 1986 who found that juveniles had wider chemoreception profiles when they recorded age-specific response patterns for sugars and amino acids in *B. glabrata*. They suggested that the wider response profiles of the juvenile snails was to ensure survival for these delicate juveniles so that they are not wiped out by adverse environmental conditions such as shortage of some kinds of food or intense competition from other organisms on particular types of food. Furthermore, Kpikpi (1990) has shown that juvenile snails have a broader chemoreception profile than adults. He has shown that out of 17 sugars tested on *B.globosus* adults, only four (23.5%) were effective as attractants and arrestants. He has also shown that *Bulinus truncatus* snails responded significantly to 6 out of 17 sugars tested (35.3%). In the present study, adult snails of *B. truncatus* and *B. pfeifferi* were found to have a broader chemoreception profile than the juveniles. These results however do not disprove or substantiate the earlier suggestion in that Thomas *et al*,(1980a & b) worked with pure chemicals and the present work deals with natural products that contain multiple factors. To account for these observations, it could be suggested that the wider response profiles of the adult snails might be due to the fact that the adult snails have apparently additional chemoreceptors for adult life, for example, recognition of sex pheromones in sexually mature adults.

The natural product which emerged as the most potent attractant/arrestant was sugar cane (*Saccharum officinarum*). For the purpose of study, this finding is of considerable interest

since both juveniles and adults of *B. truncatus* and *B. pfeifferi* responded significantly to this natural product. The ideal attractant should be effective for all ages of the target snails for efficiency. This aspect of the response which cuts across the different age groups is similar to those for ripened mature pawpaw (*Carica papaya*). These two natural products in addition to sweet potato and cassava were also found to elicit strong response and could therefore be considered as potential candidates for further investigations into their suitability for use in the slow release formulations as envisaged by this study.

The discovered attractants in this study contain high levels of carbohydrates. Sugar cane and mature ripened pawpaw have high levels of sucrose. Sugar cane juice contains mainly 83% of water and 15% sucrose (Priestley,1979). Sucrose is the main sugar present in sugar cane. Sucrose forms one molecule of glucose and one molecule of fructose by hydrolysis. Cassava contains 20-30% starch and 75-80% water. Sweet potato contains 65-80% starch and 10% sugars. The two main or perhaps sole components of the starch are amylose and amylopectin which are present in a ratio of 1:3. The main sugars present in potato are sucrose, glucose, and fructose in approximately equal amounts, although fructose predominates in cold stored immature potatoes, and sucrose also accumulates preferentially in potatoes exposed to gamma radiation. The starch content of boiled potato tissue is lower than that of the corresponding fresh tissue. This may be due in part to swelling of the starch and resulting cell rapture and leaching. Thus sucrose , among others, is the sugar found to be common to the discovered attractants (sugar cane, pawpaw and sweet potato) which cut across the different age groups of the two snails under study.

Apart from raw cocoyam, the plant materials which acted as statistically significant repellents for *B. truncatus* juveniles included boiled ripe plantain,boiled unripe banana and raw unripe plantain. Only raw unripe plantain acted as a statistically significant repellent for juveniles of *B. pfeifferi*. None of the test materials acted as significant repellent for the adults of the two snail species. Apart from raw cocoyam the discovered repellents have the following in common: they are all 1). fruits of the genus *Musa* and 2). either unripe, boiled or both. As indicated earlier sugars, mainly sucrose, have been identified to be present in high levels in the discovered attractants. It is also known that unripe/green fruits of *Musa spp* contain only 1-2% sugars and this increases to about 20% when the fruit is fully ripe (Priestley,1979). When heat is applied to food stuffs, browning occurs. The extent of this browning depends on a variety of factors such as temperature, concentration, and presence of other materials. There is the possibility that browning reactions can give rise to toxic materials (Priesley, 1979). It may be therefore suggested here that this toxicity can cause the snails to move away from such materials . It can also be suggested that the repellent effects on only juvenile snails in this study could be attributed to the fact that the juveniles are more sensitive to the toxic effects of the boiled materials than the adults.

CHAPTER THREE

SEARCH FOR MOST EFFECTIVE COMBINATION OF BIOACTIVE FACTORS AND BAMBOO CYLINDER FOR SNAIL TRAPPING

3.1 INTRODUCTION

The combination of bioactive factors and bamboo cylinder design for trapping snails was first investigated by Kpikpi (1990) under field conditions. The present study was laboratory based and involved the testing of the effective bioactive natural products identified in the bioassay studies (Chapter two). The objectives were to identify the most effective method of presenting the bioactive factors identified in chapter two prior to deployment under field conditions. The experiment was carried out under simulated natural conditions (SNC) in large tanks containing water, aquatic plants and snails.

The natural products tested under (SNC) were sugar cane (*Saccharum officinarum*), mature (ripened) pawpaw (*Carica papaya*) Sweet potato (*Ipomea batatas*) and cassava (*Manihot esculenta*). These test materials were used in their raw state since there was no statistically significant difference between responses produced by the raw and its corresponding boiled state. The bioactive natural products for this study were chosen on the basis of their effectiveness as attractants for both adults and juveniles of *B. truncatus* and *B. pfeifferi*. Further experiments were conducted to determine the optimum quantity of bioactive materials to be used in the traps as well as the form in which the materials were to be deployed.

3.2 MATERIALS AND METHODS

3.2.1. Snail Breeding

About 2,500 snails were bred within one and half months, adopting the methods used in Chapter 2 2.1. The young snails were transferred into each of twelve (66 x 38 x 21cm) tanks filled with 42.6 litres of tap water. The snails were routinely fed with lettuce every two days (Plate 3). The medium in which the snails were kept was changed once every week.

3.2.2. Simulated Natural Condition (S.N.C)

Two 1,108 litre concrete tanks (Plate 7) filled with 646 litres of tap water was prepared as follows: The bottom of each tank was covered with sand to a depth of 3cm. Macrophytes such as *Pistia sp* and *Alternanthera sp* were added to the aquaria to cover about two-thirds of the surface of the water. The water in the tank was kept at a temperature of 29±1° C and P^H and conductivity ranges of 4.10 - 5.80 and 95.3 - 125.9μSm/cm2 respectively. 400 young adult snails of 5-6 weeks old of each species of *B. truncatus* and *B. pfeifferi* were introduced into each aquarium and allowed to acclimatise for 7 days. Two large leaves of fresh lettuce were added to each aquarium every other day during this period (Plate 5). They were deprived of lettuce for a period of 24 hours before the experiment.

3.2.3 Preparation of test materials

The bioactive natural products used for the experiments were sugar cane (*Saccharum officinarum*), mature (ripened) pawpaw (*Carica papaya*), sweet potato (*Ipomoea batatas*) and cassava (*Manihot esculenta*) - all in their raw state. Each raw test material was tested in two forms i.e *as whole lumps* and also *as crushed*. Whole test materials were obtained

by cutting test materials into cylindrical forms with diameters similar to those of the lumen of the bamboo cylinders. Crushed materials were prepared by chopping the materials into smaller sizes and grinding them using pestle and mortar

In both cases i.e. whole and crushed, the test material was presented in the traps in three different ways : 1/4 full (i.e. 3.8cm of whole test material or 35ml in the case of crushed test material), 1/2full (i.e. 7.5cm of whole test material or 70ml of crushed test material), and 2/3 full (i.e. 10cm of whole test material or 94ml of crushed test material). Whole and crushed forms of the test materials were measured with a ruler and a measuring jar respectively.

3.2.4 The S.N.C experiment

The traps consisted of a hollow bamboo stem which opened at one end only. The traps measured 15 cm length with a mean diameter of 3.2 ± 0.102 cm (n=50) . The inside of the cylinders were cleaned with water and a brush to remove the pith. The bamboo traps were each washed thoroughly with tap water several times prior to and after each experiment. The traps were set up by adding appropriate quantities of test materials and controls and fastening them to a wooden grid (Plates 4&6). Stones were used as control material and control traps were also filled appropriately . For each test material ten test traps and ten control traps were used. The location of test traps and control traps were alternated to allow the snails equal chances of moving towards the test or control traps. Each trap was fixed vertically with the aid of two pieces of string, i.e. one around the top end of the trap and the other around the bottom end. The experiments were performed under a photoperiod of 12 hours of light and 12 hours of dark. The traps were completely

PLATE 3: Snail breeding for studies under simulated natural conditions

immersed and the aperture of each trap was 1 cm below the water level in the tank. The number of snails of *B. truncatus* and *B. pfeifferi* caught in each trap was counted and recorded after 24 hours. Paired t-tests (Bailey, 1981) were used to determine the levels of significance. Traps and bioactive factor combinations which caused more snails to be attracted to the test traps as compared with control traps, are deemed to be more effective under simulated natural conditions (SNC).

3.2.5. Trap orientation

Sugar cane (*Saccharum officinarum*) in its whole form was identified as the best bioactive material. It was therefore chosen for orientation test. The objective of this test was to investigate any possible effect of trap orientation on the efficiency of trapping units , i.e. whether traps set in one position would catch significantly more snails than those set in other positions. Three kinds of orientations were investigated. These were vertical orientation and horizontal orientation and orientation at 60° to the vertical. Twelve replicates were used for each kind of orientation. The half-filled traps were alternated in the following order: vertical, horizontal and an angle of 60° (fig. 3.1). The traps were left in position for 24 hours and snails caught were counted and recorded. ANOVA was used to determine the levels of significance. The same experiment was repeated using pawpaw which was identified as the next most effective bioactive material.

PLATE 4: Bamboo cylinders on wooden grid used in studies under simulated natural conditions

PLATE 5 Snails being fed on lettuce in concrete tanks prior to the test

PLATE 6: Student setting traps under simulated natural conditions

PLATE 7: Large concrete tank containing water, water plants, snails and Bamboo cylinders with test materials, under simulated natural conditions

3.3 RESULTS

3.3.1 Effectiveness of bioactive natural products under SNC

Snails found in and on a trap are deemed to be caught by the trap. All the traps with bioactive natural products caught significantly more snails than the control traps. They can be arranged in the following order of effectiveness: sugar cane > pawpaw > cassava > potato (figures 4A & 4B ;Table 9). With the exception of potato, all the bioactive factors attracted more snails of *Biomphalaria* than those of *Bulinus*. Work on young adult snails of *B. truncatus* and *B. pfeifferi* under SNC have revealed a concordance between bioassay (Chapter two) and SNC (Chapter three) results.

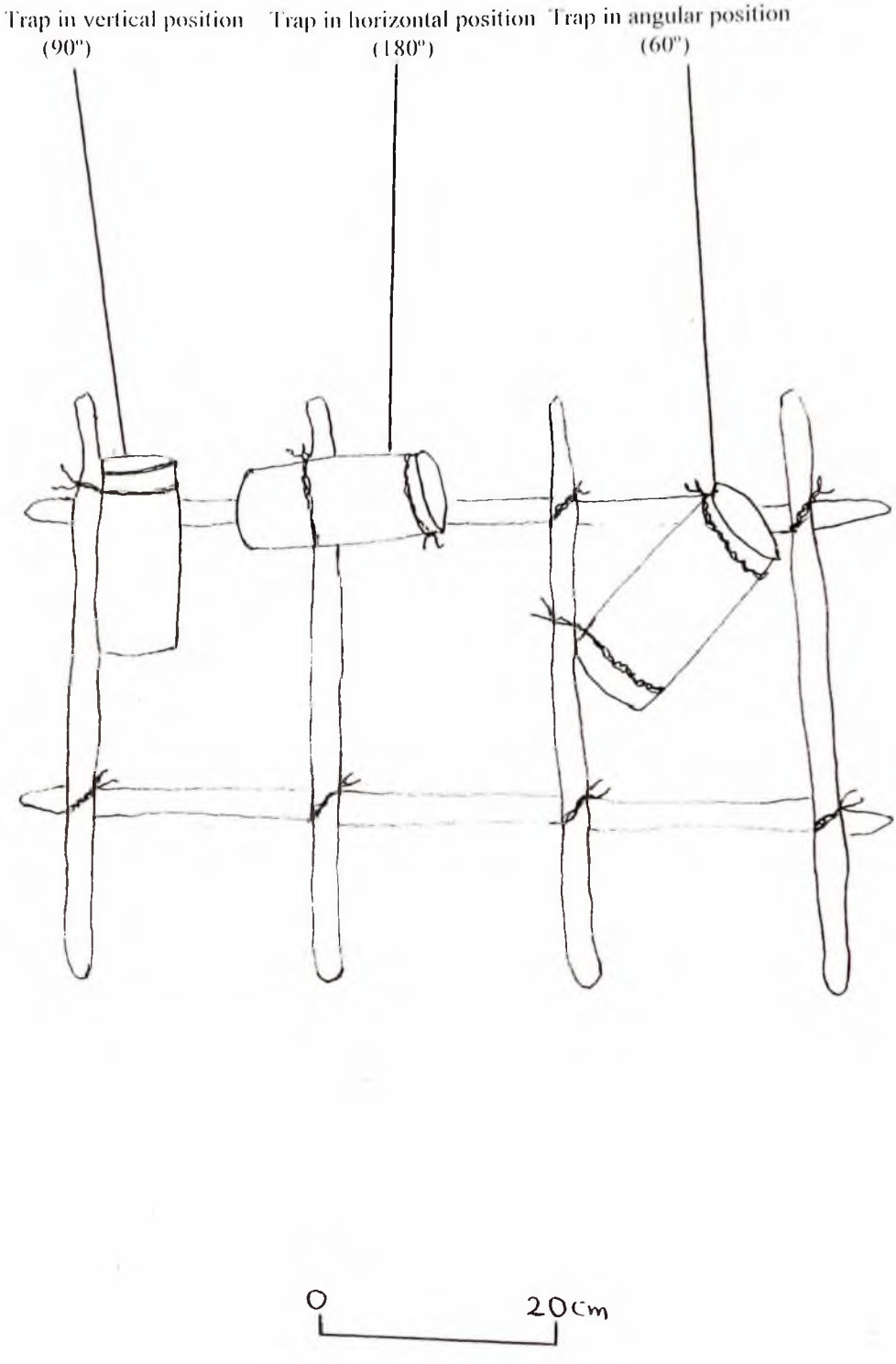
3.3.2 Effect of form of bioactive natural products on effectiveness under S.N.C.

Traps with whole forms of bioactive natural products caught more snails than those with corresponding crushed forms. Traps containing whole forms of potato and pawpaw caught significantly more snails as compared with those with the crushed forms ($p=0.023$ and $p=0.046$ respectively). Whole forms of cassava and sugar cane caught more snails than their crushed counterparts but the differences are not statistically significant ($p=0.63$ and $p=0.32$ respectively) (Table 10).

3.3.3. Effect of quantities of test materials used on effectiveness of trapping units.

Although actual numbers of snails caught using the different quantities of test material varied, no significant differences were found between any of the quantities used (Table 9; Fig. 4 A & table 10; Fig. 4B). Thus any quantity of the test material equal or greater than a quater of the trap volume can be used.

Figure 3.1: A sketch of trap positions in orientation test.



3.3.4 Effect of orientation on trapping efficiency

None of the three positions in which the traps were tested caught significantly more snails than the others (Tables 11 & 12, Figs. 4C & 4D) (ANOVA).

TABLE 9: RESPONSE OF SNAILS TO QUANTITIES OF WHOLE TEST MATERIALS

	Total snails in10 1/4full traps	Total snails in 10 1/2full traps	Total snails in 10 2/3full traps
CASSAVA	68	99	93
POTATO	47	111	104
PAWPAW	105	70	89
SUGAR CANE	105	112	102
TOTALS	325	392	388

TABLE 10: RESPONSE OF SNAILS TO QUANTITIES OF CRUSHED TEST MATERIALS

	Snails in 10 1/4full traps	Snails in 10 1/2full traps	Snails in 10 2/3full traps
CASSAVA	64	75	93
POTATO	72	78	79
PAWPAW	47	58	44
SUGAR CANE	100	80	88
TOTALS	283	291	304

TABLE 11 RESPONSE OF SNAILS TO TRAP ORIENTATION USING SUGAR CANE

Trap no	Total snails in 90 ° traps	Total snails in 60° traps	Total snails in 180° traps
1	4	2	11
2	1	0	5
3	5	6	1
4	1	6	5
5	5	1	2
6	5	5	7
7	2	4	5
8	8	3	4
9	6	12	5
10	6	4	10
11	2	3	4
12	4	14	16
TOTALS	49	60	75

90° = vertical; 60° =angle , 180° =horizontal

ORIENTATION TESTS USING WHOLE SUGAR CANE

ROW	VBR	VBP	VTOT	HBR	HBP	HTOT	ABR	ABP	ATOT
1	0	4	4	1	10	11	0	2	2
2	1	0	1	0	5	5	0	0	0
3	2	3	5	0	1	1	2	4	6
4	0	1	1	2	3	5	2	4	6
5	2	3	5	1	1	2	0	1	1
6	1	4	5	3	4	7	3	2	5
7	1	1	2	0	5	5	1	3	4
8	1	7	8	0	4	4	1	2	3
9	1	5	6	2	3	5	2	10	12
10	2	4	6	4	6	10	3	1	4
11	1	1	2	2	2	4	2	1	3
12	2	2	4	5	11	16	6	8	14

```
1TB >
I

ANALYSIS OF VARIANCE
SOURCE      DF        SS        MS        F       P
FACTOR       2      28.4      14.2     1.07   0.356
ERROR       33     439.2      13.3
TOTAL       35     467.6
                                   INDIVIDUAL 95 PCT CI'S FOR MEAN
                                   BASED ON POOLED STDEV
LEVEL        N      MEAN     STDEV  --+---------+---------+---------+----
VTOT        12     4.083     2.193   (---------*---------)
HTOT        12     6.250     4.202             (---------*----------)
ATOT        12     5.000     4.178        (----------*---------)
                                    --+---------+---------+---------+----
POOLED STDEV =     3.648             2.0       4.0       6.0       8.0
MTB >
MTB >
MTB >
MTB >
MTB >
```

VBR = No. of Bulinus truncatus caught in vertical (90°) traps.
VBP = No. of Biomphalaria pfeifferi caught in vertical traps.
VTOT = Total no. of B. truncatus and B. pfeifferi caught in vertical traps.
HBR = No. of B. truncatus caught in horizontal (180°) traps.
HBP = No. of B. pfeifferi caught on horizontal traps.
HTOT = Total no. of B. truncatus and B. pfeifferi caught in horizontal traps.
ABR = No. of Bulinus truncatus caught in angular (60°).
ABP = No. of B. pfeifferi caught in angular traps.
ATOT = Total no. of B. truncatus and B. pfeifferi caught in angular traps.

TABLE 12: RESPONSE OF SNAILS TO TRAP ORIENTATION USING PAWPAW

Trap no.	Total snails in 90° traps	Total snails in 60° traps	Total snails in180° traps
1	2	4	6
2	3	5	4
3	5	4	5
4	11	4	16
5	10	4	5
6	4	6	3
7	1	7	5
8	1	7	3
9	3	4	2
10	8	6	3
11	5	6	5
12	4	4	3
TOTALS	57	61	60

90° = vertical; 60° =angle ; 180° =horizontal

ORIENTATION TESTS USING WHOLE RIPE PAWPAW

ROW	VBR	VBP	VTOT	HBR	HBP	HTOT	ABR	ABP	ATOT
1	1	1	2	1	5	6	0	4	4
2	2	1	3	2	2	4	1	4	5
3	1	4	5	3	2	5	1	3	4
4	1	10	11	5	11	16	1	3	4
5	2	8	10	2	3	5	1	3	4
6	2	2	4	0	3	3	0	6	6
7	1	0	1	1	4	5	4	3	7
8	1	0	1	2	1	3	5	2	7
9	2	1	3	1	1	2	2	2	4
10	4	4	8	3	0	3	4	2	6
11	2	3	5	4	1	5	3	3	6
12	3	1	4	2	1	3	4	0	4

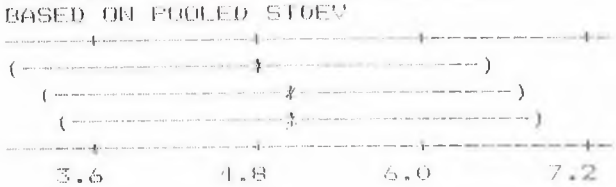
TB >
TB >
TB >
TB >
TB >

NALYSIS OF VARIANCE

OURCE	DF	SS	MS	F	p
ACTOR	2	0.72	0.36	0.04	0.959
RROR	33	285.17	8.64		
OTAL	35	285.89			

INDIVIDUAL 95 PCT CI'S FOR MEAN
BASED ON POOLED STDEV

EVEL	N	MEAN	STDEV
'OT	12	4.750	3.306
'OT	12	5.000	3.668
'OT	12	5.083	1.240



OOLED STDEV = 2.940

ITB >
ITB >
ITB >
ITB >
ITB >
ITB >

BR = No. of Bulinus truncatus caught in vertical (90°) traps.
BP = No. of Biomphalaria pfeifferi caught in vertical traps.
OT = Total no. of B. truncatus and B. pfeifferi caught in vertical traps.
BR = No. of B. truncatus caught in horizontal (180°) traps.
BP = No. of B. pfeifferi caught on horizontal traps.
OT = Total no. of B. truncatus and B. pfeifferi caught in horizontal traps.
BR = No. of Bulinus truncatus caught in angular (60°).
BP = No. of B. pfeifferi caught in angular traps.
OT = Total no. of B. truncatus and B. pfeifferi caught in angular traps.

Figure 4A: Determination of efficiency of trapping units under simulated natural conditions (SNC).

1/4FT = 1/4 full test traps
1/4FC = 1/4 full control traps
1/2FT = 1/2 full test traps
1/2FC = 1/2 full control traps
2/3FT = 2/3 full test traps
2/3FC = 2/3 full control traps
SugarcaneC = crushed sugarcane
SugarcaneW = whole sugarcane
CassavaC = crushed cassva
CassavaW = whole cassava

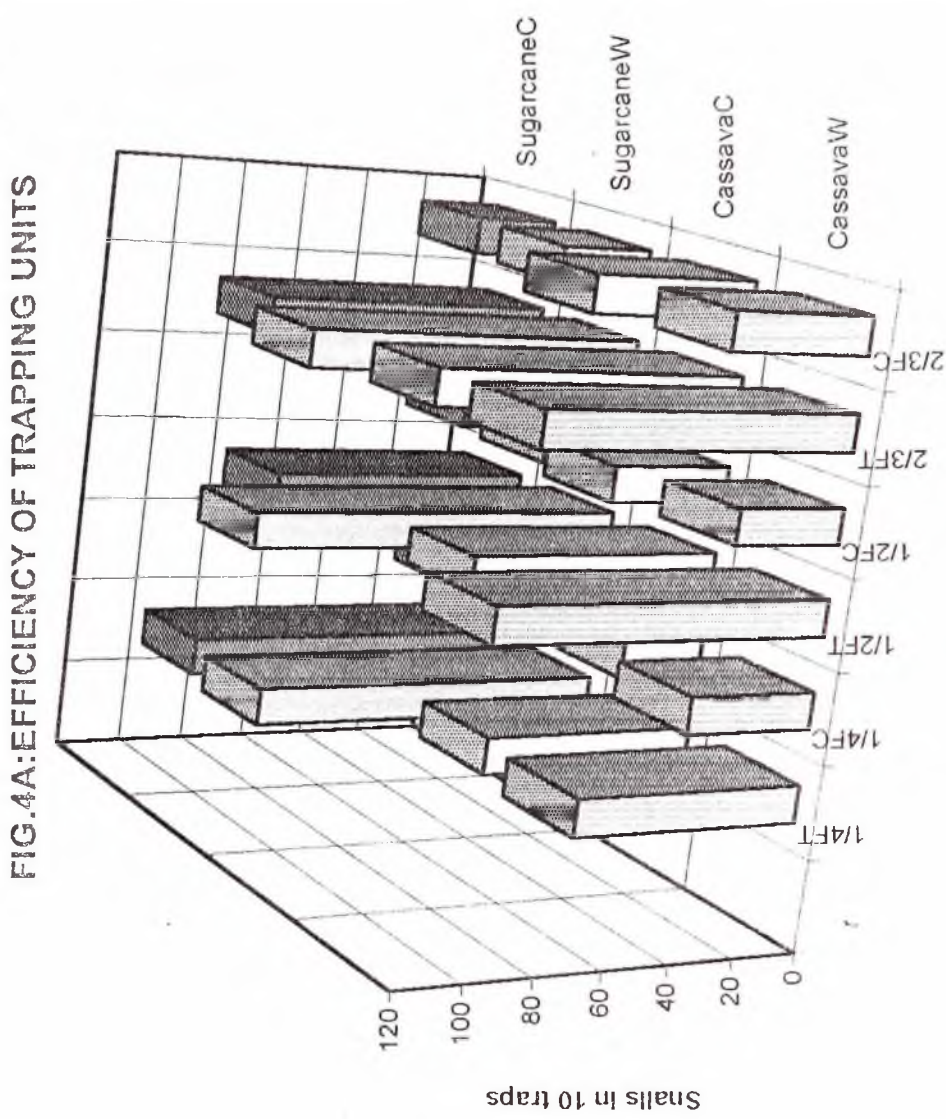


FIG.4A:EFFICIENCY OF TRAPPING UNITS

Figure 4B: Determination of efficiency of trapping units under simulated natural conditions (SNC).

1/4FT = 1/4 full test traps

1/4FC = 1/4 full control traps

1/2FT = 1/2 full test traps

1/2FC = 1/2 full control traps

2/3FT = 2/3 full test traps

2/3FC = 2/3 full control traps

Sw.potatoC = crushed sweet potato

Sw.potatoW = whole sweet potato

PawpawMC = crushed mature pawpaw

PawpawMW = whole mature pawpaw

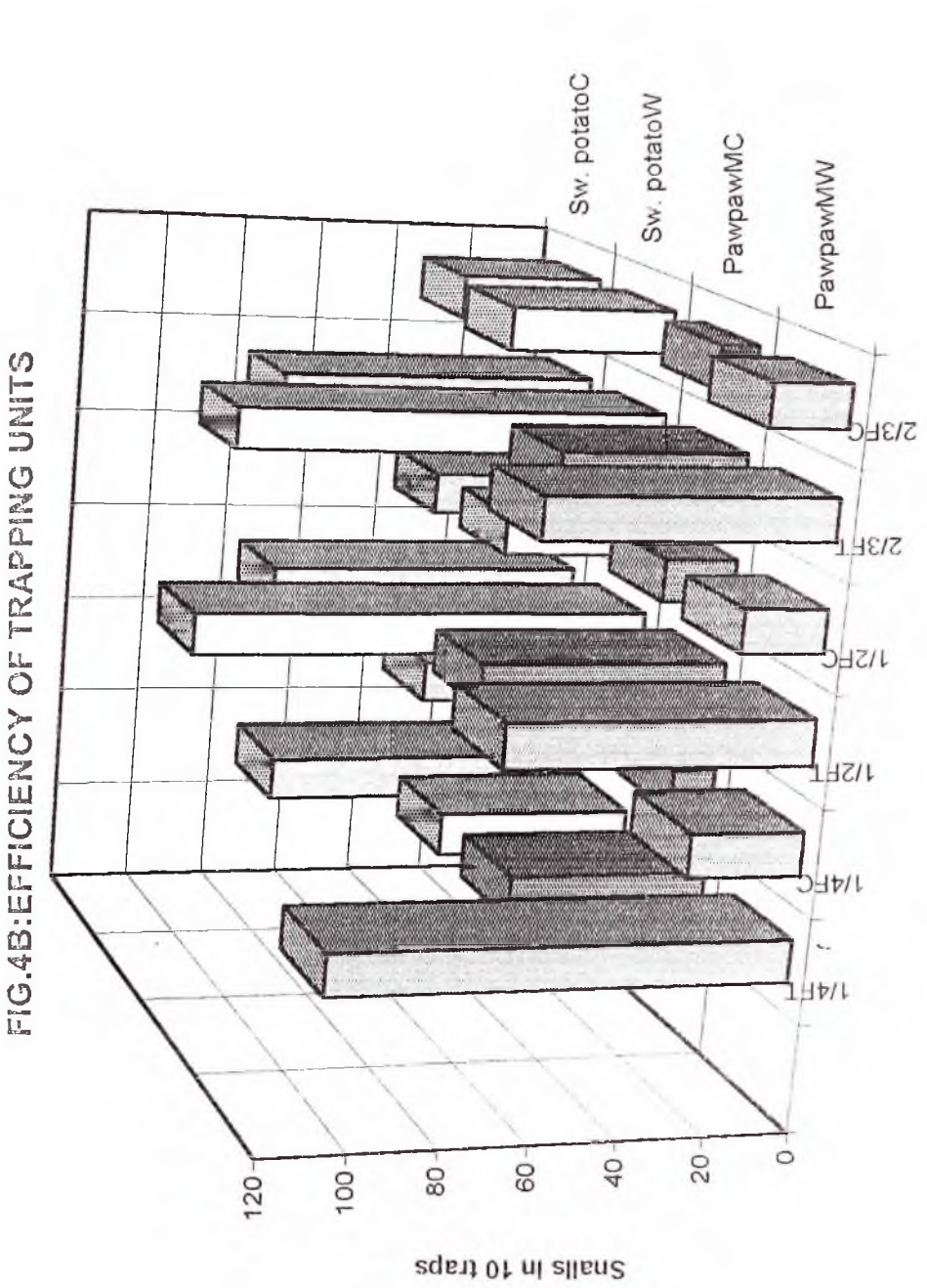


FIG.4B:EFFICIENCY OF TRAPPING UNITS

Figures 4C & 4D: Determination of effect of trap orientation on trapping units under simulated natural conditions (SNC).

VERTICAL = traps in 90 position

ANGLE = traps in 60 position

HORIZONTAL = traps in 180 position

B. PFEIFFERI = Biomphalaria pfeifferi

B. ROHLFSI = Bulinus truncatus

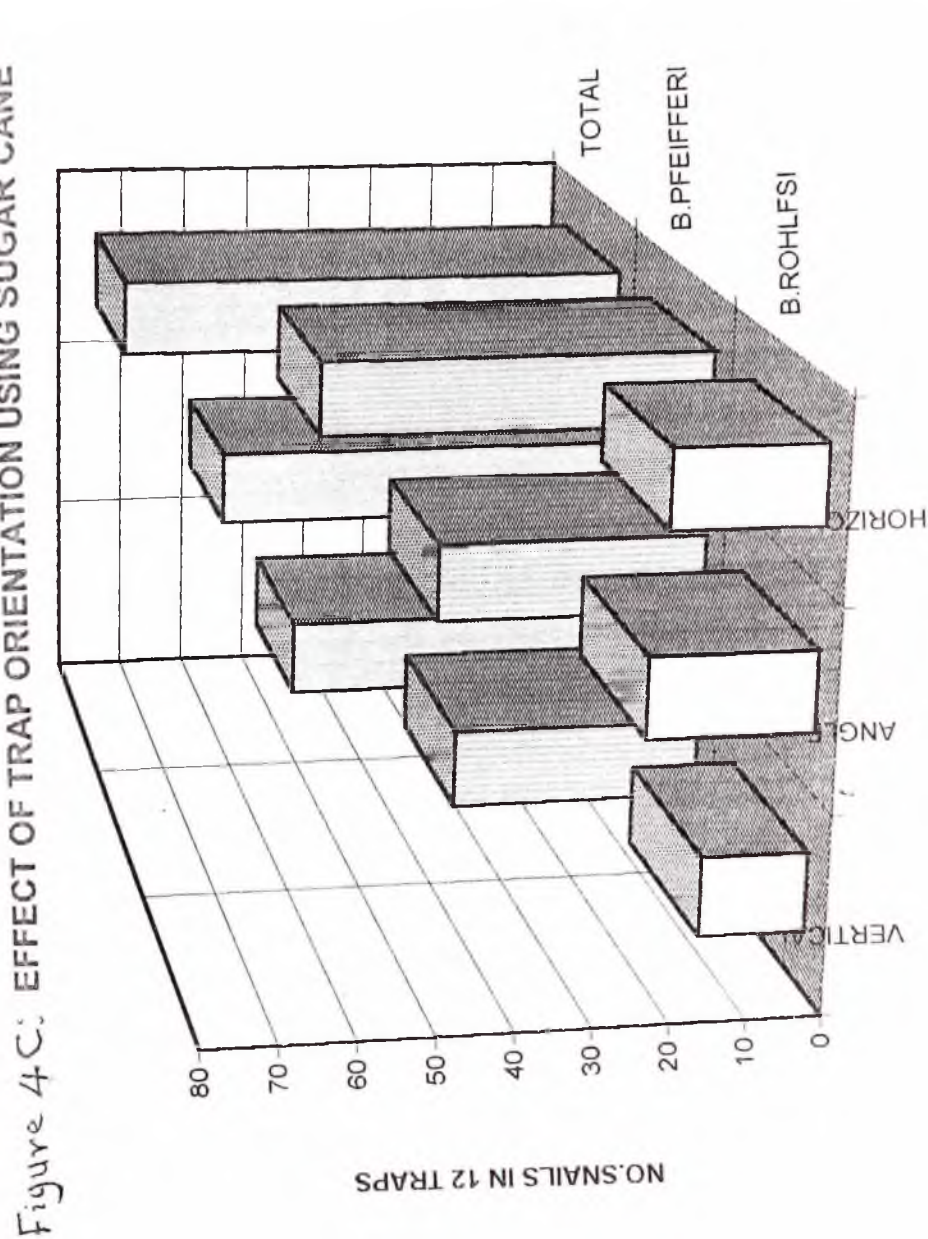


Figure 4C: EFFECT OF TRAP ORIENTATION USING SUGAR CANE

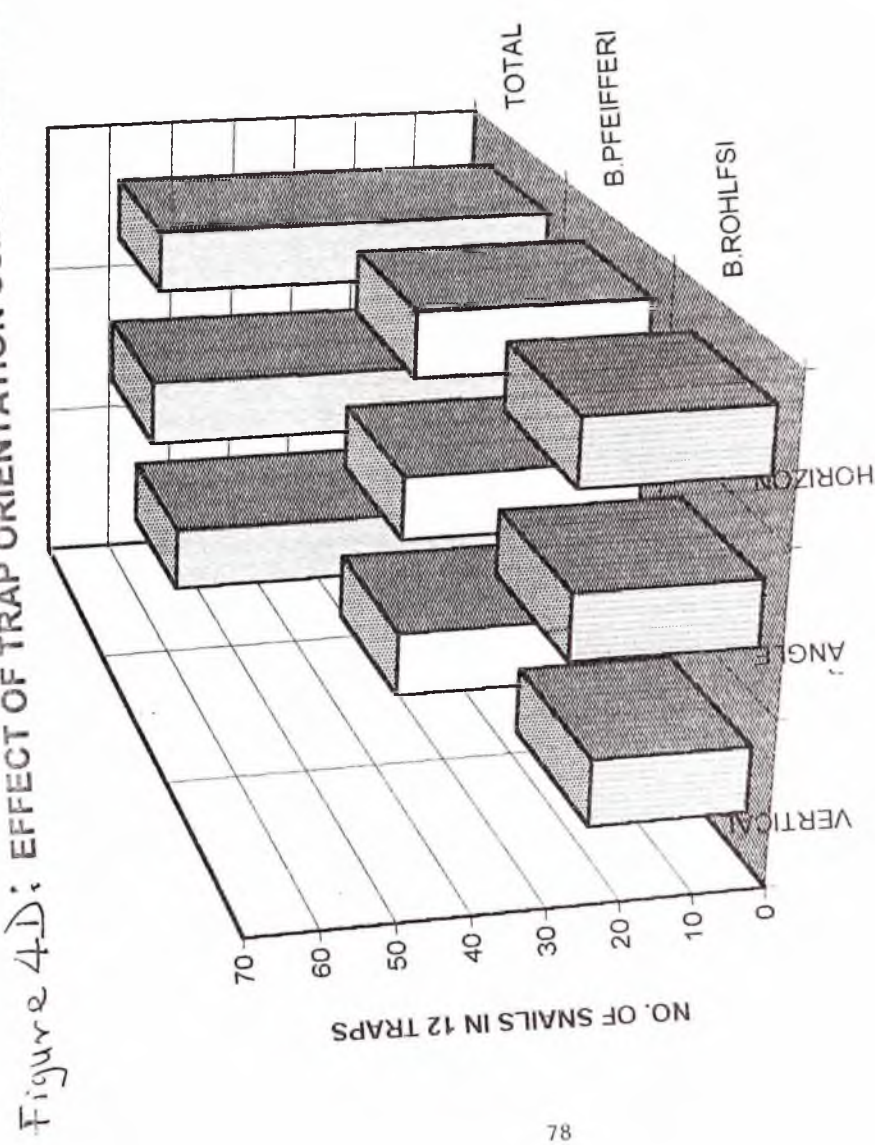


Figure 4D: EFFECT OF TRAP ORIENTATION USING PAWPAW

3.4 DISCUSSION

The studies conducted under SNC have revealed a concordance between the results of bioassay study (using olfactometer) and that of simulated natural conditions (SNC). Thus the bioactive materials identified in phase 1 (chapter 2) i.e. sugar cane, pawpaw, potato and cassava also acted as significant attractant to snails of *Biomphalaria pfeifferi* and *B. truncatus* under S.N.C.

It could be seen from figure 4 that the snails were more responsive to test traps than control traps in the cases of both whole and crushed test materials. Sugar cane elicited the highest response, followed by pawpaw, sweet potato and cassava in the case of whole test materials. This is in agreement with the results obtained from phase 1 (chapter 2) of this study. Traps loaded with whole test materials caught higher numbers of snails than those loaded with crushed test materials. The differences between numbers of snails caught by whole and crushed forms of potato and pawpaw were statistically significant whilst in the cases of cassava and sugar cane there were no significant differences between the number of snails caught by whole and crushed forms. It can be suggested here that in the case of whole materials, molecules were released slowly, thereby creating a diffusion gradient along which the snails travel to the traps. Conversely, the diffusion gradient was disturbed in the case of crushed materials since most of these materials floated out of the traps and were

almost evenly distributed in the water. Thus, little or no diffusion gradient was formed for snails to travel on to the traps, hence the low catch of snails.

It can be seen in figure 4 and table 11 that the quantity of bioactive factors introduced into the traps had no marked effect on trapping efficiency. Likewise, orientation of traps has no significant effect on trapping efficiency (table 12). These findings are very important for the purpose of this study in that any minimum amount of bioactive natural product presented to the traps and the trapping unit left in any position will produce a positive result. This will help reduce to the bearest minimum the quantity and therefore the financial cost of bioactive natural products and time that would have to be spent in setting the traps in a specific position. Combination of whole sugar cane and bamboo cylinder produced the most effective trapping units that would be evaluated under field conditions in Phase 3.

CHAPTER 4

FIELD EVALUATION OF MOST EFFECTIVE TRAPPING UNIT

4.1 INTRODUCTION

The use of broad spectrum molluscicides in the fight against schistosome host snails has generated certain problems such as the destruction of non-target organisms, and the chemical pollution of water bodies. These problems have prompted the Scientific Working Group of the WHO (1977) to recommend that the prime aims of research concerned with control should be the development of cheaper methods of control with increasing target specificity. In the same year, Cardarelli et al., (1977) had investigated the possibilities of using controlled release technology to increase the efficiency of molluscicide applications. Subsequently, Thomas et al, (1980b) has suggested that the efficiency of a controlled release formulation (CRF) could be enhanced by combining attractants, arrestants and phagostimulants with snail toxicants. The use of specific attractants, arrestants and phagostimulants would allow target snails to be removed selectively, with minimal adverse effects on the environment. With this objective in mind, the chemoreception niche of the South American schistosome host snail *Biomphalaria glabrata* (Say) has been extensively characterised, with the aim of identifying those chemical factors which could be used as kairomones in the proposed CRF's (Thomas & Assefa, 1979,Thomas et al, 1980b, Thomas et al 1986 (A & B).

Similar work on the chemoreception niches of *Bulinus globosus* and *B.truncatus* have revealed that these snails have different chemoreception niches when tested in two separate bioassay systems under laboratory conditions (Kpikpi 1990). He discovered that maltose

acted as a potential attractant, arrestant and phagostimulant to *B. globosus* in the field in Lake Kpong - Ghana. This result is in agreement with results of laboratory experiments. He also found a similar concordance between laboratory and field results with British freshwater snails in the Lewes Brooks.

In the present study, similar work on juvenile and adult *Bulinus truncatus* and *Biomphalaria pfeifferi*, using natural products, has revealed that these snails showed strong preferences for a few natural products i.e. sugar cane, pawpaw, potato and cassava which were tested in two separate bioassay systems under laboratory conditions (chapters 3 & 4).

The question which needed to be answered next was whether natural products would exert the same effects on the schistosome host snails in their natural environment. In order to answer this question, plans were made to evaluate the efficacy of the most effective discovered natural product i.e. sugar cane in the Weija lake near Accra, Ghana. The present chapter describes the field studies. The implications of these findings to slow release technology are also considered.

4.2 THE STUDY AREAS

The study area was located on the Weija Lake near Accra (Latitude 5° 30', Longitude 0°20'). The Weija lake was created by the construction of a dam on river Densu in 1977. The reservoir lies within 0°20'N and 5° 30N, 5° 45 N (fig. 4.1). It is located 17km west of Accra and covers approximately 3361 hectares of land at maximum water level. The lake has a shoreline of 48km. The normal surface elevation is estimated at 14.37km with a

maximum of 15.24km (Nukunya *et al.* 1979, as cited by Zuta, 1994). The reservoir provides water to western Accra, supports an irrigation programme and some fishery. The Weija lake area is low lying with undulating topography and isolated ridges. Climatic conditions are tropical with temperatures averaging 27 °C. Rainfall is moderate and seasonal averaging 65mm annually. Peak rainfall occurs in June and September with dry periods spanning December through March.

The ethnic composition of the riparian population is heterogeneous, comprising Ga-Adangbe, Ewe, Akan and Northerners. They are mostly peasant farmers, freshwater fishermen and traders. The squatter communities which comprise of unapproved, unplanned settlements located less than 1km to the lake , characteristically are inhabited by migrant freshwater fishermen. These communities have no sanitary facilities and pipe-borne water supply. A single, multiple-purpose water contact site for the village serves for docking of canoes, bathing, swimming, washing and domestic water collection. Occupational and recreational contact with water at this site is therefore very intense and unrestricted for all ages (5 years and above) and both sexes. Moreover there is gross contamination of the lakeshore bushes near this water contact site with faecal matter (which ultimately gets washed into the contact site by run-off water during rains). Host snails encountered at this site included *B. truncatus* and *B. pfeifferi*. These intense and unrestricted water contact activities coupled with the presence of the host snails promote the transmission prevalence of schistosomiasis in the communities near the Weija lake.

In a preliminary investigation of the prevalence of schistosomiasis in the Weija Lake 15 years after impoundment, Zuta (1994) has shown that the prevalence rates of S. haematobium generally decreased with increasing distance from the Weija Lake. He noted that the prevalence rates of *S. haematobium* increased significantly from the resettled townships to the squatter communities which are closest to the lake. High prevalence rates (50 to 89.4%) were recorded within the squatter population. The highest prevalence rate of urinary schistosomiasis observed for the whole study (89.4%) was recorded at New Galilea, a squatter community. The next highest prevalence rate of 80.0% was recorded at Tomefa where the field study of the present work was undertaken.

The choice of this site was determined by the availability of sizeable populations of the two snail species, i.e. *B. truncatus* and *B. pfeifferi* in the water body. The choice of trap location was influenced by the availability of aquatic vegetation as schistosome host snails are commonly found in the ecotone zone of water bodies which have well developed aquatic vegetation. The aquatic vegetation in the study area consisted mainly *of Ceratophyllum demersus*, *Pistia stratoites*, *Echinochloa pyramidalis and Ipomeoa aquatica*. The traps were set among these water plants.

4.3 MATERIALS AND METHODS

Larger versions of the bamboo cylinder used in Phase 2 (Chapter 3) were constructed. The traps consisted of a hollow bamboo cylinder which open at one end. The traps measured 25cm in length with a mean diameter of 5.9 ± 01 cm. The inside of the cylinders were

cleared with a long brush and water to remove the pith. A string was attached to the trap to allow fastening to macrophytes when introduced into the water body. The study area was divided into three parts, zones A, B&C for easy comparison.

4.3.1 The Field Experiment

4.3.1.1 Optimum trapping Duration

The aim of this experiment was to determine the optimum duration for leaving the traps in the water. This will be shown by the span over which the highest number of snails were caught.The most effective bioactive factor, whole sugar cane, was cut into cylindrical forms measuring 12 cm in length with a diameter of 3 cm. The bioactive factor was introduced into 100 traps which were placed in the water body. Twenty traps were removed for inspection at the following time intervals to determine the optimum trapping duration: (a) after 12 hours, (b) after 24 hours (c) after 36 hours

(d) after 120 hours (5 days) and (e) after 168 hours (7 days).

4.3. 1.2 Field Evaluation

The objective of this aspect of the experiments was to investigate the efficacy of the discovered bioactive factor under field conditions. The trapping period which yielded the highest number of snails per unit time from the previous experiment was adopted here (Fig. 5). Using polystyrene, an inert material in traps as controls, the best performance traps with sugar cane as bioactive factor, were tested simultaneously for the optimum duration determined, (i.e. 12 hour trapping period) The experiment was replicated three times.

4.4 RESULTS

4.4.1 Optimum duration of trapping

The trapping period which yielded the highest number of snails per unit time was 12 hours (Chart 5). The trapping period which produced the highest total number of snails was 120 hours (5 days) (Fig. 5). The 12 hour trapping period was used in field evaluation.

4.4.2. Field evaluation

The results show that significantly more snails were caught in test traps as compared with control traps in all the three replicates ($p<0.05$) (Figs. 6A, 6B, 6C).

A statistically significant number of snails of *B. truncatus* were caught in test traps as compared with control traps ($p<0.05$). But in the case of *B. Pfeifferi* the number of snails caught in the test traps was not significant as compared with control traps ($p>0.05$). Similar observations are shown by all the three replicates (Figs. 6A, 6B, 6C). The implications are discussed below.

Figure 5: Determination of optimum trapping duration under field conditions.

BR BP = total number of B. pfeifferi & B. truncatus caught.

TOTAL SNAILS/HR = total number of B. pfeifferi & B. truncatus caught per hour.

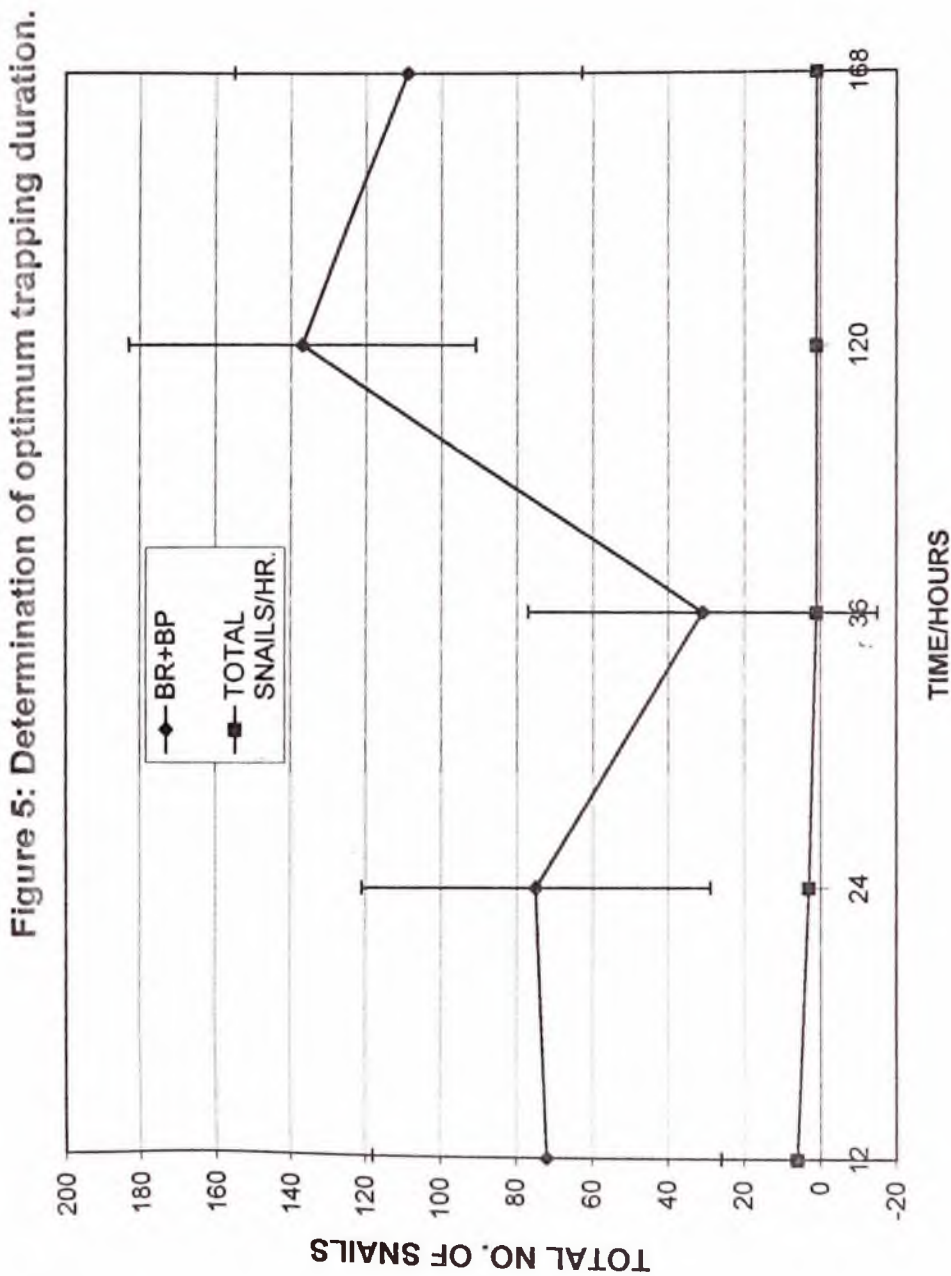


Figure 5: Determination of optimum trapping duration.

Figures 6A, 6B & 6C: Determination of efficacy of sugarcane under field conditions.

BRTEST = Bulinus truncatus caught in test traps

BRCONT = Bulinus truncatus caught in control traps

BPTEST = Biomphalaria pfeifferi caught in test traps

BPCONT = Biomphalaria pfeifferi caught in control traps

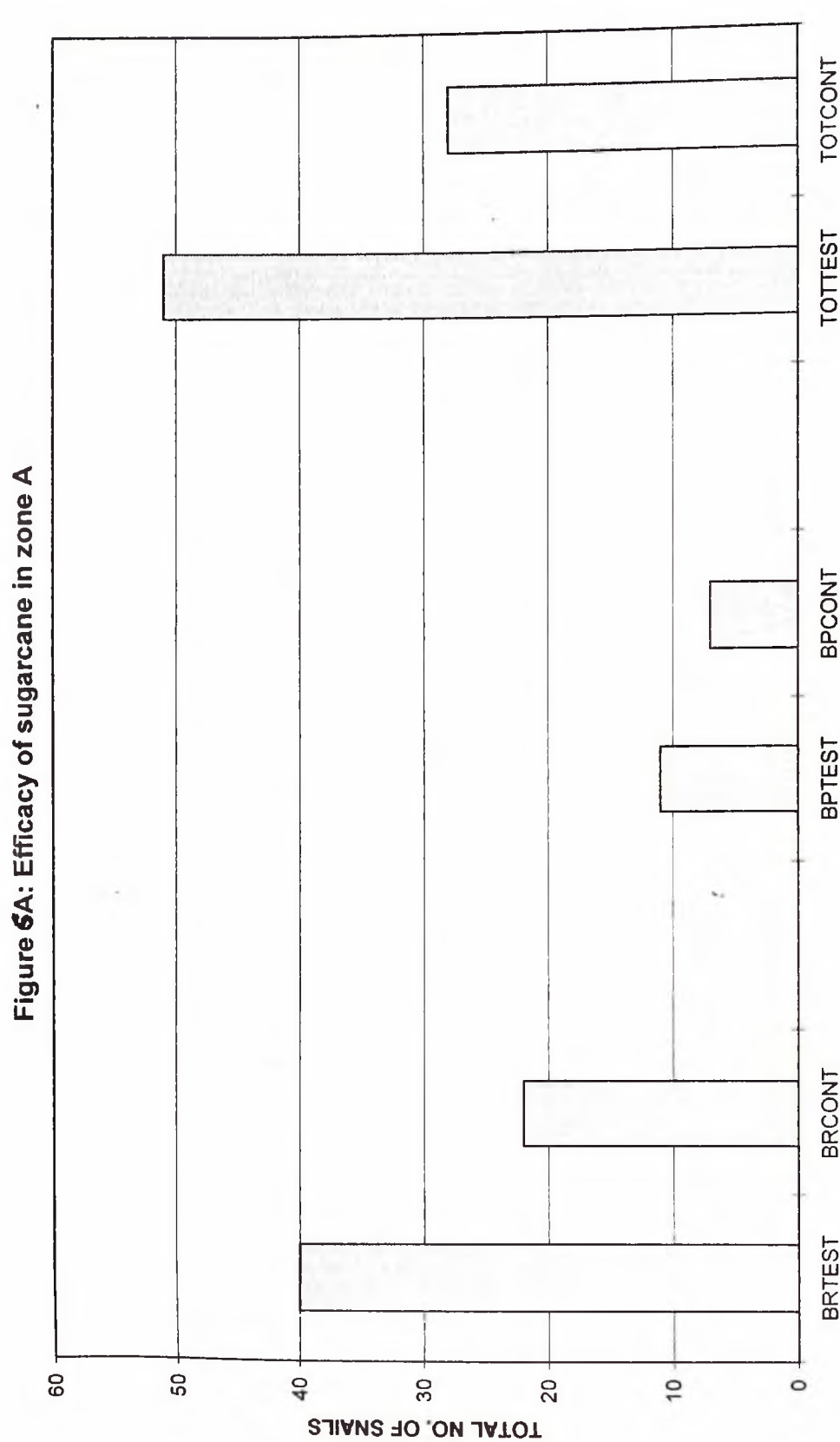


Figure 6A: Efficacy of sugarcane in zone A

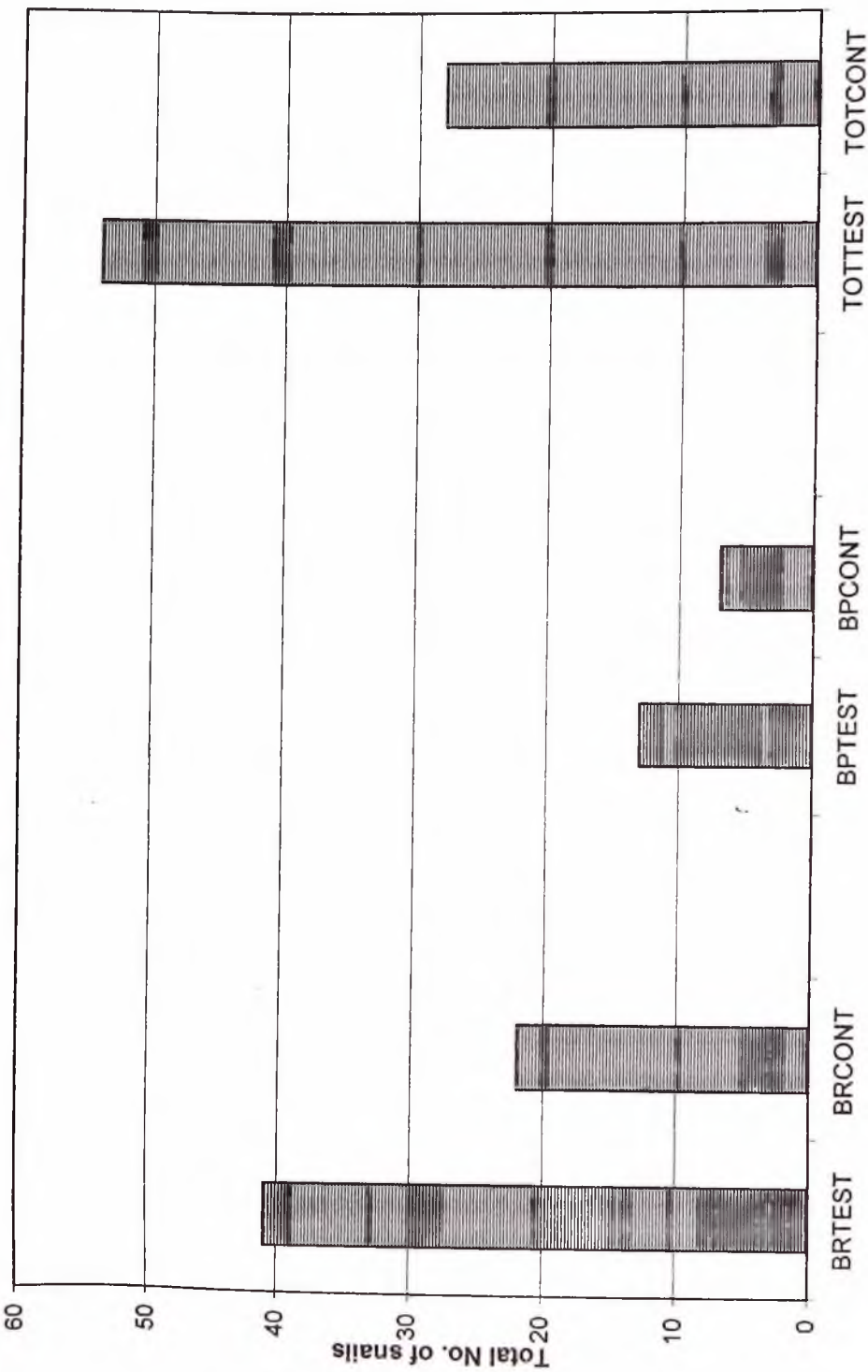


Figure 6B: Efficacy of sugarcane in zone B

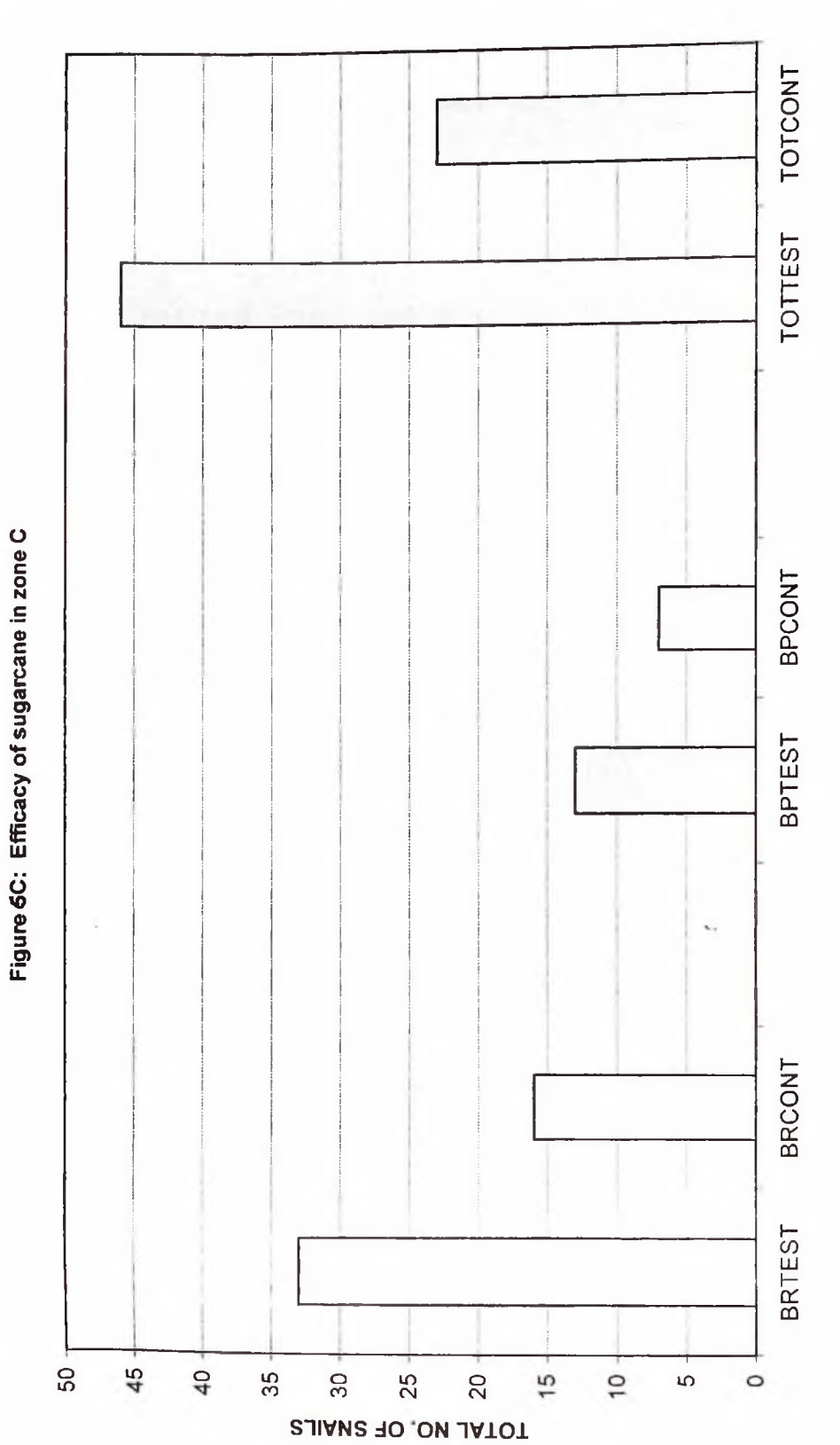


Figure 6C: Efficacy of sugarcane in zone C

4.5 DISCUSSION

The highest total number of snails caught in traps was produced by 120 hour (5 days) duration of trapping.The lowest total number of snails was produced by a 36 hour trapping period . The effective or optimum trapping duration was measured by the highest total number of snails caught per unit time and this was produced by 12 hour trapping period (Fig.5). The results of all the field experiments show that far more snails of *B. truncatus* were caught than *B. pfeifferi*. Zuta, (1994) and Odei, (1978) have shown that the prevalence rate of *Schistoma haematobium* transmitted by *B. truncatus* is higher than the prevalence rate of *Schistosoma mansoni* transmitted by *B. pfeifferi* in the communities located around the Weija lake.

In using bamboo cylinders in trapping, two important problems could probably be addressed. Firstly, the system could be able to hold the test materials and release them at slow rate such that some of the active molecules could still be present in the traps at the end of the test period. Secondly, the bamboo cylinder could provide a rigid framework in which the snails could be trapped. In considering alternatives, the bamboo is cheaper in terms of cost and availability. This is because in Ghana, naturally occurring goods or materials are cheaper than synthetic ones, and also bamboo is very common in Ghana.

The choice of Weija Lake as a suitable site for the testing of the bioactive factor was influenced by the abundant availability of the two snails in the water body as shown by a preliminary study. The choice trap location was influenced by the availability of aquatic vegetation.

The present studies came under field conditions. The results indicate that schistosome host snails can be selectively trapped in their natural environment using bioactive natural material as baits. A second possibility in the process is the incorporation of toxic factors in the trapping units. These could be synthetic chemicals, plant molluscicides or microbial agents. The use of the kind of traps employed has a major advantage in that if molluscicides were added to the traps, the contamination of aquatic environment will be minimal while target snails would enter the traps and be killed. Another advantage is the identified trapping units shown by these studies are cheap and easy to come by and therefore can easily be embraced and adopted by the local people. This is because the use of traps for example to catch fish, crabs etc. has become part of the daily activities of the members of the fishing communities located near water bodies.

This approach of combining bioactive materials and bamboo traps may be a viable one towards the control of schistosome host snails. Thus the bamboo traps can be used as snail

control tools and sugar cane which emerged as the most effective bioactive factor in this study can be combined with toxicants for selective removal of schistosome host snails

CHAPTER FIVE

SUMMARY AND CONCLUSIONS

The present investigations have revealed some important features of the chemoreception niches of two African host snails of schistosomiasis, *Bulinus truncatus and Biomphalaria pfeifferi*. This information was used to trap snails under both laboratory and field condition. In the present chapter, an attempt will be made to summarize these findings and point out how they might be utilized in the control of the snail host of schistosomiasis.

5.1 Diffusion olfactometry

This bioassay was used to study the distance chemoreception behaviour pattern of the snails. The two indices, generated by this bioassay (the attractant index and arrestant index) were used to identify bioactive natural factors which had a significant effect on the behaviour pattern of the schistosome host snails. This revealed that the two host snails had different chemoreception niches (although there was some degree of overlap).

Five out of the twenty-six natural products tested (19.2%) were found to act as statistically significant attractants for *Bulinus truncatus* adults. These were: sugar cane > raw potato > raw pawpaw > boiled pawpaw > boiled cassava.

Three out of the twenty-six natural products tested were found to act as statistically significant attractants for *B. truncatus* juveniles in the following order of importance: Ripe pawpaw (Boiled) > Ripe pawpaw (Raw) > Sugar cane.

Biomphalaria pfeifferi showed a similarly selective response to the natural products tested. The list of natural products which acted as statistically significant attractants and arrestants for *Biomphalaria pfeifferi* adults were presented in Table 7(chapter two). A comparison of the attractant and arrestant indices of *Bulinus truncatus* and *Biomphalaria pfeifferi* shows that the latter had a broader chemoreception profile than the former. The chemoreception profiles of the adults of the two snail species were shown to be broader than those of the juveniles. The chemoreception profiles of the juveniles were found to fall within those of the adults. It was only in cases of sugar cane and pawpaw that there was an overlap in responses shown by the two snails. Thus for *B. truncatus* adults, sugar cane and pawpaw acted as attractants as well as arrestants, but the responses to sugar cane were much stronger as compared with controls ($P < 0.001$). Sugar cane and pawpaw acted only as attractants for the juveniles of *B. truncatus*. For *B. pfeifferi* adults, sugar cane and pawpaw acted as attractants and arrestants with sugar cane producing much stronger response as compared with controls ($P < 0.01$). For *B. pfeifferi*, pawpaw acted only as an attractant while sugar cane acted as an attractant as well as an arrestant. From these results, one can conclude that chemoreception niches of these two schistosome host snails are different.

5.2 Effective combination of bioactive natural products and bamboo cylinder under SNC

5.2.1 Effectiveness of bioactive natural products under SNC

Snails found in and on a trap are deemed to be caught by the trap. All the traps with bioactive natural products caught significantly more snails than the control traps. They can be arranged in the following order of effectiveness: sugar cane > pawpaw > cassava > potato. With the exception of potato, all the bioactive factors attracted more snails of *Biomphalaria* than those of *Bulinus* ($p<0.05$).

5.2.2 Effect of form and quantities of bioactive materials on effectiveness of trapping units under SNC

Traps with whole forms of bioactive natural products caught more snails than those with corresponding crushed forms. Traps containing whole forms of potato and pawpaw caught significantly more snails as compared with those with crushed forms ($p=0.023$ and $p=0.046$ respectively). Whole forms of cassava and sugar cane caught more snails than their crushed counterparts but the differences are not statistically significant. Although actual numbers of snails caught using the different quantities of test material varied, no significant differences were found between any of the quantities used.

5.2.3 Effect of orientation on trapping efficiency

None of the three positions in which the traps were tested caught significantly more snails than the others. Based on the above findings, any quantity of the test material equal to or greater than a quarter of the trap volume can be added to bamboo cylinders and the traps oriented in any position.

5.3 The evaluation of the efficacy of bioactive natural products under field conditions

The optimum duration of trapping period measured by the highest total number of snails caught per unit time was found to be 12 hours. Test traps with sugar cane caught significantly more snails than control traps with polystyrene. The results of all the field experiments show that far more snails of *B. truncatus* were caught than *B. pfeifferi*. This may be due to difference in populations of *B. truncatus* and *B. pfeifferi* in the Weija lake. For example with the discovery of earlier workers (Zuta, 1994; Odei, 1978) and the preliminary studies carried out prior to the field experiments, it has been found that the population of *B. truncatus* in the Weija lake is higher than that of *B. pfeifferi*. This observation is in agreement with the fact that the prevalence rate of *Schistosoma haematobium* transmitted by *B. truncatus* is higher than that of *Schistosoma mansoni* transmitted by *B. pfeifferi* in the communities located around the Weija lake (Zuta, 1994).

There were two major choices involved in evaluation of test products in the natural environment of the snails. These were: (1) the choice of bioactive natural factors and (2) the method or assay system to be used. Each of these are considered in turn.

5.3.1 The choice of test factors

The characterisation of the chemoreception profiles of the two snail species (Chapters 2 & 3) led to the identification of some bioactive natural products as attractants and arrestants for the snails. Ideally, the most potent of these factors should be selected for further testing under field conditions. Bioactive natural products which acted as attractants and/or arrestants for all age groups of the two snails should be preferred to those which had a significant effect on only one species or one age group of the snails.

However two other features (in addition to potency) of the natural test materials which influence the final choice for further evaluation were cost and availability of the bioactive natural products in the areas where schistosomiasis is endemic.

One of the major problems of snail control is the high costs of the molluscicides. This is partly because they are manufactured outside the countries in which the disease is endemic and therefore have to be imported. Since these countries are in general poor, the cost of any molluscicides should be a key consideration in any research programme. Thus a good choice of bioactive natural factor would be one which combines effectiveness with low cost, as recommended by WHO (1977).

One bioactive natural factor which came closest to meeting all three features outlined above was sugar cane. It was found to act as significant attractant/arrestant to both adults and juveniles of the two snails tested in this study. Sugar cane, was thus chosen for the field tests.

Sugar cane, which emerged as the most potent attractant for both age groups of the two snail species and also known as a cheap crop which is commonly grown near water bodies and is easily available in abundance throughout the year, seems to meet most of the requirements for use in target specific molluscicides.

5.3.2 The choice of method for evaluating test materials

In the design of the trapping units, two vital problems had to be avoided. Firstly, there was the requirement that the system be able to hold the test factors and release them at slow rate such that a diffusion gradient could be formed along which the snail would navigate to the test material. Secondly, there was the need for a rigid framework in which the snails could be trapped. In considering alternatives, the cost of these materials was also taken into consideration and this prompted Kpikpi (1990) to design bamboo traps in the modified form which was used in this study. This is because in Ghana naturally occurring materials are cheaper than synthetic ones.

5.3.3 Towards slow release technology

The present studies have identified natural bioactive factors for host snails under laboratory conditions and evaluated the efficacy of the most effective one under field conditions. Sugar cane is effective as an attractant for *B. pfeifferi* and *B. truncatus* under field conditions. The results indicate that schistosome host snails can be selectively trapped in their natural environment using bioactive natural products as baits. Thus a combination of a bioactive factor and bamboo cylinder is a possible way of collecting snails.

A second possibility identified from these studies is that the bioactive natural products could be refined to exclude repellants and increase the relative proportion of the attractants and arrestants. This would enhance the efficacy of naturally occurring bioactive factors.

The next stage in the process is the incorporation of toxic factors in the trapping units. These could be synthetic chemicals, plant molluscicides or microbial agents. The use of the kind of traps employed has a major advantage in that if molluscicides were added to the traps, the contamination of aquatic environment will be minimal while target snails would enter the traps and be killed. Thus there will be minimal adverse effect on the non-target organisms in the environment. It is therefore recommended that this line of research using traps be pursued further. This is because fishermen, who are usually victims of the disease, can incorporate these methods of control into their fishing activities, because they use traps, for example, to catch fish, crabs and prawns.

The present preliminary studies of trap function in the field and the underlying mechanisms suggest that there is scope for transforming the present trapping units into effective snail control tools apart from the fact that they can be good sampling devices.

It is suggested however, that a much better understanding of the mechanisms used by the snails to locate chemical sources and chemoanalysis of the effective bioactive natural products identified in this study, will also be needed for the development of more effective traps. Also, since numbers of snails caught in traps were not high enough to make a real impact on snail populations, more potent bioactive materials and improved trap design would be required.

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