STUDIES ON THE USE OF BIOACTIVE MATERIALS IN MOLLUSCAN TRAPS FOR THE CONTROL OF SCHISTOSOME HOST SNAILS

BY

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This work is dedicated to God Almighty for His divine protection and guidance, and to my wife Josephine, my mother Mrs. Salome Adobea Baidoo, and also, in memory of my late father, Mr. T.A. Baidoo who passed away just when this research work was started.

'Everything that happens in this world happens at the time God chooses. He sets the time for birth and the time for death, the time for planting and the time for pulling up, the time for killing and the time for healing, the time for tearing down and the time for building,

He sets the time for sorrow and the time for joy.' - Ecclesiastes 3 : 1-4

DECLARATION

I do hereby declare that except for the references to other people's work which I have duly acknowledged, this work is a result of my own original research, and this thesis, either in whole, or in the part has not been presented for another degree elsewhere.

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Abstract

As an aid to the control of the schistosome host snails, effective bioactive materials namely cassava, cocoyam, and sweet potato (Domeh, 1998), sugarcane and peels, all in their raw and processed states were tested in simulated natural environment experiments. The experiments were conducted using 'biopots' traps designed using a pot with few small windows created on the sides to allow easy diffusion of test materials to reach the water body to attract the snails. It was observed that the materials retained their effectiveness for both *Bulinus truncatus* and *Biomphalaria pfeifferi* snails in the following order:

cocoyam (1 day fermented) > sweet potato (7 days fermented) > cassava (7 days fermented) > cassava (1 day fermented) > sweet potato (1 day fermented).

The effectiveness of one of the top attractants for the snails (i.e. sugarcane) was found to be unaffected by the addition of small quantities of the toxicant bayluscide (< 100μl, 300μl, 500μl, and 700μl of 0.6ppm bayluscide per 6.495gm of sugarcane) when tested under laboratory conditions.

Higher quantities, however, reduced the attractant effects of the bioactive material. Similar findings were obtained when the tests were conducted under field conditions (i.e. < 1,424.5μl, 4,274.5μl, 7,124.5μl 0.6ppm bayluscide per 92.579gm of sugarcane (an attractant) with bayluscide (a toxicant) in the newly developed 'biopots traps'. The duration of the traps is a factor to be considered. The details of the experiments leading to these discoveries and the results are presented in this thesis.

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Schistosomiasis

The parasite worm which causes schistosomiasis, also known as bilharzia or bilharziasis was first discovered by German pathologist Dr.Theodor Bilharz while conducting an autopsy in a Cairo hospital (Appleton, 1996). There are now 76 countries in which schistosomiasis is endemic, with more than 600 million people at risk of infection and some 200 million infected (WHO TDR programme report, 1989). The disease is widely distributed in Africa, Caribean Islands,Latin America, the Eastern Mediterranean, S.E. Asia, and parts of the Western Pacific region.

Schistosomiasis is particularly associated with water development projects such as dams and irrigation schemes because the parasite's intermediate host snails breed in freshwater lakes and streams. In Egypt and the Sudan, where large irrigation systems have existed for many years, schistosomiasis ranks as a public health problem of the first order (Liese, 1986). After an epidemiological survey, Khallaayoune et al (1995), pointed out that in Morocco, the extensive irrigation networks have contributed to the creation of suitable habitats for the intermediate hosts of schistosomiasis. They also explained that transmission of the disease was not restricted to irrigated areas alone but also occurred in natural foci such as temporary swamps (merja), marshy plains and residual water in pre-Saharan palm groves in the south of the country. The causative agents are trematode flatworm (flukes) of the genus *Schistosoma*, transmitted from fresh-water infected snails. The disease has proved very difficult to control. The World Health Organisation has done much to stimulate research into the basic problems related to the control of schistosomiasis, and now there have been improvement in the efficacy of drugs available for treating people suffering from the disease. Liese (1986) reported that through UN/WHO sponsored projects, praziquantel which is now the most favored drug for treating schistosomiasis, originally costing US$4 per treatment has been reduced to roughly US$0.32 per treatment. Praziquantel has proved very effective in schistosomiasis control in China, where the drug has been used in the treatment of more than one million cases (Fu et al, 1988). The drug is now being used by many countries including Ghana to control schistosomiasis. It must be emphasized however, that, snails control, health education and provision of pipe-borne waters are also very important components of the control strategy. An integrated approach to control schistosomiasis is thus needed to avoid the risk of re-infection.

To effectively control schistosomiasis the disease needs to be given a top priority in national health policies just as malaria. Akogun (1991), explained that in Nigeria although schistosomiasis is the second most prevalent parasitic disease, the control of other diseases such as river blindness, sleeping sickness and malaria is given a more serious consideration. Schistosomiasis remains a serious problem in tropical countries although new effective antischistosomal drugs have been produced for treatment. The problem can be attributed, at least partly to the reinfection of treated individuals living in endemic areas as they continue to have contact with infected water bodies during their day to day activities. Chimbari et al (1995), also pointed out that, under normal circumstances not all infected individuals get treated in a community and as such there is always a reservoir through which water bodies can be reinfected.

1.2 Schistosomiasis in Ghana and Africa.

The association of schistosomiasis incidence with development projects in Africa has been documented by many research scientists, (Stephenson, 1947), (Witenberg and Saliternik, 1957), (McCullough, 1965), (Nelson, 1972), (Betterton, 1984), (Liese, 1986), (Madsen et al, 1987).

Ouma (1995), stated that the disease was present virtually in all African countries where an estimated 150 million persons are infected. In Africa, dams are constructed for hydro-electricity and irrigation projects. Unfortunately the creation of these dams in many countries has provided very suitable environments for the schistosome intermediate host snails. Some of these occurences that have been reported in some countries are found in, Senegal river basin (Diaw, 1995), irrigation projects all over Morocco (Khallaayoune et al, 1995), Lagdo, Benue Division, Northern province of Cameroun (Tsafack et al, 1995), irrigated areas in Khartoum state (Hilali et al, 1995), and lake Kariba, which was created to generate hydroelectric power for Zambia and Zimbabwe (Mungomba et al, 1995).

In a recent study involving the situation of potential intermediate hosts of schistosome parasites in Burkina Faso, it was reported that, of 496 positive biotopes of the hosts identified, 40.89% were located in man-made reservoirs, 33.8% in rivers, 19.64% in temporary ponds, 3.44% in irrigation channels and 2.23% in natural lakes (Poda et al, 1997). Public health authorities in such affected African coutries have not taken enough precautions during the onset of such developmental projects to prevent the spread of the disease in affected communities. The disease has become endemic in many African countries, and its control has really been a very hard task. Nelson (1972), stated that the Aswan dam in Egypt is an example of such development in a country where control of schistosomiasis has been largely ineffective in spite of a great deal of effort over many years. Appleton (1996), reported that in South Africa, schistosomiasis occurs in most of Zimbabwe, Swaziland, Mozambique, Northern Province, North west Mpumalanga and Kwazulu-Natal. Witenberg and Saliternik (1957), explained that in Israel, apart from changes in hydrological conditions (for instance creation of water reservoirs, introduction of wide and complicated irrigation systems, numerous fish ponds), creating habitats for the snails vector, visits by Coptic pilgrims from Egypt, and Egyptian laborers extensively employed in orange groves were important ways of introducing schistosome parasites into Israel.

Bilharziasis is endemic throughout most of the Sudan, and attributed to the development of the Gezira irrigation scheme (Stephenson, 1947). Schistosomiasis has been found to be endemic in Borno State (northern Nigeria), due to the construction of the South Chad irrigation project (Betterton, 1984). A survey conducted in the Federal capital territory in Nigeria (Achillea and Asuma, 1979) on freshwater snails revealed that, on the whole freshwater molluscan fauna was relatively poor. However, there was an indication that schistosomiasis was present at the northwest part of the capital territory, through the centre towards the southeastern part of the territory. Bozdech (1972), studied schistosomiasis prevalence in Accra (Ghana) and Kaduna (Nigeria), and reported that the prevalence was higher in Kaduna than in Accra.

Schistosomiasis in Ghana has been extensively studied over the years (Paperna, 1968, 1965, and 1969), (Odei, 1961, 1965, 1983), (McCullough,1959, 1962, 1965), (Bozdech, 1973), (Kuma, 1979), (Amankwa et al, 1994). Bilharziasis has been endemic in Ghana for a long time (Odei, 1961). He stated that, the earliest report was in the 1895

Annual Report of the Colony of the Gold Coast, in which it was stated that a patient had been admitted to hospital as a result of 'bilharzia haematobia' infection.

Paperna (1968, 1969), reported that schistosomiasis was very prevalent among inhabitants of the local communities at the lower reaches of the Volta river, in the Eastern region of Ghana, and a few villages of the inland area of the Volta Region. He also reported after conducting research among school pupils in Tafo area, Pokuase area and Accra plains. Earlier, Odei (1961) had reported that *S. haematobium* infection was largely confined to two extensive areas, one in the Central Region of southern Volta Region, around Ho and Hohoe districts. The prevalence of schistosomiasis is largely associated with the presence of aquatic habitats which create congenial atmosphere for the schistosome host snails. In the drier regions of Ghana the snail habitats are mostly intermittent or seasonal streams, dams, ponds, lagoons, marshes and swamps (Odei, 1965). He explained that, in the secondary forest areas they are mainly swamps, choked and sluggish streams, while in the forest areas they are usually permanent streams and pools. Also, the barraging of water for fishing, livestock watering and irrigation, may create more snail habitats. It is believed that schistosome parasites existed in some areas of the Volta even long before the dam was constructed. In 1983, Odei reported that the freshwater lagoons in the Osudoku and Battor areas which were associated with the river Volta, and which were filled up during the flood period, were the main sources of host snails and schistosomiasis transmission in the lower Volta in the pre-Volta dam period. He reported however that, around the same period, that is before Akosombo dam was built across the river Volta in 1964, schistosomiasis was not very common in some communities. This was because conditions prevailing there could not support the establishment of aquatic weeds or snails on the river beds; and that during this period the river Volta did not contain the major transmission foci for schistosomiasis transmission even though a lot of fishing activities took place in it. In Ghana urinary and intestinal types of schistosomiasis are both prevalent. McCullough (1965), referring to the prevalence and distribution of intestinal schistosomiasis in Ghana explained that the disease was confined to Tarkwa and Bogoso in the south-west of the country, and to a few areas in the north-east around Bawku and at Wiaga near Navrongo. He also stated that the disease could be found at Wa in the northwest and at Nyive and Atikpui villages near Ho in the Volta region.

The general prevalence of the disease can be attributed to the following major factors:

(a) creation of suitable habitats for the snail hosts of the schistosome parasites,

(b) human activities that enhance water contact; this includes occupational, recreational and transport

(c) Lack of complete knowledge about the disease among the general public.

(d) Lack of social infrastructure such as latrines and pipe-borne water.

A number of dams that have been established for economic developments such as production of hydroelectricity and irrigation schemes continue to pose health hazards. There have been reports of incidence of schistosomiasis associated with these projects. The Tono irrigation scheme constructed in 1977, in the north-eastern part of Ghana was an exaample of an Agricultural developmental programme which resulted in increasing prevalence rate of schistosomiasis in the Kassena Nankana district (Amankwaa et al, 1994).

They reported that the Tono scheme area represented an area of very high endemicity for both urinary schistosomiasis was already endemic in the area prior to the construction of the scheme, but the prevalence of intestinal schistosomiasis was related to the irrigated agriculture creating suitable habitats for vector snails. McCullough (1965), suggested that habitats of *Biomphalaria* were increasing very fast in Ghana, and that they were man-made. Citing examples of such instances, he pointed out that near Kintampo, in Ghana, *Biomphalaria,* together with other molluscs had been found in a series of burrow-pits bordering a road that was constructed just 10 years ago. Also that, in north-east and north-west of Ghana, *Biomphalaria* have become established in dams and fish-ponds, many of which had been recently constructed.

Odei (1983) explained the factors that led to schistosomiasis outbreak after the construction of both Akosombo and Kpong dams. These dams were established for hydroelectric power. He stated that before the Akosombo dam was constructed, seasonal flooding and scouring of the bed of the river Volta by silt-laden flood water prevented establishment of weeds on the river bed. Also the rate of flow of the river prevented the establishment of host snails in it. In the estuary the influx of seawater did not favour establishment of aquatic weeds or snails. He reported that the establishment of the dam led to the desilting of the water thereby making it transparent and shallow with a slower rate of flow. This enhanced sunlight penetration and therefore the proliferation of submerged and rooted aquatic macrophytes which the snails mostly associate with.

Human activities of the inhabitants of the affected communities with the river increased the spread of the disease. The major occupation of the riparian communities is fishing and clam digging in the river. Some of the inhabitants depend on the river for transport to other villages or communities. Children in such places also enjoy swimming in the water for recreational purpose. The lack of pipe-borne water in such communities makes them depend on the rivers for water for their domestic use. They also lack social infrastructure such as toilet facilities and therefore continue to contaminate and infest the water bodies with parasites through faecal waste materials and urine when deposited near the water bodies. All these increase the rate of water contact and for that matter the danger of getting infected by the schistosome parasites which is found in infested waters. The inhabitants of some affected communities have not yet received enough education on the mode of spread of the disease. In almost all the affected communities along the Volta River, however, a lot of education by both the 'Volta Basin Research Project' (VBRP) and the health sector of the Akosombo hospital has been going on and together with chemotherapy as well as focal mollusciciding, some amount of progress is being achieved with regards to reduction of incidence of the disease.

1.3 Biology of the schistosome parasite

The principal schistosomes that affect humans are of three species, *Schistosoma haematobium*, *Schistosoma mansoni* and *Schistosoma japonicum*. All species have developmental stages in freshwater snail intermediate hosts, from which free-swimming cercariae are shed to penetrate the skin of the definitive human host when in contact with water. In general, fast-running water is not suitable either for the snail intermediate host or for the cercariae. After successful penetration of a person's skin the cercariae transform to schistosomula, which migrate through veins and lymph vessels to the lungs. From there they migrate to the liver, developing into young male and female worms in the portal blood vessels. After 4-6 weeks, mating takes place and the worm-pairs move to their destination, which in urinary schistosomiasis is the vessels of the bladder and, in the intestinal form, those of the intestines. Some eggs, produced by the female worm, work their way through the vessel walls and are shed in urine or faeces, completing the life cycle if they reach water, where another free-swimming form (the miracidium), hatched from the eggs, can encounter the snail intermediate host. Many other eggs are retained in the tissues, where they provoke an inflammatory reaction.

It is this reaction that is responsible for the disease. The mere presence in the bloodstream of the adult schistosome (which remain joined and continue to shed eggs for several years) does not give rise to a pathological response. However, the degree of morbidity and the intensity of infection are determined by the number of adult worms present in the human host and shedding eggs that may be deposited in organ tissue.

1.4 The schistosome intermediate host

The snails which carry schistosome are members of the family *Planorbidae* (in Africa and South America) or *Amnicolidae* (in Asia), and the principal species which act as hosts belong to the following genera in Africa: *Biomphalaria* and *Bulinus* (aquatic snails); in South America: *Biomphalaria* (aquatic); in Asia: *Oncomelania* (amphibious) The bilharzia intermediate host snails occur mostly in permanent water bodies, e.g. rivers, streams, lakes, farm dams, and irrigation canals. Should these dry out, the snails can survive for up to six months by aestivating in the bottom sediments and then emerging when the habitat re-fills. To do this they lower their metabolic rate considerably and, provided the sediments remain aerobic, they survive well. When re-filling occurs, the sediments quickly become anoxic and the aestivating snails resume activity. Being hermaphrodites the snails are capable of storing sperm from earlier copulations, to use it either for cross-fertilisation with a partner or with their own eggs. Both *Bulinus* and *Biomphalaria* have a life span of about $1^1/_2$ years. Under favorable conditions they reach maturity in about 6 weeks but at times they start egg laying as late as in the 4th month of their life. *Bulinus* lay egg clutches appearing as flat oval packets of 5-20 eggs arranged in one layer, glued together with a yellowish jelly. An individual *Bulinus* may lay up to 50 egg clutches during its life. *Biomphalaria* lays on the average 5 similar egg clutches during its lifetime, each containing up to 30 eggs. The snails lay eggs mostly on stems and undersurface of leaves of living plants. They may also lay eggs on decaying plants, on fallen leaves and tree branches. *Bulinus* and *Biomphalaria* are active day and night. They incessantly crawl on the bottom, on plants and on various objects immersed in water, scraping their radula and swallowing the scrapings rich in vegetable and microorganisms and organic debris. They also eat all kinds of rotting wood. The snails are tolerant of water varying widely in its physical and chemical characteristics but the optimal temperature is about 25°C, conductivity about 300µs/cm and pH 6-8. They are not found in water flowing faster than about 0.3m/sec. In their aquatic habitats the host snails are mostly found associated with some particular aquatic macrophytes (Madsen, 1995). Witenberg and Saliternik (1957), reported that in Israel the main host plant of *Bulinus* and *Biomphalaria* was the deciduous *Potamogeton nodusus.* Others include *Ceratophyllum demersum, Nitella translucens, Potamogeton pectinatum, Cynodon dactylon* and *Panicum spp.* They explained that these plants provide shelter, aeration, and sites for oviposition. In a study of aquatic weeds in the Volta lake of Ghana, Paperna (1969) reported that *Ceratophyllum* and to lesser extent, *Pistia* and *Scirpus* supported large populations of *Bulinus truncatus rohlfsi*, and that, the number of snails greatly declined when the weeds disappeared from many sites in the lake. In another study involving lake Kariba, in Zambia, Mungomba et al (1995) reported that the presence of vegetation in general was favorable to the intermediate host snails. They noted that the snails were more abundant in patches with vegetation compared to barren ones. Thomas (1995), suggested that because of the close mutualistic linkages between certain pulmonate snail hosts of schistosomiasis and macrophytes, bioengineering measures aimed at snail control should include those directed at the macrophytes.

1.5 Effects of schistosomiasis

In humans adult *S. haematobium* lives in the veins and venules draining the kidneys and urinary system while *S. mansoni* lives in those draining the intestine. The adult worms do not cause damage to tissues but their eggs. The pathology in schistosomiasis is mainly due to the immunological, and histological reaction of the host's tissues to parasite eggs retained in them. Acute schistosomiasis is non-specific febrile syndrome associated with temporary itching. There is also marked eosinophilia and often hepatomegaly or splenomegaly. In general however, those infected with schistosomiasis pass into a chronic stage without the acute stage being particularly noticed. In urinary schistosomiasis, calcification of eggs deposited in the walls of the ureters and bladder causes localised thickenings which obstruct the flow of urine from the kidneys, a condition called hydro-nephrosis. Other histopathological features of urinary bilharzia are bladder ulcers and polyposis and there is also a link with bladder cancer. There is also a relationship between heavy infections in children and impaired growth, psychological development and performance at school. The pathological consequences of *S. mansoni* infection are enlargement of the liver and spleen, damage to the intestine and hypertension of the abdominal and oesophageal veins. The early phases of egg excretion are associated with general symptoms of weakness, lassitude, giddiness, weight loss and diarrhoea. According to WHO TDR eleventh programme report (1993), anthropological studies indicate that the disease may have significant social impact, such as associated with haematuria in women.

1.6 Control of schistosomiasis

In general, there is no single foolproof method for controlling the disease. There are however different methods that contribute in one way or the other towards reduction of schistosomiasis prevalence. The use of molluscicides and several other biocontrol methods, for instance, helps in killing the snails, thereby reducing the number of potential intermediate hosts, and for that matter the number of infective cercariae.

Chemotherapy will reduce the number of worms present in individuals who are infected. Health education and the provision of pipe-borne water and toilet facilities can also reduce water contact by the people living in communities where the water bodies are located. Morgan (1977), reported that in St. Lucia, when pipe-borne water supplied to five villages in which the disease was highly prevalent, there was reduction of human contact by 82 per cent and a corresponding significant reduction in prevalence and incidence of schistosomiasis. Appleton (1996), pointed out that the current opinion is to aim at reducing morbidity rather than aiming at controlling or eliminating the disease, and this can be achieved through an integrated approach, with all the methods mentioned above being used concurrently. Liese (1986) suggested that the public health services (PHS) arm of a national health system which is generally responsible for epidemiological surveillance as well as implementation of disease control strategies with a preventive rather than therapeutic effect, needs to be strengthened to help reduce morbidity of schistosomiasis. In Ghana, starting from an epidemiological research programme in 1971-78, the control strategy included focal mollusciciding, provision of community water supplies and health education, and selective population chemotherapy using metrifonate.

1.6.1 Chemotherapy

The development of new drugs for effective chemotherapy has been one of the remarkable achievements towards the reduction of morbidity of schistosomiasis. Metrifonate was introduced in the early 1960s and is still widely used, although the required 3-dose treatment leads to logistical problems and difficulties of compliance for the complete course of treatment, (Liese, 1986). He stated that hycanthone, introduced in 1965, was the first single dose drug, but has since been superceded by oxamnique (in 1973) and praziquantel (in 1977). Both these drugs provide single oral dose chemotherapy with high rate of cure.

It must be pointed out however, that, metrifonate though cheap, is ineffective against *Schistosoma mansoni,* and in areas endemic for onchocerciasis, it can induce the violent Mazzoti-type reaction which reduces compliance with the therapy. According to WHO (1993) report, treatment with praziquantel resulted in egg clearance of 60-90% in infected people, and in a reduction in egg loads of more than 95% among those who remained with patent infection after treatment.

In China, treatment with praziquantel at a dosage of 60mg/kg, divided into three doses (over 1 day) showed a 97.7% success in treatment (Fu et al, 1988). They reported that the side-effects produced by praziquantel treatment were mild and transient in most cases, and depended on the dosage of drug given. In their research work, out of 98% of patients who completed the regimes prescribed, 25-50% were free from any adverse effects. Side-effects mainly involved nervous, digestive and cardiovascular systems. 24.1-49.1 complained of dizziness, 3.5-27.3%, of headache, and 12.5-23.7%, of fatigue. Insomnia, muscular tremor, sweating, numbness in limbs and blurred vision were also noted in a few cases. In a WHO (1989) report, a field trial of schistosomiasis in Cote d'Ivoire, using praziquantel brought about the temporary cessation of *S. haematobium* transmission, although *S. mansoni* proved more difficult to control.

In spite of the tremendous successes in the use of praziquantel there has been a report of an unexpected failure in the use of the drug, in the findings of a Senegalese-Dutch study (WHO (1992)).

The study which was conducted in a village near Richard Toll, a town on the Senegal River, following an outbreak of schistosomiasis in the region, showed that only 53 of the 298 (18 percent) showed cure and there were complaints of severe side-effects. According to the report, the head of the research team, Belgian epidemiologist Bruno Gryssels, believed that this unusual result was not due to schistosome resistance to the drug, but attributed it to an extremely rapid reinfection. The main limitation on the use of praziquantel for control is the high re-infection rate in endemic areas, even after mass treatment, as well as the high cost of the drug (Gryssels and Polderman, 1991). They therefore recommended that repeated treatment be given where necessary.

1.6.2 Snail host control

Snail control is one of the more feasible means of interrupting parasite transmission in most areas (Ndamba et al, 1995). Until about 1970, snail host control (mainly by mollusciciding) was the primary procedure for schistosomiasis control (WHO, 1984 report). Snail control may be achieved principally through chemical, environmental or biological means (Madsen, 1990, 1992), (Ndamba et al, 1995). In areas of intense transmission where chemotherapy is being applied, it is usually describe desirable that snail control activities be coordinated with the treatment regime (WHO report, 1984). According to WHO (1993) report on Tropical Diseases Research, snail control has limited transmission of schistosomiasis in some places , notably in China, but failed to do so everywhere partly because it is expensive and difficult to sustain.

1.6.2.1 Environmental control of the snail host

It has been reported that the environment in which the schistosome intermediate host snails live can be altered in one way or the other to affect their continuous survival, thereby helping to control these snails (Madsen, 1997; Jordan & Webbe, 1982). These methods of environmental manipulation include increasing the current speed, fluctuating water level in canals or reservoirs (including drying them out completely), possible reconstruction and re-dimensioning of canals (cement-lining), and removal of aquatic plants (Madsen, 1997). By application of engineering measures, the speed of water currents can be increased to exceed 0.3m/s, which is normally the limit at which the snails can cope up with, to make the medium unsuitable for their survival. A possible setback of this method may be erosion of canal banks. In small canals it may be possible to fluctuate water level so that for some period, the snails will face dessication and die. Aquatic plants may also be removed by this method. This may however work for only situations where the snails are found primarily along the banks. In situations where submerged aquatic plants are found at great distances from the shore, water level fluctuations may not significantly affect snail populations, since they will still be covered by some amount of water at all times.

Aquatic macrophytes provide surfaces for snails to graze on and on which to deposit their egg masses. They also protect the snails against high current speeds as well as predators. Removal of aquatic plants will therefore affect snails population. Limitation of use of this method may be a possible erosion of canal banks. Concrete lining of canals would help in increasing the current speed within the canals.

1.6.2.2 Biological control

Although the use of molluscicides remains the most effective way of controlling the schistosome intermediate host snails (McCullough, 1986), the high cost involved in its use as well as its non-specificity to targets (WHO,1984), thus killing fish and other aquatic organisms, together with its impact on the environment (Andrews, Thyssen and Lorke, 1983) suggest that the use of biological control will be a very useful alternative to control the snail hosts. Biological control employs the use of living organisms, whether introduced or otherwise manipulated to reduce the density of the target species (Madsen, 1995). These organisms may be predators, parasites, pathogens or competitors. In its broadest sense, the use of pheromones, genetic manipulation and fertility control may also be considered as biological control. Several hundred species, ranging from fish to fungi have been considered as potential competitors or predators (including parasites and pathogens), but their efficiency has rarely been tested outside laboratory model systems (McCullough, 1981). Madsen pointed out that it is highly unlikely that one method of biocontrol can be used to control the schistosome intermediate host snails, under all conditions. He therefore suggested that all the methods should be tested and sometimes combining some of the methods if possible. Biological control demands regular inspection of sites, as well as mass production and introduction of the control agents. As a result of this, it is mostly viewed as labour intensive.

1.6.2.2.1 Microbial pathogens, predators and parasites

Pathogens are microorganisms that often kill their host with subsequent liberations of millions of individual microbes (Madsen, 1995). In a WHO (1984) report, invading pathogens may cause atrophy of organs, aplasia, necrosis and tissue liquification, alterations in tissue enzymes and haemolymph components, metaplasia, hyperplasia and neoplasia. Physiological changes in fecundity, growth, locomotion and feeding behavior may also occur in snails infected with particular pathogens. A number of microbial pathogens from freshwater snails have been described. This includes fungal organisms such as *Catenaria sp.,* which invades and destroys egg masses of *Biomphalaria glabrata;* protozoa such as *Glaucoma paeedopthora* which infects eggs and kills the embryo of *Biomphalaria sp.* and *Bulinus (Physopsis) sp., Hartmanella quadriparia* which affects reproduction and survival during aestivation of *Biomphalaria* and *Bulinus sp.*; and bacteria such as *Bacillus pinotti* destroys *Biomphalaria glabrata*. McCullough (1981) explained that virtually all the mentioned studies were laboratory-based, and no successful field trials had yet been achieved. According to WHO (1984) report, the number of microbial pathogens described from freshwater snails is relatively small, due to limited interest in this field.

Predators of freshwater snails are identified virtually in every major group of the animal kingdom (Michelson, 1957). Madsen (1995), however stated that most of these predators are polyphagous, and pointed out that specific snail predators comprise certain cichlid species larvae of the sciomyzidae species and certain species of leeches. McMahon et al (1977) reported that the fish *Astatoreochromis alluadi* had a significant long term effect on snail populations in artificial dams in Kenya. Other malacophagous fish species include *Serranochromis sp.*, *Tilapia melanopleura* and *Clarias sp.* (McCullough, 1981). Appleton (1996) also cited examples of predators as fish, notably some barbels and cichlids, insects such as the larvae of the marsh flies (family Sciomyzidae), the Giant waterbugs (family Belostomitidae) and leeches of the family Glossiphiniidae, but pointed out that although they have all been tested, none has been found suitable for field trials. McCullough (1981) expressed that almost all observations on fish/snail host interactions have been carried out in East and Central Africa, but none could be accepted as conclusive, thus the need for much more detailed investigations.

The most prominent parasites of freshwater snails are trematodes (Madsen, 1995). Trematode infections may decrease the rate of replacement of snail populations by approximately 10-20% (Brown et al, 1988). It has been reported (WHO, 1984), that *Angiostrongylus cantonensis*, a rat nematode has been proposed as a biological control agent, but unfortunately it also causes serious human disease in the Pacific region and as such its use has not been recommended. According to McCullough (1981), *Ribeiroia marini guadeloupensis*, a trematode capable of sterilizing the snail hosts (*Biomphalaria glabrata)* is one trematode parasite that has been studied in detail by French scientists in Guadeloupe. He mentioned that due to target specificity as well as it being competitively economical, the method was attractive and deserves to be explored. In comparing the efficiency of *Ribeiroia guadeloupensis* and *Malignities tuberculate* (competitor) as control agents for biocontrol, Pointier (1989) found out that the trematode was more efficient in the short term.

1.6.2.2.2 **Inter –molluscan competition**

Competitors are organisms which affect the abundance of the target species through competition for shared resources (Madsen, 1995). McCullough (1981) stated that the attraction of the method for snails control is based on the principle of competitive exclusion/displacement, whereby it is believed that if two species are sufficiently similar in their biological profile, then one (the stronger and hopefully, the introduced species) will eliminate the other weaker (the target species or control its population size). The use of competitors has been considered as the most promising canditates for the biological control of the pulmonate intermediate hosts (Madsen, 1990), (Appleton, 1996). According to Appleton (1996), WHO (1984), considerable success has been achieved, particularly with three South American ampullariids (*Marisa cornuarietis, Pumice glace,* and *P. paludosa)* and two thiariids (*Malignities tuberculate* and the Asian *Tarebia* *granifera).* Other pulmonate snails that have been used in biological control include *Helisoma duryi* , WHO (1984), McCullough (1981), (Madsen, 1992) and *Physa spp.* (McCullough, 1981). Results of a test performed Pointier et al (1989) showed that there was a rapid colonization of *Thiara tuberculate* a competitor snail, when it was introduced into two groups of water-cress beds containing *Biomphalaria glabrata;* the populations of the competitor snails increased appreciably whilst populations of *B. glabrata* declined considerably, when sampling was done after 1 year. Approximately a year later (i.e. approximately 2 years after starting the test), *B. glabrata* had totally disappeared from samples taken. They explained that a similar result was obtained when *B. straminea* was used in place of *B. glabrata,* and following this success programme, *T. tuberculate* was introduced into all other water-cress cultures in Martinique. According to WHO (1984) report, results of medical surveys carried out recently in the Island of Martinique, have all shown that transmission of intestinal schistosomiasis has been totally interrupted, and explained that the situation correlated with replacement of *B. glabrata* by *B. straminea.* Pointier and McCullough (1989) stated that, there was no evidence that *Thiara* snails cause any adverse environmental impact. If more research studies are done on the use of competitor snails for the control of schistosome intermediate host snails, it may be very good substitute to the use of molluscicides, which though very effective has it's own limitations.

1.6.2.3 The use of molluscicides

In general there are two forms of molluscicides, namely synthetic molluscicides, and plant molluscicides. However, Appleton (1995) stated that although many plant molluscicides have been screened, only one, *Phytolacca dodecandra* or Endod has been adequately tested, although it has not even been registered for use in its country of origin

This implies that most of the molluscicides that have been used successfully in schistosomiasis control programmes are the synthetic type. Not until 1960, two chemicals were widely used as molluscicides. These were NaPCP and CuSO4. NaPCP was particularly used in Brazil and Japan, whilst CuSO4 was particularly used in Africa. These chemicals however had their defects. NaPCP was unstable in the presence of sunlight and also posed immense health hazards to spray men. The use of CuSO4 required that the vegetation of the area be cleared before it became effective, and also could not destroy snail eggs. In 1960, the first specially formulated molluscicide, Bayluscide (Bayer 73) was produced. At present it is the only molluscicide which is commercially available (Madsen, 1992). Bayluscide is the ethanoalamine salt of niclosamide, and with specific gravity of 1, which is the same as water, it disperses readily when sprayed. Apart from the snails it is also toxic to their eggs and the miracidium and cercariae of schistosome. The chemical has been used in many control programmes. Webbe (1964) reported that Bayluscide application to the Mirongo River produced a very satisfactory degree of control of *S. mansoni* transmission. Also, in the Rahid irrigation scheme, Sudan, the use of the chemical in a focal mollusciciding achieved a great success in control. Madsen (1992) pointed out that the effectiveness in the use of molluscicides depends on water velocity, presence of submerged or floating aquatic macrophytes, and permanence of habitats. There are thus different mollusciciding strategies. In flowing watercourses such as natural streams, canals and drains in irrigation schemes the recommended methods include spraying, partial treatment, controlled spillage, focal/contact point treatment, dam-and-flush treatment, and drip-feed dispensing. For lentic water bodies such as farm storage dams and pools in rivers, total volume treatment, focal/contact point treatment and slow-release formulations can be used. As a result of the high cost of Bayluscide (i.e. both product and operational cost) most developing countries in which the disease needs to be controlled cannot afford the cost of its use. It is highly desirable that extensive research be carried out to rely on production of plant molluscicides which may be of a lower cost. Alternatively, new methods involving the judicious use, that is, smaller quantities of Bayluscide with maximum results must be developed. Currently, much emphasis is on the possibility of using bioactive substances that can act as attractants, arrestants, or phagostimulants in snail control (Thomas and Assefa (1979), Kpikpi and Thomas (1986), Kpikpi et al (1995), Kpikpi and Thomas (1992, Thomas et al (1985). It is envisaged that a toxicant can be incorporated into these attractants and arrestants to form a slow release system, which essentially will make use of smaller quantities of Bayluscide as compared to using Bayluscide alone.

1.7 The objectives of the present study:

The present study was undertaken with the following objectives: a) designing an effective schistosome host snail trapping unit with sugarcane,

b) performing simulated natural environment experiments to determine the efficacy of some identified bioactive substances namely, fermented cocoyam (fermented *Xanthosoma muffafa)*, fermented cassava (fermented *Manihot esculenta)*, and fermented sweet potato (fermented *Ipomoea batatas)*,

c) finding out the efficacy of a combination of bioactive material and a toxicant (bayluscide) in simulated natural environment tests, for snails control, and

d) field trials of (i) best trapping unit identified (ii) most potent bioactive substance, and (iii) combination of bioactive material and toxicant.

CHAPTER TWO

DESIGNING AN EFFECTIVE SUGARCANE TRAPPING UNIT

2.1 Introduction

Chemoreception studies involving *Biomphalaria glabrata* (Thomas et al, 1979), *Bulinus rohlfsi* (Thomas et al, 1985), *Bulinus (Physopsis) globosus (Morelet)* and *Bulinus rohlfsi (Clessin)* (Kpikpi, 1991), suggest that schistosome host snails, like other gastropod molluscs are sensitive to chemicals. This property enables them to detect other organisms or targets in the aquatic medium in which they live. Bioassay tests using diffusion olfactometers, (Kpikpi and Ansa, 1994), (Dogbey, 1995), and (Domeh, 1998), showed that some naturally occurring materials, in their raw or processed forms, were capable of attracting schistosome host snails. They showed that some of these substances actually have properties which seem to keep the snails attached to them for a considerable period (that is, arrested). These bioactive substances were categorised as attractants, arrestants and phagostimulants. Attractants are substances which cause snails to spend significantly more time on their sides as compared with their controls. Those which make the snails to spend significantly more time on them, as compared with controls are deemed to have an arrestant effect (Dogbey, 1995). Phagostimulants are those substances which stimulate feeding behaviour. It has been suggested that these bioactive substances with significantly high attractant and arrestant indices for the schistosome host snails can play a major role in selective removal of the schistosome host snails from an infested aquatic body. These substances can be presented in an aquatic medium in the form of traps to attract or arrest the snails.

Sugarcane *(Saccharum officinarum)*, among other bioactive substances tested emerged as the most potent bioactive substance. When presented as chunks (whole) in bamboo cylinders, it attracted more snails (both *Bulinus truncatus* and *Biomphalaria pfeifferi*) than controls in tests conducted in simulated natural environment tests (Dogbey,1995).

The main objective of the present study was to design a more effective trapping unit with sugarcane (*Saccharum officinarum*) as the bioactive factor. The different forms of presentation in an aquarium in which the tests were conducted were:

(i) sugarcane in single units (ii)sugarcane in grids (designed with poles support) (iii)sugarcane in grids (designed with calabash) (iv) sugarcane peels, woven together to form a mat.

All the experiments were performed under simulated natural environment using *Bulinus truncatus* snails. The details of the findings are discussed in this chapter.

2.2 MATERIALS AND METHODS

2.2.1 Snails breeding

Bulinus truncatus snails used were obtained from Weija lake. They were bred in separate glass tanks filled with tap water and kept in the laboratory where they received approximately 12 hours of artificial light and darkness. The average temperature of the water medium was 26±1°C. The snails were fed regularly with fresh lettuce leaves. The water was changed weekly and replaced with fresh water. Within a period of 1 month, 1500 healthy snails (both juveniles and adults) could be obtained for the test.

2.2.2 Preparation of aquarium

The aquarium, which was situated outside the laboratory where direct sunlight could be obtained during daytime was constructed with concrete. It has a cylindrical shape, with a diameter of 130 cm and height of 77 cm, giving a total volume of 2.01 x 10^6cm^3. The bottom of the aquarium was filled with soil obtained from the beach of Weija lake to a height of 15 cm. It was filled with tap water to about 2/3 full (0.67x10^6 cm^3) and then aquatic plants (*Ceratophyllum demersum*) added to cover about 2/3 of the total surface. *Ceratophyllum* affords the snails with shelter and points of attachment. The system was allowed to generate sufficient oxygen through photosynthesis to aerate the water medium.

Plate 2a; Aquarium, situated outside the laboratory in the open

2.2.3 **Preparation of traps**

2.2.3.1 TRAP 1 SINGLE UNITS OF SUGARCANE

This trap was designed using single units of sugarcane and calabash. The test material was prepared by first peeling off the sugarcane and then cutting it into smaller pieces at the nodes of the sugarcane to obtain the single units. A total of 20 calabashes of similar shapes and sizes were obtained. The sides of the calabashes were cut to create four small windows. This was to allow diffusion of the chemical components of the test material which was fixed on a stick and firmly placed in the calabash, into the water medium. Each of these calabashes was then fastened to a pole by strings to act as a support and to enable the test material be placed at a desired depth in the water.

2.2.3.2 TRAP 2 SUGARCANE PEELS MAT TRAP

The sugarcane was first cut into pieces at the nodes, and then carefully peeled. The peels were used to prepare the trap.The peels were woven together by string to form a mat (Plate 2b(i)). The ends of each peel mat were then fastened to a pole by means of string (Plate 2b(ii)). This arrangement allowed the test materials and their controls to be well positioned below the surface of the water. In a similar manner, controls were prepared by using polystyrene.

2.2.3.3 TRAP 3 SUGARCANE GRID WITH POLES

The grid is a combination of several units of sugarcane pieces. This was prepared by cutting the sugarcane at the adjacent joints to obtain pieces, measuring 13 cm in

(i) (ii)

Plate 2b (i) Sugarcane peels mat Trap;
(ii) Sugarcane peels mat Trap fastened to wooden pole supports

length. The sugarcane pieces were then peeled, after which each piece was split into two halves. Four of the half pieces were stuck together by pushing a needle shaped piece of wood through them to form a grid (Plate 2c). The grid measured 18 cm in length. 10 grids were made and the ends of each grid were fastened to wooden supports, shown in Plate 2b(ii), by strings. This was to help position the test materials in the aquarium. The control traps were made of polystyrene, and were also placed in the aquarium in the same manner.

Plate 2c; Sugarcane grid

2.2.3.4 TRAP 4 SUGARCANE GRID WITH CALABASH

The sugarcane grid was prepared using the same method described in Trap 2. However, instead of using poles as support, the whole grid was placed inside a calabash which had windows created on the sides. A string was then tied at two ends at the mouth of the calabash so that the calabashes can be hung on a wooden bar placed across the mouth of the aquarium (Plate 2d).

Plate 2d; The Calabash Traps

2.2.4 THE SIMULATED NATURAL CONDITIONS EXPERIMENTS

All the experiments were conducted in the aquarium in the open (Plate 2a). At the start of the experiment, 1000 healthy snails of *Bulinus truncatus* were released into the aquarium and a period of 7 days allowed for them to acclimatize to the aquarium condition, before the tests were performed.

The experiments were performed for a duration of 3 days and 3 nights (i.e. 72 hours). Readings were taken every 12 hours from the start of experiments. All the test materials and controls were placed in the aquarium, completely submerged below the water surface. For traps that had poles as support, the lower ends of the poles were pushed into the aquarium, while for the traps without poles, they were hung from wooden bars across the mouth of the aquarium. In order to obtain readings from the experiments, the traps were removed one after another and inspected for snails. These were counted and their numbers recorded. In each experiment 10 experimental traps and 10 control traps were used. The temperature of the aquarium, water conductivity, dissolved oxygen concentration, and the pH of the water were all monitored throughout the experiments.

Paired t-tests (Bailey, 1981) was used to determine whether there were significant differences in number of snails trapped in controls. The trapping index, which refers to the difference in number of snails trapped on test and snails trapped on controls gave an idea of the effectiveness of the different designs of traps.

Plate 2e; (i) A set up for S.N.C. experiments. Aquarium containing some calabash Traps

Plate 2e; (ii) Aquarium containing sugarcane grid with poles Trap

2.3 RESULTS

2.3.1 **Efficacy of the traps**

Four different designs of trapping units were used in different experiments. In Traps 1 and 4, which involved calabashes in their designs the number of snails found in the pots as well as those attaching to the outside part of the calabashes were considered trapped. In Traps 2 and 3, involving the use of poles, the numbers of snails that were found attached to the test materials were the ones considered trapped. In addition the snails that were attached to the pole supports and were found in the vicinity of the test material were considered trapped. This is because they were believed to within the concentration gradient. The results of all the experiments have been shown in Table 2.1.

In Trap 1, the largest number of snails trapped was after 60 hours, when 40 snails were counted in the test trap. The least number of snails trapped was 18, observed after 24 hours. After 60 hours however, the number of snails found in test trap reduced considerably to 28 snails.

In sugarcane peels mat trap (i.e. Trap 2) the largest number of snails caught in the test traps was 30, occurring after 24 hours and 36 hours after which the number declined till the least (13 snails), was observed after 72 hours.

In Trap 3 (sugarcane trap, with poles) the number of snails caught in the test trap increased from 32 snails to 36 snails between 12 hours and 24 hours. However, the number started declining between 36 hours and 72 hours, till the least number recorded (15 snails) was reached between 60 hours and 72 hours.

The sugarcane grid trap, (with pots) which seemed to perform best, trapped 81 snails after 72 hours. It revealed somehow a fluctuating trend, in that there was a rise in number of snails between 12 hours and 24 hours, a fall after 36 hours then an increase after 48 hours, a decrease after 60 hours, and finally an increase after 72 hours.

The efficacy of each trapping unit therefore varied within the test period. In Table 2.3, a statistical analysis of the results using t-test to compare test and control traps confirms the significant differences of the traps' performance for the different periods.

2.3.2 Efficiency of the different designs of traps

To compare the efficiency of the different trapping designs the total number of snails trapped by test traps over the 3-day period, for each design was worked out. In a similar manner, the total number of snails trapped on control traps was also calculated. The difference between results of tests and controls, as shown in Table 2.1, gives the trapping indices for different trap designs. These values are considered as indicators of efficiency of the traps. Trap D (i.e. sugarcane grid trap, with pot) recorded trapping index of 135 snails which was considered as the highest. Trap A (i.e. single units of sugarcane trap), recorded a trapping index of 66, which was the least among the four trap designs. Trap B (sugarcane peels mat trap) had trapping index of 84 while Trap C recorded 96. The results can be arranged as follows:

Sugarcane grid trap(with pot) > sugarcane grid trap(with poles) > sugarcane peels mat trap > single units of sugarcane trap.

2.3.3 Aquarium conditions

During the experimental periods, an average temperature of 27.4°C was recorded in the mornings, 30.4°C in the afternoons and 32°C in the evenings. Using a Water

Quality Checker (model U-10), an average water conductivity value of 102 μSm/cm^2 was measured. Average dissolved oxygen was 2.68 mgl^{-1}, whilst average salinity measured was 0.01%. The aquarium water had an average pH of 8.5.

Table 2.1 **NUMBER OF SNAILS TRAPPED IN SIMULATED NATURAL ENVIRONMENT EXPERIMENTS FOR DIFFERENT TRAP DESIGNS**

(1) SUGARCANE (Single units) TRAP

TIME (Hours)	**12**	**24**	**36**	**48**	**60**	**72**
TEST TRAPS	19	18	21	28	40	28
CONTROLS	15	17	6	19	16	15
TRAPPING INDEX	4	1	15	9	24	13

(2) SUGARCANE PEEL MATS

TIME (Hours)	**12**	**24**	**36**	**48**	**60**	**72**
TEST TRAPS	24	30	30	29	19	13
CONTROLS	12	11	4	4	10	10
TRAPPING INDEX	12	19	26	25	9	3

(3) SUGARCANE GRID TRAP (WITH POLES)

TIME (Hours)	**12**	**24**	**36**	**48**	**60**	**72**
TEST TRAPS	32	36	30	29	19	13
CONTROLS	12	17	24	1	2	2
TRAPPING INDEX	20	19	6	28	17	11

(4) SUGARCANE GRID TRAP (IN POTS)

TIME (Hours)	**12**	**24**	**36**	**48**	**60**	**72**
TEST TRAPS	49	51	35	69	23	81
CONTROLS	29	27	16	48	13	40
TRAPPING INDEX	20	24	19	21	10	41

Table 2.2 COMPARING A TRAP DESIGN OVER A 3-DAY PERIOD (pooled)

TEST TRAPS	SINGLE UNITS	PEEL MATS	GRID WITH POLES	GRID WITH POTS
	154	145	154	308
CONTROLS	88	61	58	173
TRAPPING INDEX	66	84	96	135
SIGNIFICANCE LEVEL	**	*	-	***

* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$

Table 2.3 TRAPPING INDICES OF THE DIFFERENT TRAP DESIGNS OF SUGARCANE OVER 72 HOURS

TIME (Hours)	SINGLE UNITS				SUGARCANE PEEL MATS				GRID TRAP (WITH POLES)				GRID TRAP (IN POTS)			
	T	C	(T.I)	(L.S)	T	C	(T.I)	(L.S)	T	C	(T.I)	(L.S)	T	C	(T.I)	(L.S)
12	19	15	4	-	24	12	12	-	32	12	20	***	49	29	20	*
24	18	17	1	-	30	11	19	**	36	17	19	**	51	27	24	*
36	21	6	15	***	30	4	26	***	35	24	11	**	35	16	19	*
48	28	19	9	-	29	4	25	*	21	1	20	*	69	48	21	-
60	40	16	24	*	19	10	9	*	15	2	13	**	23	13	20	*
72	28	15	13	-	13	10	3	-	15	2	13	**	81	40	41	-

T = NUMBER OF SNAILS TRAPPED BY TEST MATERIALS,

C = NUMBER OF SNAILS TRAPPED BY CONTROLS,

(T.I) = TRAPPING INDEX = T-C,

(L.S) = LEVEL OF SIGNIFICANCE, (* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$).

Table 2.4 TRAPPING UNITS AND THEIR SUPPORTS

DTN (hours)	Trapping Unit 1				Trapping Unit 2				Trapping Unit 3				Trapping Unit 4			
	T	C	Ts	Cs	T	C	Ts	Cs	T	C	Ts	Cs	T	C	Ts	Cs
12	19	15	5	6	24	12	24	32	32	12	18	14	49	29	0	0
24	18	17	5	7	30	11	12	26	36	17	19	28	51	27	0	0
36	21	6	8	6	30	4	27	30	35	24	10	9	35	16	0	0
48	28	19	5	10	29	14	20	23	21	1	13	2	69	48	0	0
60	40	16	6	8	19	10	40	28	15	2	4	2	23	13	0	0
72	28	15	4	8	13	10	24	10	15	2	4	2	81	40	0	0
Total Number of snails trapped in 3 days	154	88	33	45	145	61	147	149	154	58	68	57	308	173	0	0

Trapping unit 1 = Single units of sugarcane trap,

Trapping unit 2 =Sugarcane peel mats,

Trapping unit 3 =Sugarcane grid (with poles) trap,

Trapping unit 4 =Sugarcane grid (with pots) trap,

T = number of snails trapped on test trap; Ts = number of snails on supports of test traps;

C =number of snails trapped on controls, Cs = number of snails on supports of controls:

DTN = Duration.

Table 2.5 A comparison of trapping indices (Domeh, 1998 & the present studies)

Total number of snails caught in 10 traps	Domeh (1998) (transparent plastic bottle traps)	Present studies			
		T1	T2	T3	T4
Test traps	150	154	145	154	308
Control traps	38	88	61	58	173
Trapping Index	112	66	84	96	135
Significance Level	***	**	*	-	***

* = P < 0.05; ** = P < 0.01; *** = P < 0.001

T1 = Single units

T2 = Peel mats

T3 = grid with poles

T4= grid with pots

FIGURE 2a; EFFICACY OF SUGARCANE (SINGLE UNITS) UNDER SIMULATED NATURAL ENVIRONMENT

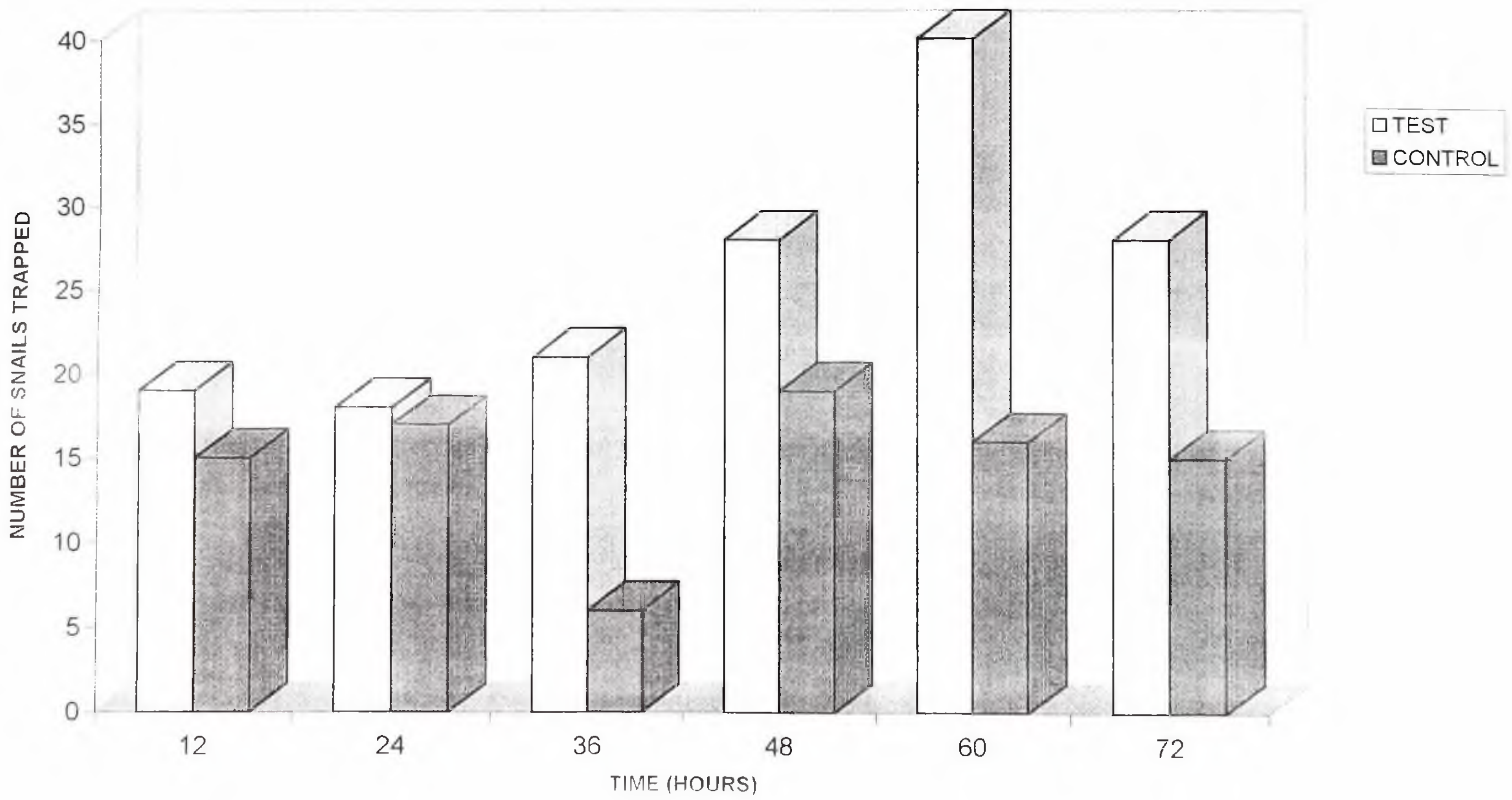


FIGURE 2b; EFFICACY OF SUGARCANE PEEL MATS UNDER SIMULATED NATURAL ENVIRONMENT

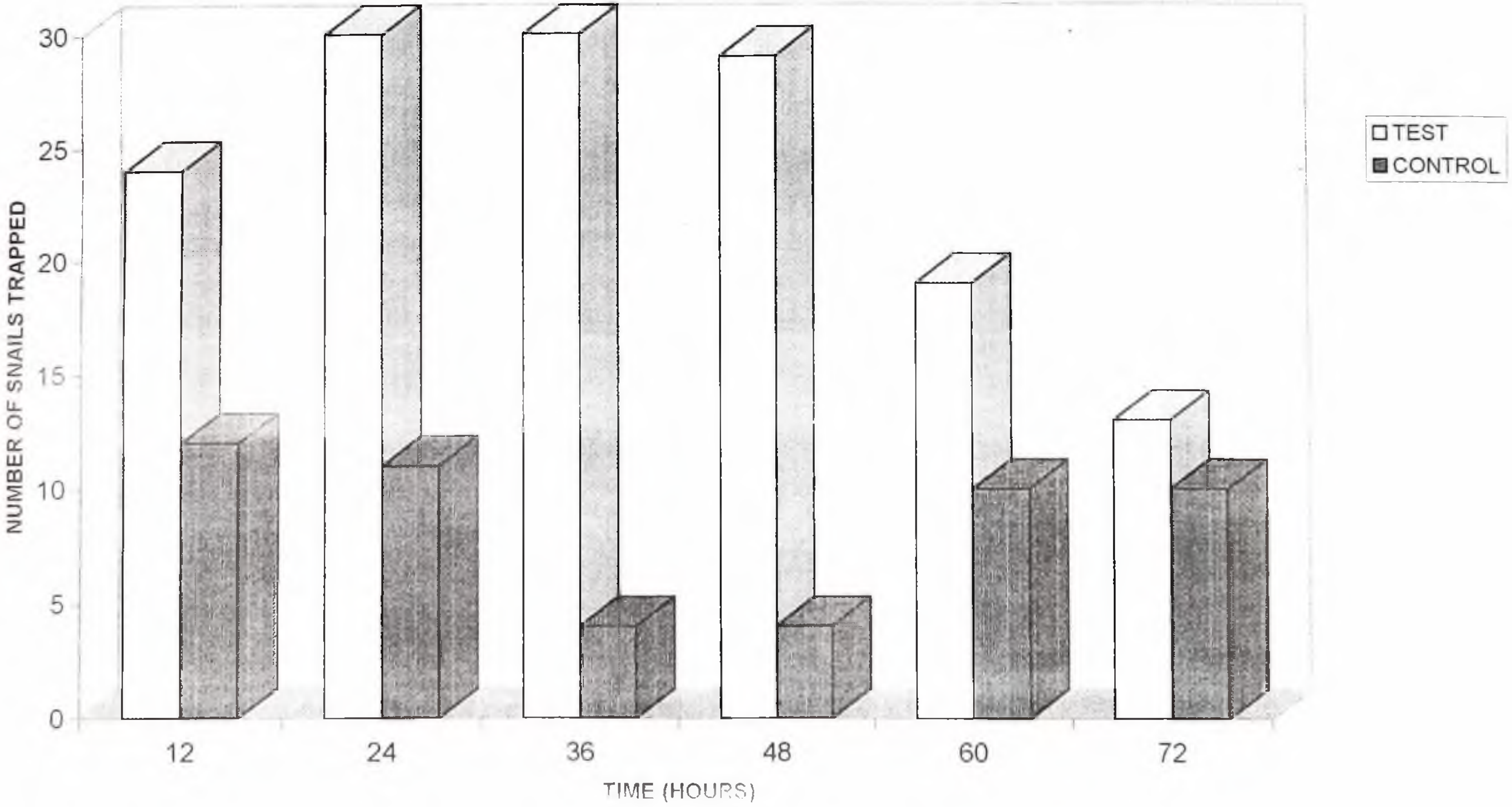


FIGURE 2c; EFFICACY OF SUGARCANE GRID TRAP UNDER SIMULATED NATURAL ENVIRONMENT

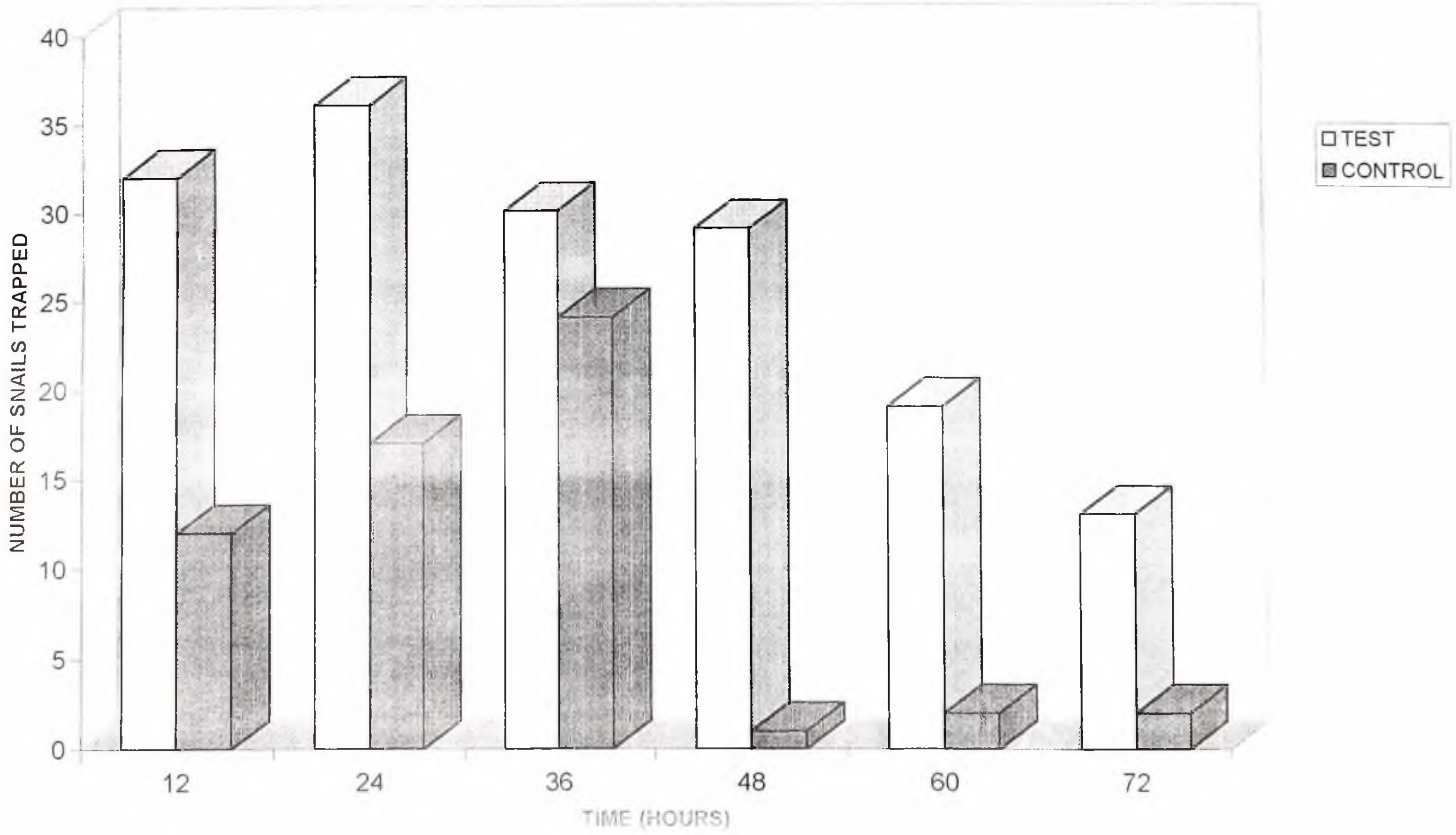


FIGURE 2d; EFFICACY OF SUGARCANE GRID IN POTS UNDER SIMULATED NATURAL ENVIRONMENT

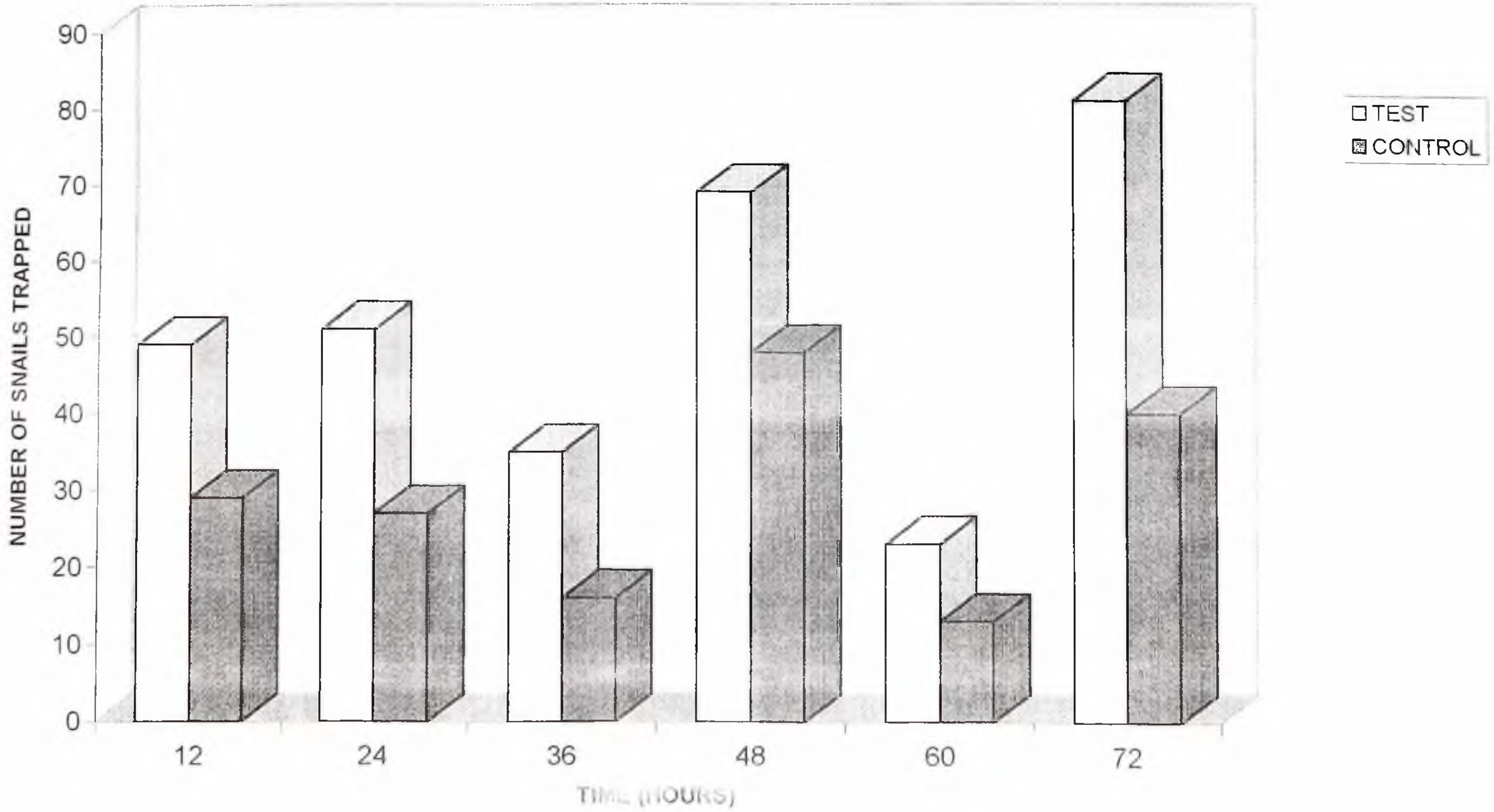


FIGURE 2e; A COMPARISON OF TRAPPING INDICES (DOMEH, 1998; & PRESENT STUDIES)

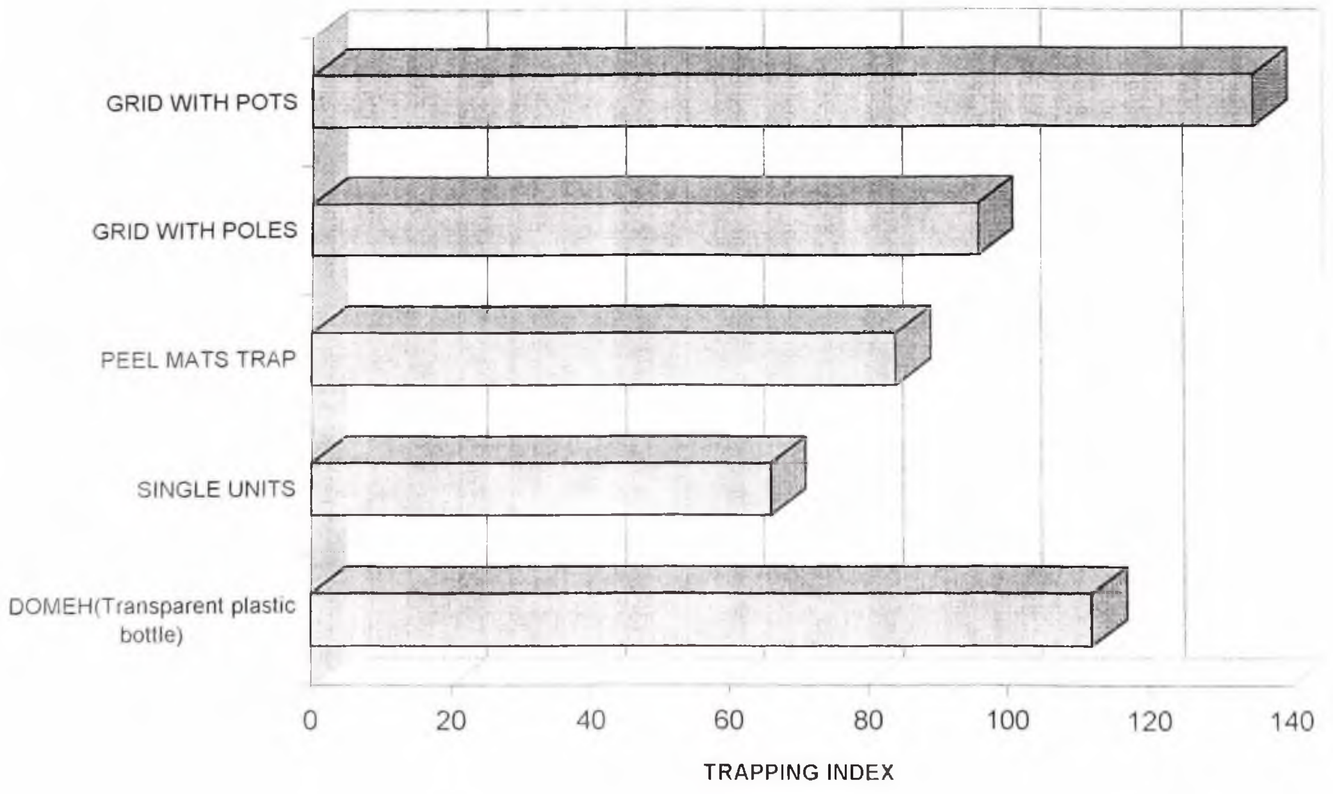
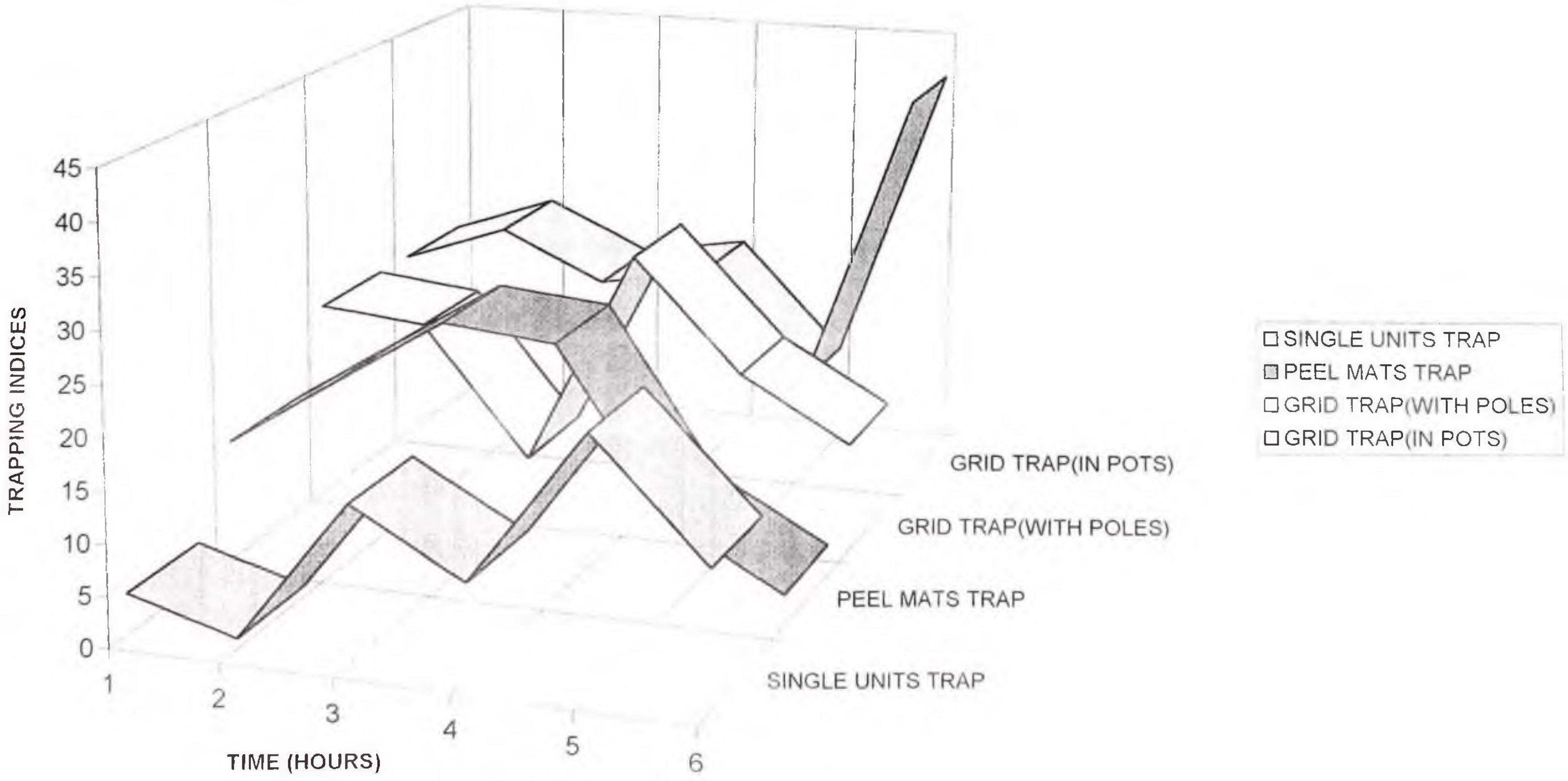


FIGURE 2f; A COMPARISON OF TRAPPING INDICES FOR DIFFERENT TRAP DESIGNS



2.4 DISCUSSION

Results that have been obtained from the present studies showed that significantly more snails were caught in the test traps than the control traps ($P < 0.05$). These results are similar to those recorded by Dogbey (1995) and Domeh (1998) in similar studies, when some naturally occuring bioactive materials were combined with traps and tested under simulated natural environmental conditions. Different trap designs were used by these researchers. Dogbey (1995) used the bamboo trap in his studies. This trap was designed and first used by Kpikpi (1990). The traps that were used by Domeh (1998) consisted of transparent plastic spring water bottle (ASTEK FNSU bottles) with holes created on the sides.

The results from the studies suggest that snail trapping can now be considered as a feasible tool both for research, for example snail sampling and control. Researchers who are interested in obtaining large samples of the schistosome host snails from infested water bodies can depend on the use of snail traps.

The effectiveness of sugarcane in snail trapping as observed from the present studies conforms with observations recorded in previous studies (Dogbey, 1995; Domeh, 1998). They both discovered that sugarcane had the best performance in terms of attracting/ arresting the schistosome host snail, *Bulinus truncatus* to the test trap than the other naturally occurring bioactive materials tested under simulated natural environmental conditions. The difference in the number of snails caught in test traps as compared with those in control traps is referred to as the trapping index. The trapping index gives an idea of the effectiveness of the trap. The higher the trapping index value, the more effective the trap is considered

In a comparison of trapping indices in the present studies with similar studies by Domeh (1998), shown in Table 2.5, there is an indication that one of the four different trap designs used in the present studies, the grid with pots, recorded a higher trapping index (135) than the design used by Domeh (1998) which had a trapping index of 112. The other 3 trap designs however recorded lower trapping indices (i.e. 66, 84, and 96 respectively) than Domeh's design.

The implication in this observation is that at least one of the trap designs in the present studies has improved the trapping index, and can be regarded as a better trapping unit.

Results shown in Table 2.2 & Figure 2e show that the 'grid with pots' trap design gave the highest trapping index and can therefore be regarded as the most effective trap design in the present studies. The high performance of this design may be attributed to the presence of the pot which provides an enclosure to keep the diffusion gradient that was set up by the sugarcane, and to prevent it from dispersing easily. The snails that were trapped could also be prevented from moving away. Kpikpi (1990) explained that the bioactive natural products established a diffusion gradient in the water medium with the test factor as a source. He pointed out that it is along this gradient that the snails are attracted to move towards the test material. In the other traps there were no enclosures around the test materials, and probably the diffusion gradient created might have dispersed so quickly. The snails that were attracted or arrested could also have moved away easily, especially since there was no barrier to prevent them from moving away from the test material. The grid with pots trap design is the only one which caught the highest number of snails during the 72-hour period (Figure 2f), which suggests that the diffusion gradient persisted for a longer period. The effectiveness of the other trap designs can probably be increased by forming a barrier or enclosure around them. The trap design should however not be completely covered so that the molecules of the test material can diffuse and spread in the water medium.

Sugarcane and the pots which form the basic components of the most effective trap design can both be obtained locally, and at any time of the year in Ghana. They are sold in almost every market centre in Ghana. In terms of cost they are relatively not expensive, and can easily be afforded. The assembling of the traps does not take so much time. Approximately, 20 traps can be assembled by one person in a day. Although the pots are used by some people as drinking vessels, the demand-for it for this purpose by the people of Ghana is relatively low, since many people generally depend on plastic drinking cups. The quantities of sugarcane used in the traps are comparatively small and will not affect consumption by humans. The peel mats trap which recorded a trapping index of 84 was the least expensive in terms of cost. This is basically due to the fact that the peels of sugarcane are normally not consumed by people but rather discarded It however takes a comparatively longer time to weave pieces of the peels together so as to obtain a mat.

The trapping duration appears to have an effect on the efficiency of the traps. The trapping indices recorded in Table 2.1 indicate that snail capture peaked between 36 – 72 hours for all the traps. The diffusion of sucrose that is present in sugarcane into the water medium and the subsequent establishment of a diffusion gradient along which the snails are attracted probably needed some time to occur. It appears that the longer the period allowed, the stronger and wider the diffusion gradient becomes, thereby attracting more snails. This may account for the snail capture peaking between 36 – 72 hours.

Secondly, the sugarcane probably got fermented in the aquarium water after 36 hours to produce some additional factors that enhanced the attractant effects of the sugarcane. Perhaps if the enclosure factor is added to all the trap designs the highest trapping index may occur during the 72-hour period or thereabout, as it occurred in the 'grid with pots' traps. This seems to suggest that the trapping units may be able to capture snails even for periods beyond 72 hours.

In a field work however it has been cautioned that if traps are left in an aquatic medium for a longer period, any pure effects of the test traps which have been measured under laboratory conditions may be altered (Kpikpi et al, 1996). They pointed out that when a carbohydrate rich material such as sugarcane is introduced into freshwater medium it becomes a substrate for the growth of bacteria, algae and other microbial organisms. The longer these materials are kept in water the greater the microflora and fauna that would result.

CHAPTER THREE

EFFICACY OF BIOACTIVE MATERIALS IN SIMULATED NATURAL ENVIRONMENT

3.1 **Introduction**

In bioassay tests using diffusion olfactometers, a number of materials have been identified as either attractants or arrestants. These bioactive materials include sugarcane (*Saccharum officinarum)*, mature (ripened) pawpaw (*Carica papaya*), sweet potato (*Ipomoea batatas*), and cassava (*Manihot esculenta)* (Kpikpi et al, 1995). Other bioactive materials identified are boiled unripe plantain (*Musa paradisiaca*), unripe banana (unripe *Musa paradisiaca var sapientum*), boiled cocoyam leaf (boiled *Xanthosoma mafaffa* leaf), and raw yam (*Dioscorea cayenensis*) (Kpikpi and Ansa, 1994).

Domeh (1998), screening processed bioactive materials for their potency as either an attractant or arrestant, identified the following as having significantly high attractant indices, and recommended their use in snail trapping: fermented cocoyam (fermented *Xanthosoma mafaffa* leaf), fermented cassava (fermented *Manihot esculenta*) and fermented sweet potato (fermented *Ipomoea batatas*). He showed that these materials, when fermented for 1 day or 7 days produced significantly high attractant and arrestant indices for juveniles and adults of both *B. truncatus* and *B. pfeifferi*. However, due to the fact that these studies were confined to the laboratory, it was deemed necessary to ascertain the efficacy of these bioactive substances in snail control. As a first step this involved the tests being performed under simulated natural conditions.

In this chapter, the results of work undertaken to test the applicability of laboratory findings under S.N.E. conditions are reported. The main objective was to help identify the most effective bioactive material. In order to do this, the test materials were all investigated using the best trapping unit identified in Chapter 2.

3.2 MATERIALS AND METHODS

3.2.1 **Snails breeding**

The same method described in Chapter 2, for breeding snails was used in this test. Two snail species were used in the experiments. These were *Bulinus truncatus* and *Biomphalaria pfeifferi*. They were bred separately in different glass tanks measuring 49cm x 36cm x 20cm and filled with tap water. Adult snails of-both *B. truncatus* and *B. pfeifferi* were collected from Weija lake and supplied with the necessary conditions for their proper growth and reproduction, already described in Chapter 2. In a period of 1 month, 1200 healthy snails (both juveniles and adults) of each species could be obtained for the experiments.

3.2.2 **Preparation of test materials**

The test materials used were cocoyam, cassava, and sweet potato, all in their raw and fermented states. These materials were used one after the other in separate experiments. In their raw states they were first peeled and divided into smaller equal pieces. They were then put into cold tap water and allowed to ferment, under ambient temperature conditions. For each test material, two different treatments were given, with regard to the period of fermentation.

One set of test materials was allowed to ferment for 1 day (24 hours), while the other set (the same type of materials) was allowed 7 days (168 hours) for fermentation. The fermented materials were removed from water and fixed on sticks so that they could be placed in pots to form the calabash trapping unit described in Chapter 2. Small windows were created on the sides of each calabash by cutting off some portions with a sharp pointed knife. Strings were then passed through two holes created at the margins of the calabash, so that they could be suspended from a point above the aquarium. Samples of these traps have been shown in Plate 2e.

2.2.3 **Traps**

The most efficient trap design identified in Chapter 2 was used for all the simulated natural environment tests. This was the grid (in calabash) traps (Plate 2e).

3.2.4 **Aquarium**

The same aquarium used for tests performed in the previous chapter was used. It was prepared as already described in Chapter 2.

3.2.5 SIMULATED NATURAL ENVIRONMENT EXPERIMENTS

A total of 2000 healthy snails (1000 *Bulinus truncatus* and *Biomphalaria pfeifferi*) were used for each experiment, with the exception of the first test in which 1000 snails of only *Bulinus truncatus* were used. The snails were made up of both juveniles and adults. They were introduced into the aquarium, and allowed a period of 7 days for acclimatisation to the aquarium conditions. During this period they were routinely fed with fresh lettuce leaves. A day prior to each test however, the snails were deprived of food for 24 hours. Four long wooden bars were placed accross the aquarium so that the trapping units could be suspended on them by means of strings. The test traps were arranged on one half of the aquarium whilst the controls, prepared in a similar manner using polystyrene in place of the test material were also arranged on the other half. Six sets of experiments, each lasting for 3 days were conducted using only one particular test material in each experiment. 10 test traps and 10 control traps were altogether set in each test. The traps were inspected for trapped snails every 12 hours, for a period of 3 days. The conductivity of aquarium water, temperature, pH, dissolved oxygen concentration, and salinity of the water were all monitored.

3.3 RESULTS

3.3.1 **Efficacy of test materials in traps**

3.3.1.1 COCOYAM (7 DAYS FERMENTED)

Results of the simulated natural environment (S.N.E.) experiments showed that generally 7 days fermented cocoyam used in test traps caught more *B. truncatus* snails than control tests (Table 3.2). The number of snails trapped however varied from one period of inspection of traps to another. There seems to be marked fluctuations in the number of snails trapped throughout the test period. The highest number of snails trapped, 43 snails, occurred after 24 hours (Fig. 3a). After this period the number declined till the lowest (9 snails) was trapped after 60 hours. It was only after 60 hours that control traps caught more snails than test traps. Cocoyam, fermented for 7 days appeared so soft that it got disintegrated and fell into the aquatic medium during the test period.

3.3.1.2 COCOYAM (1 DAY FERMENTED)

All the test traps using 1 day fermented cocoyam caught more snails (both *B. truncatus* and *B. pfeifferi*) than the control traps (Tables 3.1 and 3.2). Furthermore, fluctuations in snail numbers were found from one inspection period to another. The highest number of snails trapped for both types of snails was after 72 hours; 48 snails of *B. pfeifferi* and 75 snails of *B. truncatus* were counted. The lowest number of *B. pfeifferi* trapped was after 36 hours and 60 hours, during which periods 31 snails were trapped. For *B. truncatus*, the lowest number of snails captured (35 snails) occurred after 60 hours. The test materials being more rigid than the 7 days fermented types did not disintegrate.

3.3.1.3 CASSAVA (7 DAYS FERMENTED)

All the test traps caught more than the control traps (Table 3.1 and 3.2). For *Bulinus truncatus*, the highest number of snails trapped in test trap (76 snails) was after 48 hours. However, 12 hours after this period (i.e. after 60 hours) the lowest number of snails trapped occurred. The highest number of *B. pfeifferi* snails (51 snails) was trapped after 24 hours. The least number occurred after 60 hours. Test materials disintegrated into the aquatic medium.

3.3.1.4 CASSAVA (1 DAY FERMENTED)

All the test traps caught more snails than controls (Table 3.1 and 3.2). The highest number of *Bulinus truncatus* snails trapped occurred after 72 hours, during which 79 snails were counted. The lowest (16 snails) was after 60 hours. Similarly, the highest number of *B. pfeifferi* snails trapped (33 snails) was 72 hours and the lowest, 10 snails occurred after 60 hours. The test materials did not disintegrate.

3.3.1.5 SWEET POTATO (1 DAY FERMENTED)

All the test traps trapped more snails than the control traps (Table 3.1 and 3.2). The highest number of *B. truncatus* snails trapped was 92, and this occurred after 48 hours. The lowest number of *B. truncatus* trapped (12 snails) was after 60 hours. It needs to be pointed out that, the number of snails trapped after 72 hours was also comparatively high (i.e. 81 snails trapped). Forty one (41) *B. pfeifferi* snails, being the highest was recorded after 48 hours. Twenty-nine (29) snails was recorded after 72 hours. The lowest number of *B. pfeifferi* snails was 6, and occurred after 60 hours. The test materials did not disintegrate.

3.3.1.6 SWEET POTATO (7 DAYS FERMENTED)

All the test traps caught more snails than the control traps. Ninety-five (95) snails of *B. truncatus*, being the highest number trapped occurred after 72 hours. Similarly, the highest number of *B. pfeifferi* trapped (34) was after 72 hours. The test materials did not disintegrate.

3.3.2 **Efficiency of test materials**

Results shown in Tables 3.1, 3.2, and 3.3, all give an idea of the efficiency of the different test materials. Table 3.3 shows that a total of 612 snails (375 *Bulinus truncatus* and 237 *Biomphalaria pfeifferi*) were recorded for 1 day fermented cocoyam; this was a

36.60% of the total snails used. Also, 7 days fermented cocoyam trapped 144 snails (only *B. truncatus* used), giving 14.40% of total snails used. With the 1 day fermented cassava a total of 450 snails (296 *B. truncatus* and 154 *B. pfeifferi*) were trapped forming 22.50% of total snails used. Test results for 7 days fermented cassava, and those of sweet potato have been shown in Table 3.3. It is expected that the most efficient test material will be the one which trapped the highest number of snails within the 3-day test period. Trapping indices shown in Tables 3.1 and 3.2 were used to assess the efficiency of the test materials used in the traps. From Table 3.1, the highest value of the trapping indices, 71, occurred in a test in which 7 days fermented potato was used. From Table 3.2 the highest trapping index was 37, occurring after 24 hours in a trap that used 7 days fermented cassava as the test material. A comparison of the trapping indices for all the different bioactive materials in the present studies has been shown in Figures 3l & 3m. All the traps caught more *Bulinus truncatus* snails than *B. pfeifferi.* The difference was statistically significant ($P < 0.05$).

3.3.3 AQUARIUM CONDITIONS

Water conductivity range was between 98.8 – 112.6 µSm/cm^2. Dissolved oxygen concentration range was 1.66 – 2.20 mg/l. Salinity was 0.01%, and the pH range was 8.4 – 8.7. Aquarium temperatures measured were 27.4°C, being the average for the mornings, and 30.2°C in the afternoons. These measurements were taken with a 'Water Quality Checker, model U-10' instrument.

Table 3.1 NUMBER OF SNAILS TRAPPED IN SIMULATED NATURAL ENVIRONMENT EXPERIMENTS FOR EFFICACY OF THE TEST MATERIALS

(*Bulinus truncatus* snails used)

TIME (HOURS)		12	24	36	48	60	72
COCOYAM (7 DAYS FERMENTED)	T	35	43	28	16	9	13
	C	18	14	12	7	21	11
	(I)	17	29	16	9	12	2
	L.S.	-	***	-	-	-	-
COCOYAM (1 DAY FERMENTED)	T	71	79	51	64	35	75
	C	31	19	21	21	18	25
	(I)	40	60	30	43	17	50
	L.S.	**	***	**	***	*	***
CASSAVA (7 DAYS FERMENTED)	T	57	68	28	76	19	39
	C	17	21	14	21	10	15
	(I)	40	47	14	55	9	24
	L.S.	**	***	-	***	*	***
CASSAVA (1 DAY FERMENTED)	T	56	48	37	60	16	79
	C	20	17	16	20	10	29
	(I)	36	31	21	40	6	50
	L.S.	**	**	*	***	-	50
SWEET POTATO (1 DAY FERMENTED)	T	20	67	17	92	12	81
	C	15	25	16	22	8	26
	(I)	5	42	1	70	4	51
	L.S.	-	**	-	***	-	**
SWEET POTATO (7 DAYS FERMENTED)	T	23	70	43	74	39	95
	C	17	21	22	39	24	24
	(I)	6	49	21	35	15	71
	L.S.	-	**	**	***	-	***

T = NUMBER OF SNAILS TRAPPED IN TEST TRAPS
C = NUMBER OF SNAILS TRAPPED IN CONTROL TRAPS
(I) = TRAPPING INDICES
L.S. = LEVEL OF SIGNIFICANCE
* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

Table 3.2 NUMBER OF SNAILS TRAPPED IN SIMULATED NATURAL ENVIRONMENT EXPERIMENTS FOR EFFICACY OF THE TEST MATERIALS

(*Biomphalaria pfeifferi* snails used)

TIME (HOURS)		12	24	36	48	60	72
COCOYAM (1 DAY FERMENTED)	T	47	39	31	41	31	48
	C	14	18	15	10	11	17
	(I)	33	21	16	31	20	31
	L.S.	***	**	-	***	**	**
CASSAVA (7 DAY FERMENTED)	T	37	51	22	49	13	20
	C	13	14	7	14	3	12
	(I)	24	37	15	35	10	8
	L.S.	**	**	*	***	-	-
CASSAVA (1 DAY FERMENTED)	T	32	28	21	30	10	33
	C	10	12	11	13	5	11
	(I)	22	16	10	17	5	22
	L.S.	**	*	-	*	-	**
SWEET POTATO (1 DAY FERMENTED)	T	13	39	10	41	6	29
	C	10	9	2	9	5	9
	(I)	3	30	8	32	1	20
	L.S.	-	**	-	***	-	**
SWEET POTATO (7 DAYS FERMENTED)	T	11	30	17	28	18	34
	C	5	13	13	13	7	14
	(I)	6	17	4	15	11	20
	L.S.	-	-	-	*	-	**

T = NUMBER OF SNAILS TRAPPED IN TEST TRAPS
C = NUMBER OF SNAILS TRAPPED IN CONTROL TRAPS
(I) = TRAPPING INDEX
L.S.= LEVEL OF SIGNIFICANCE

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

Table 3.3 TOTAL NUMBER OF SNAILS (i.e. both *Bulinus truncatus* & *Biomphalaria pfeifferi*) TRAPPED IN TEST TRAPS IN SIMULATED NATURAL ENVIRONMENT EXPERIMENTS

TEST MATERIAL	*B. truncatus* Number of snails trapped in 10 traps for 3 days	*B. pfeifferi* Number of snails trapped in 10 traps for 3 days	Total number of snails trapped in 3 days	Percentage number of snails trapped in 3 days (%)
COCOYAM (1 day fermented)	375	237	612	30.60
COCOYAM (7 days fermented)	144	-	144@	14.40@
CASSAVA (1 day fermented)	296	154	450	22.50
CASSAVA (7 days fermented)	287	192	479	23.95
SWEET POTATO (1 day fermented)	289	138	427	21.35
SWEET POTATO (7 days fermented)	344	138	482	24.10

@ Only *Bulinus truncatus* used.

FIGURE 3a; EFFICACY OF COCOYAM (7 DAYS FERMENTED) IN SIMULATED NATURAL ENVIRONMENT -BULINUS

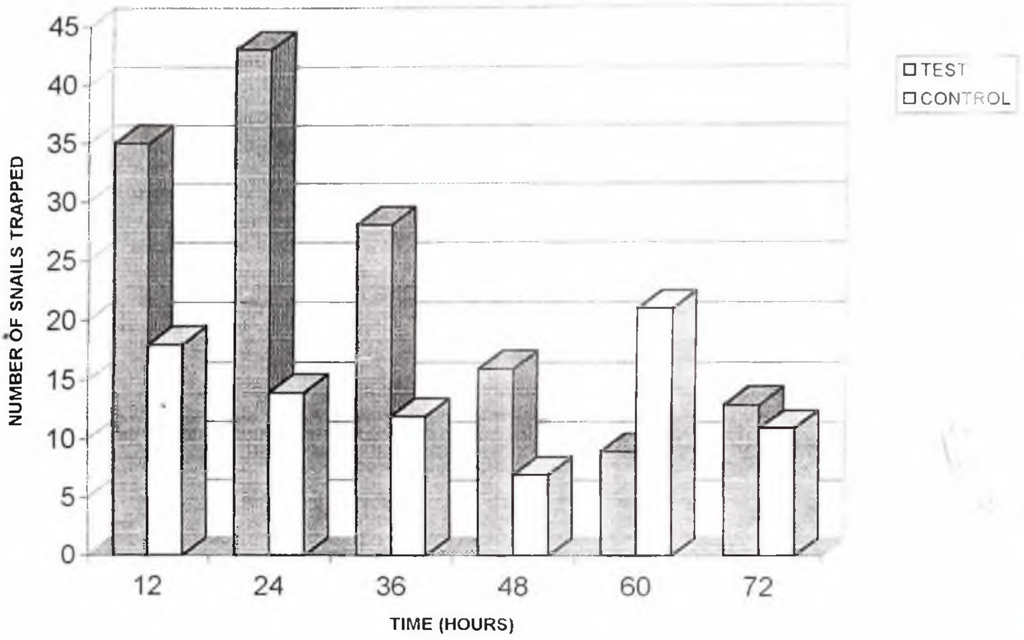


FIGURE 3b; EFFICACY OF COCOYAM (1 DAY FERMENTED) IN SIMULATED NATURAL ENVIRONMENT -BULINUS

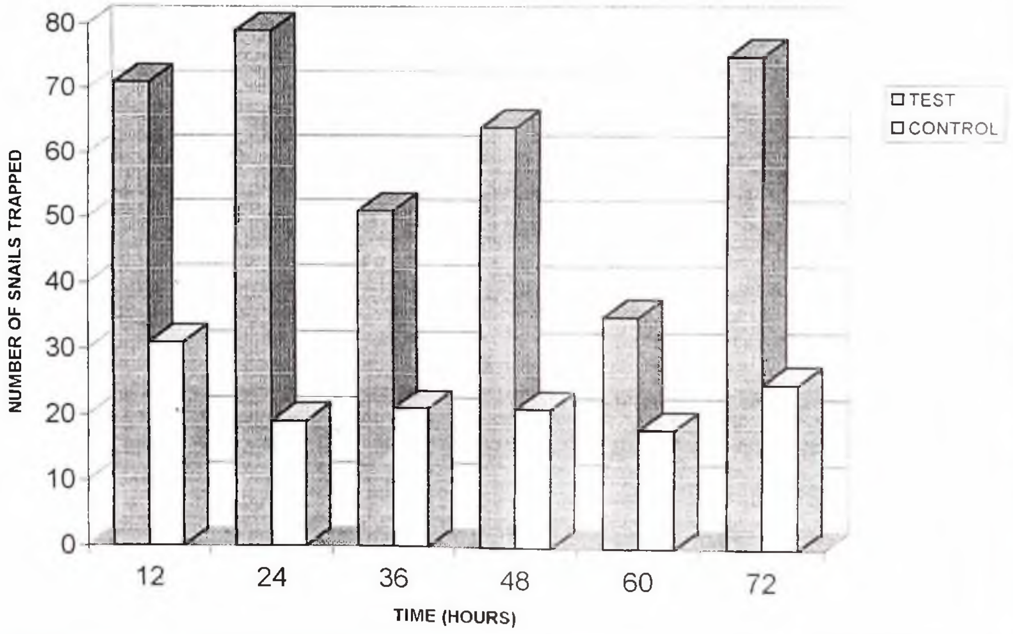


FIGURE 3c; EFFICACY OF CASSAVA (7 DAYS FERMENTED) IN SIMULATED NATURAL ENVIRONMENT -BULINUS

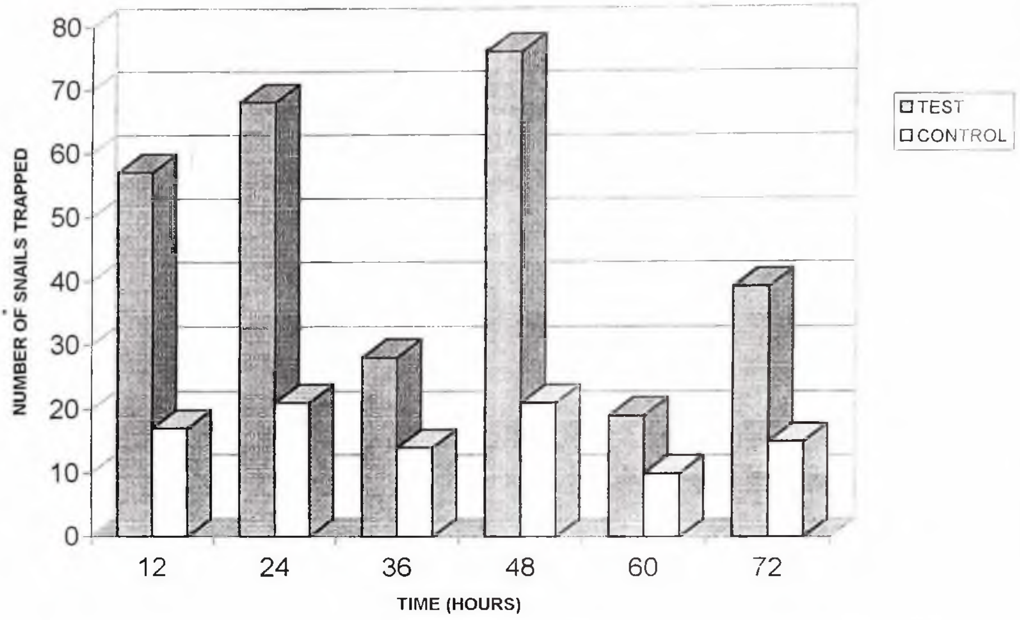


FIGURE 3d; EFFICACY OF CASSAVA (1 DAY FERMENTED) IN SIMULATED NATURAL ENVIRONMENT -BULINUS

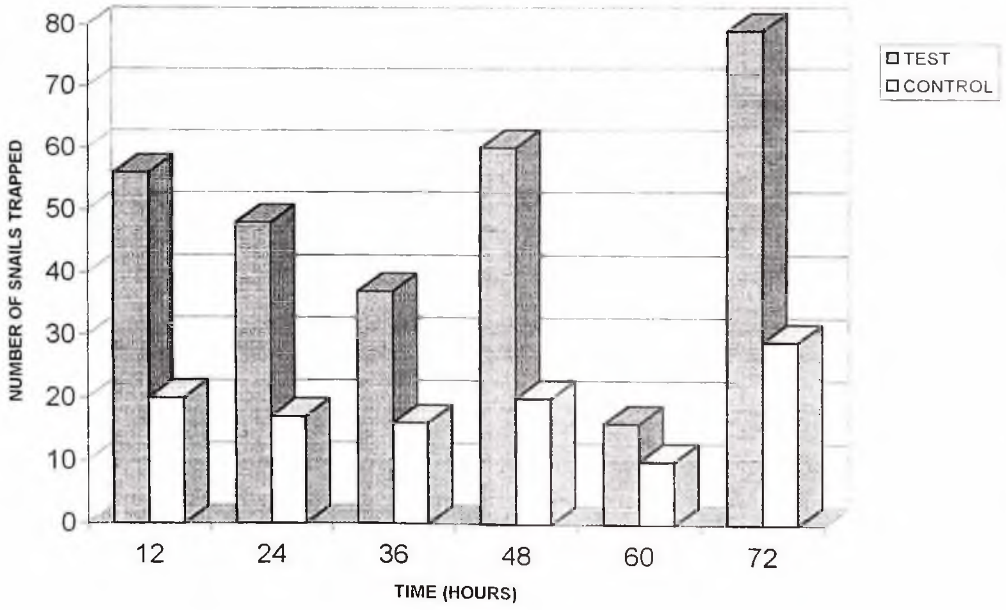


FIGURE 3e; EFFICACY OF SWEET POTATO (1 DAY FERMENTED) IN SIMULATED NATURAL ENVIRONMENT -BULINUS

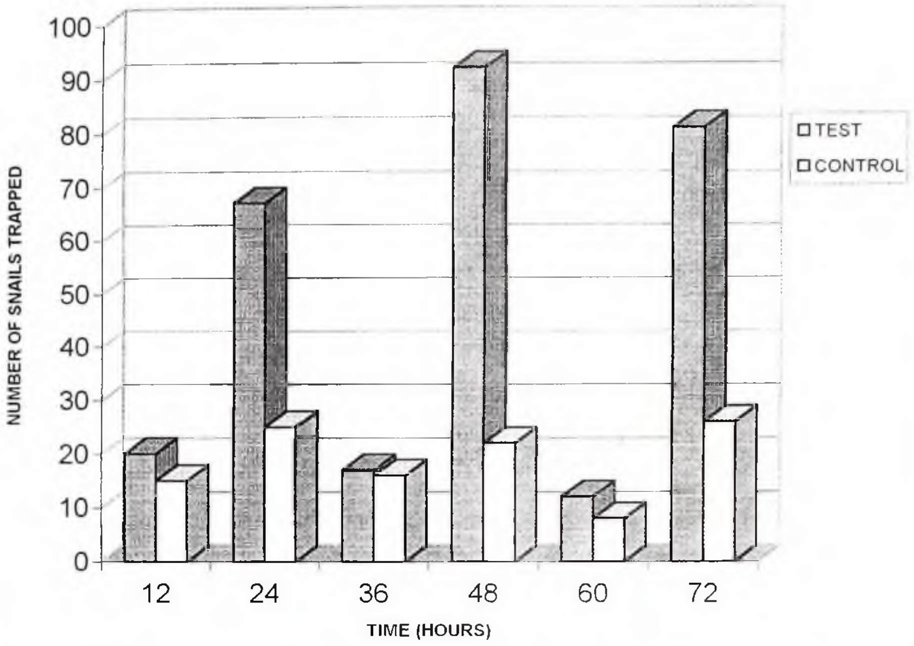


FIGURE 3f; EFFICACY OF SWEET POTATO (7 DAYS FERMENTED) IN SIMULATED NATURAL ENVIRONMENT -BULINUS

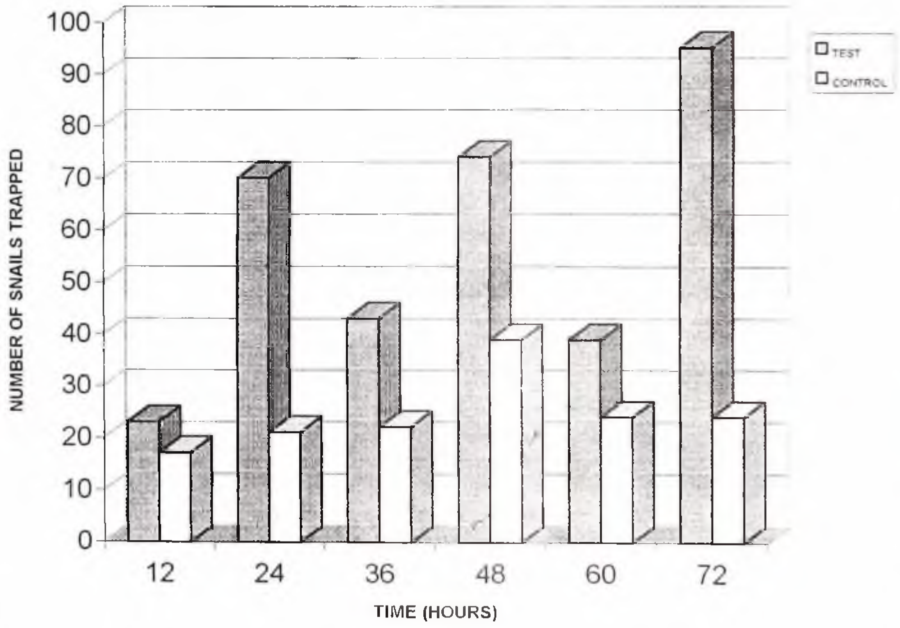


FIGURE 3g; EFFICACY OF COCOYAM (1 DAY FERMENTED) IN SIMULATED NATURAL ENVIRONMENT -BIOMPHALARIA

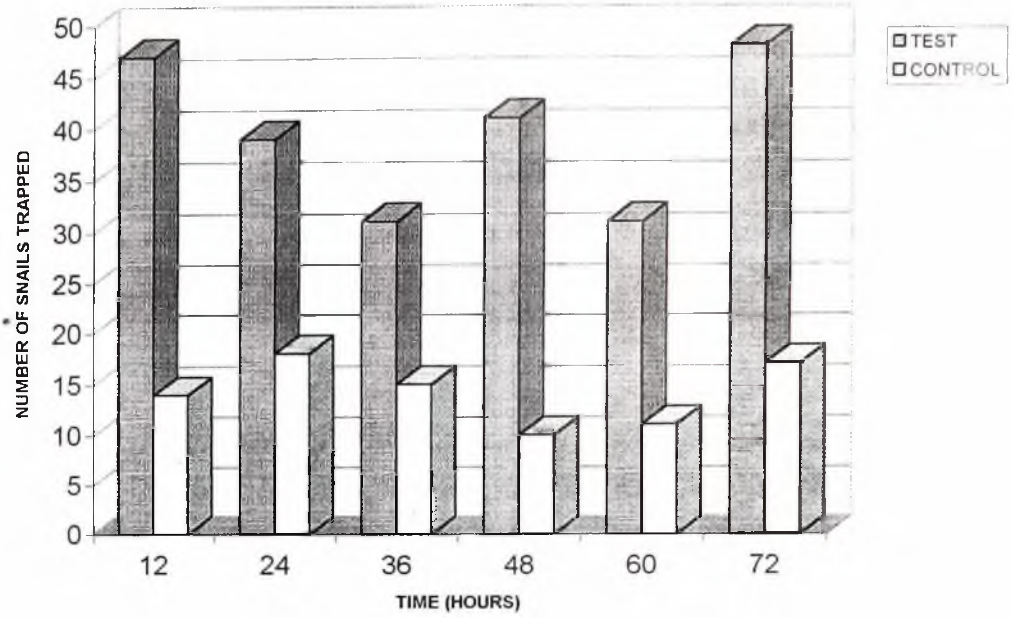


FIGURE 3h; EFFICACY OF CASSAVA (7 DAYS FERMENTED) IN SIMULATED NATURAL ENVIRONMENT -BIOMPHALARIA

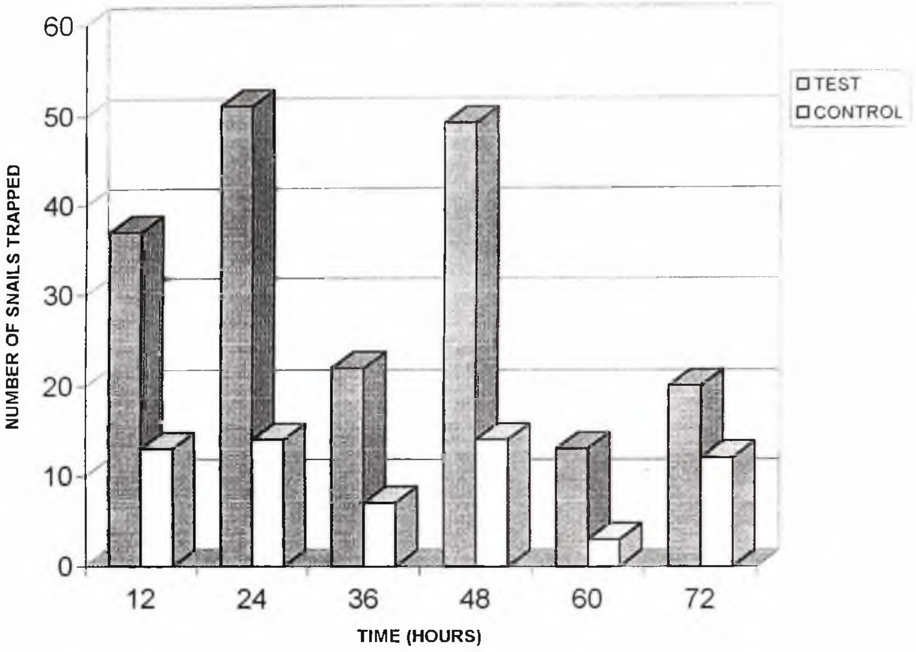


FIGURE 3i; EFFICACY OF CASSAVA (1 DAY FERMENTED) IN SIMULATED NATURAL ENVIRONMENT -BIOMPHALARIA

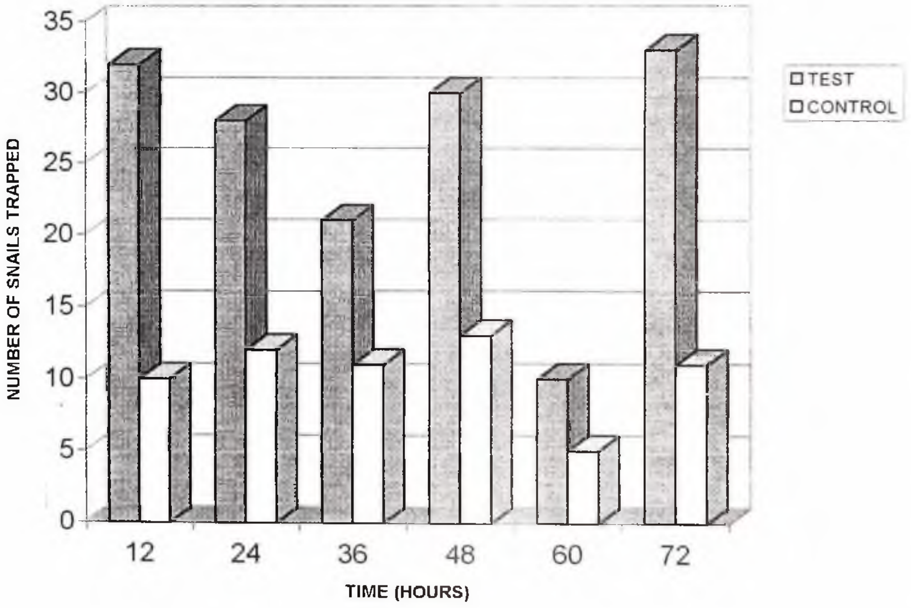


FIGURE 3j; EFFICACY OF SWEET POTATO (1 DAY FERMENTED) IN SIMULATED NATURAL ENVIRONMENT -BIOMPHALARIA

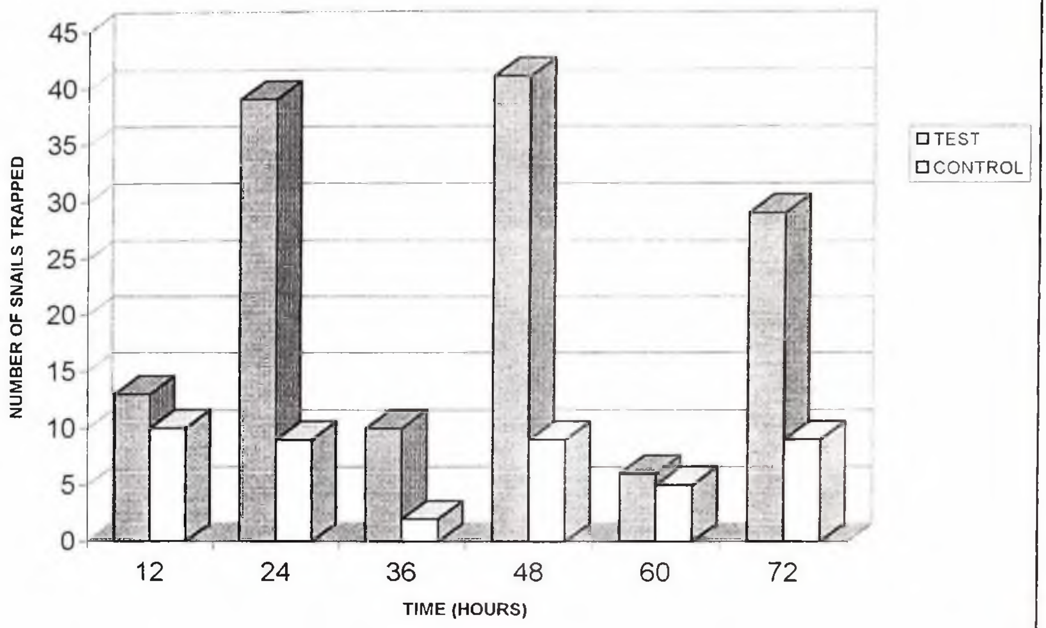


FIGURE 3k; EFFICACY OF SWEET POTATO (7 DAYS FERMENTED) IN SIMULATED NATURAL ENVIRONMENT -BIOMPHALARIA

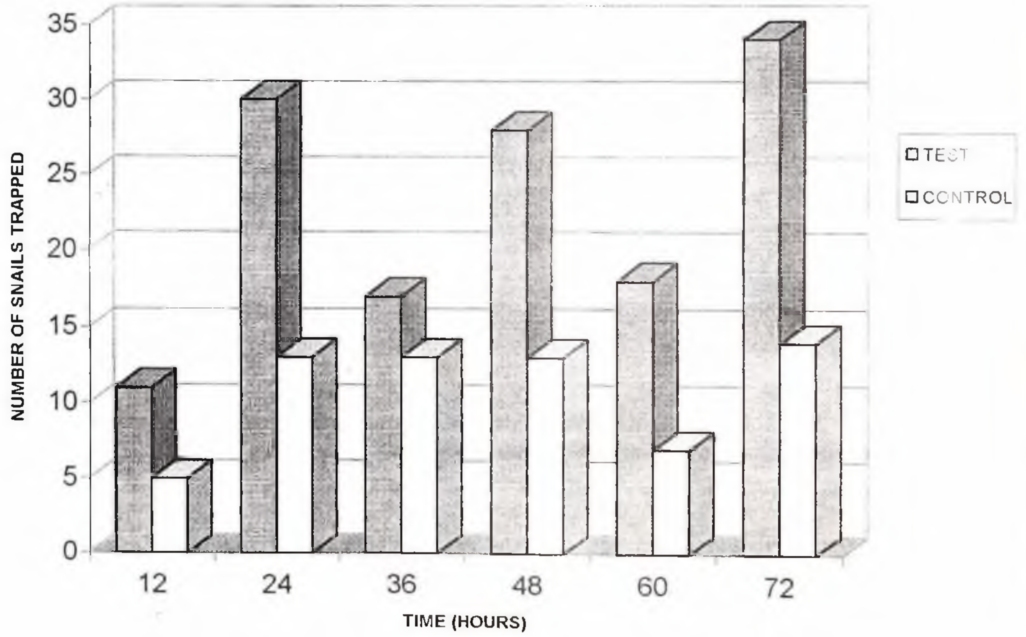


FIGURE 3I; A COMPARISON OF TRAPPING INDICES FOR DIFFERENT NATURAL BIOACTIVE SUBSTANCES -BULINUS

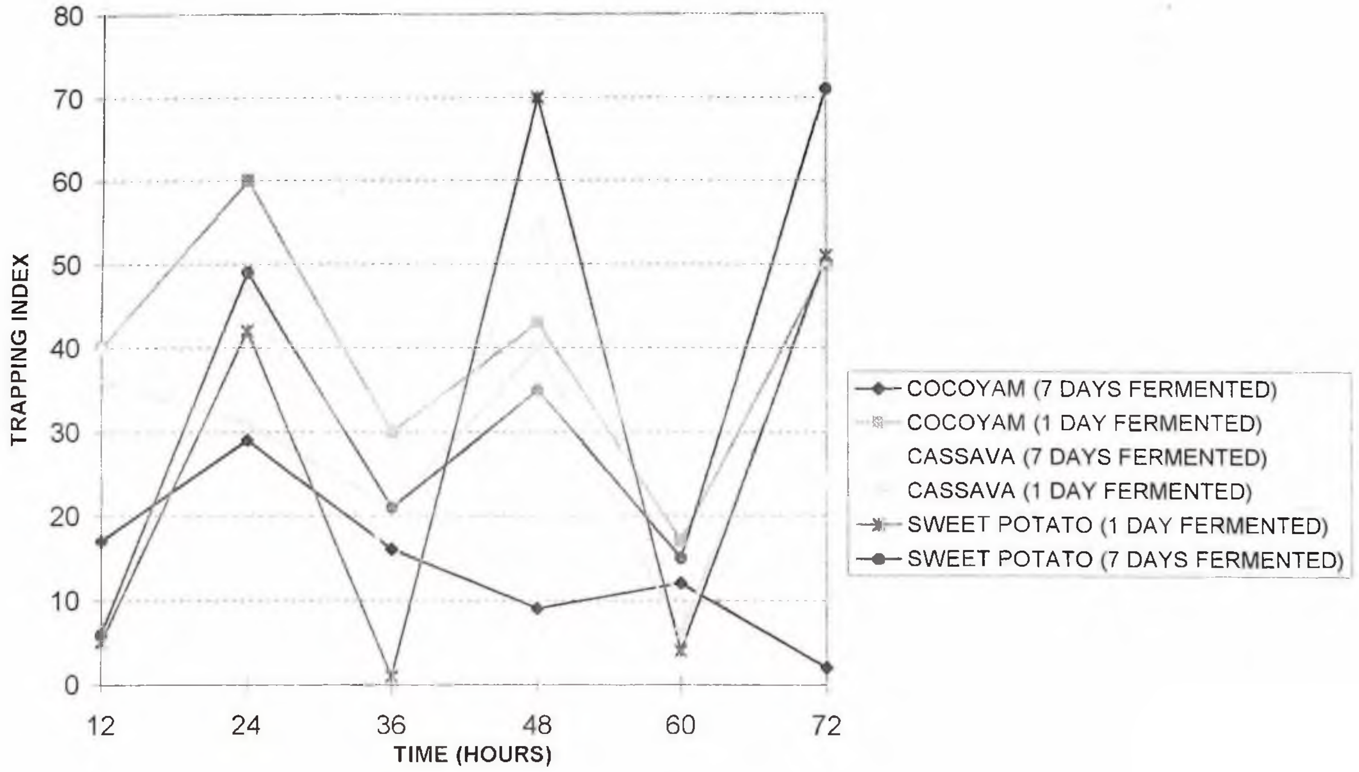


FIGURE 3m; A COMPARISON OF TRAPPING INDICES FOR DIFFERENT NATURAL BIOACTIVE SUBSTANCES -BIOMPHALARIA

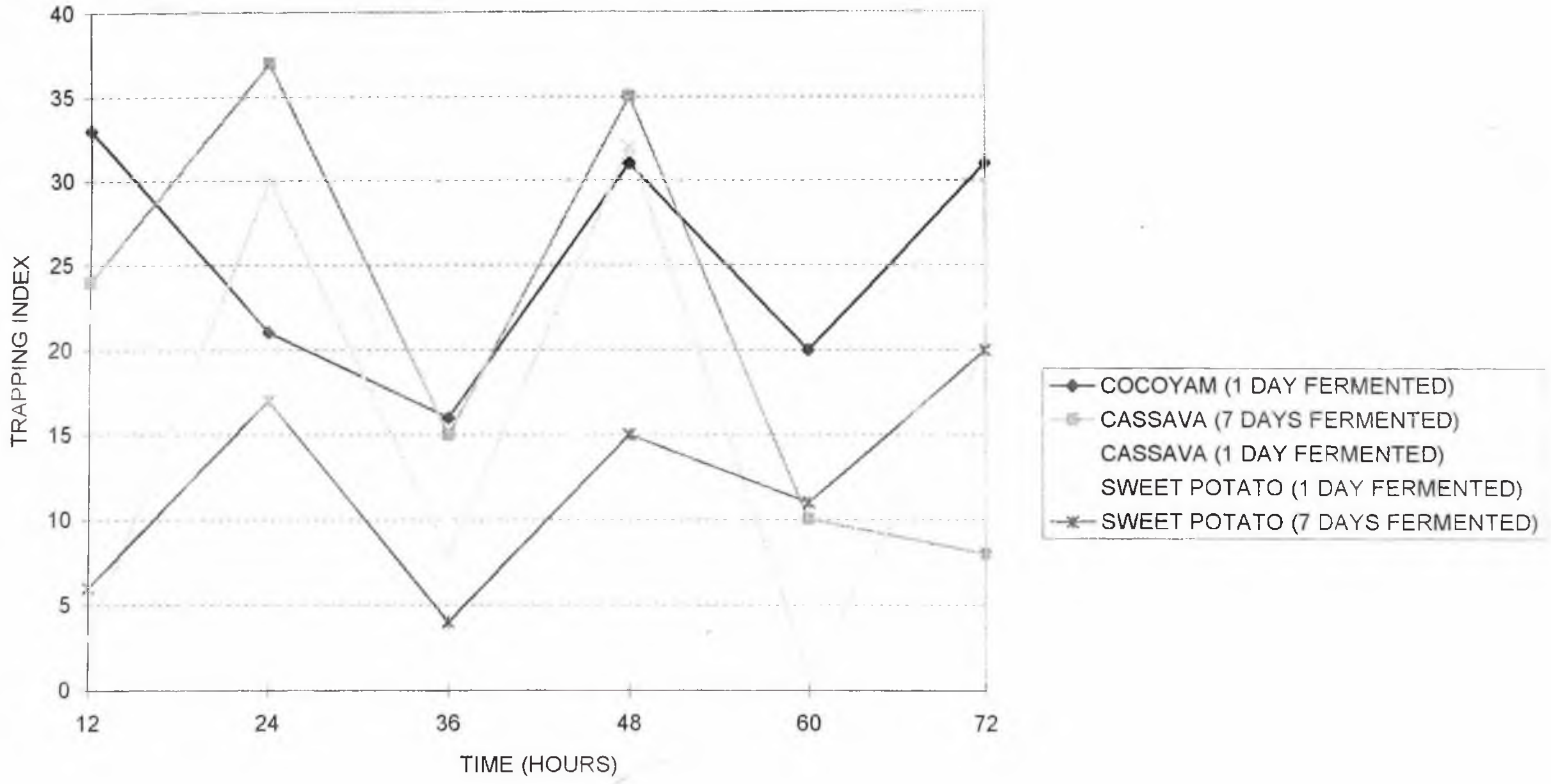


FIGURE 3n (i) A COMPARISON OF TRAPPING INDICES SHOWING THE RESPONSE OF BULINUS SNAILS TO 7 DAYS FERMENTED BIOACTIVE MATERIALS

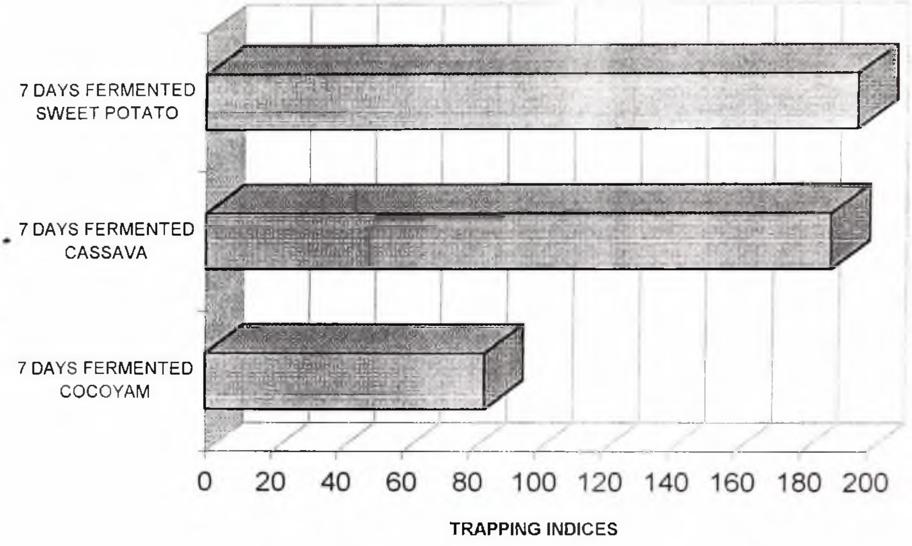


FIGURE 3n (ii) A COMPARISON OF TRAPPING INDICES SHOWING THE RESPONSE OF BULINUS SNAILS TO 1 DAY FERMENTED BIOACTIVE MATERIALS

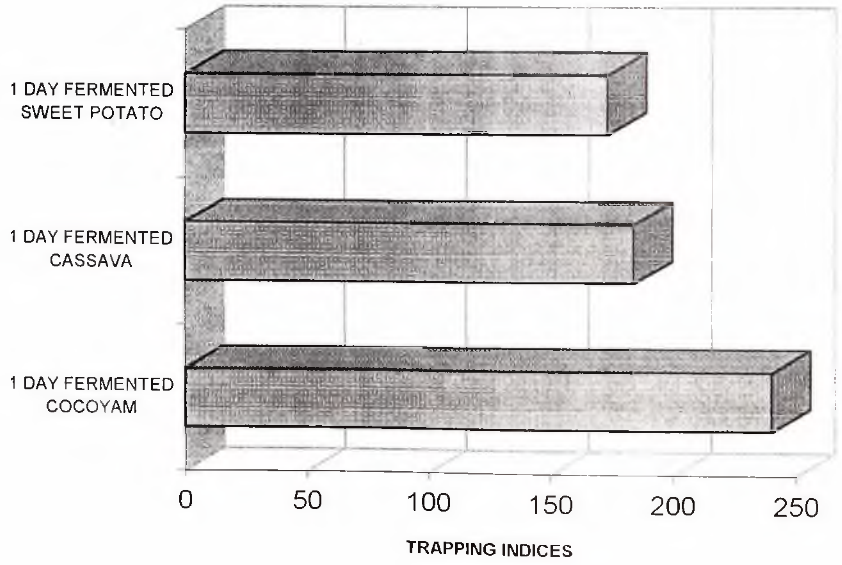


FIGURE 3(o); A COMPARISON OF TRAPPING INDICES SHOWING THE RESPONSE OF BULINUS SNAILS TO SOME BIOACTIVE NATURAL MATERIALS

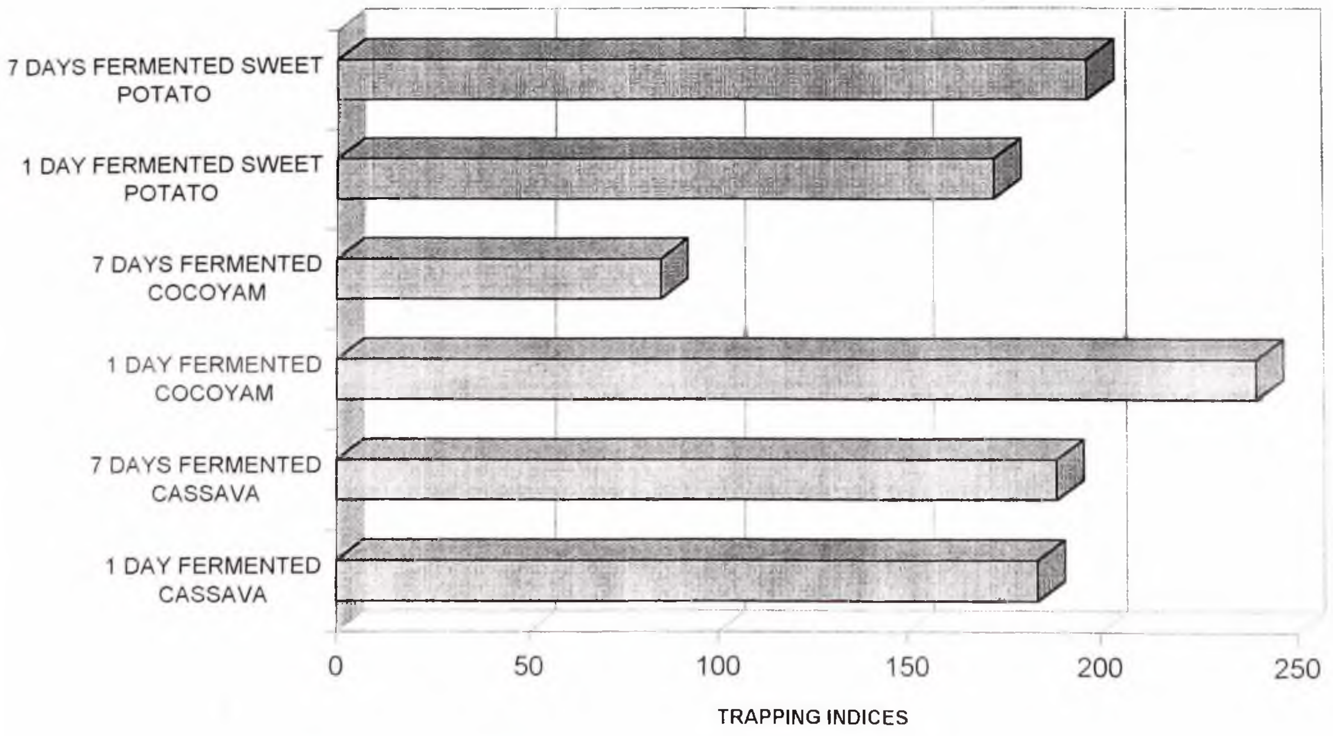


FIGURE 3p (i) A COMPARISON OF TRAPPING INDICES SHOWING THE RESPONSE OF BIOMPHALARIA SNAILS TO 7 DAYS FERMENTED BIOACTIVE MATERIALS

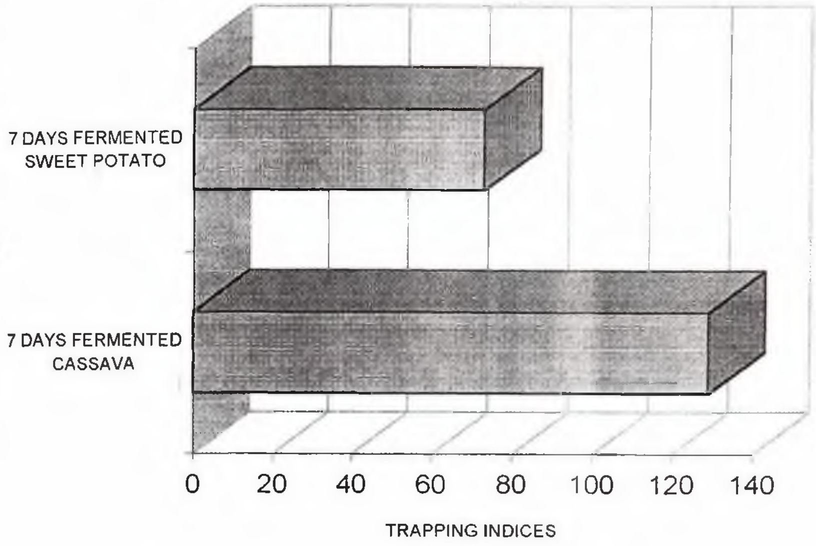


FIGURE 3p (ii) A COMPARISON OF TRAPPING INDICES SHOWING THE RESPONSE OF BIOMPHALARIA SNAILS TO 1 DAY FERMENTED BIOACTIVE MATERIALS

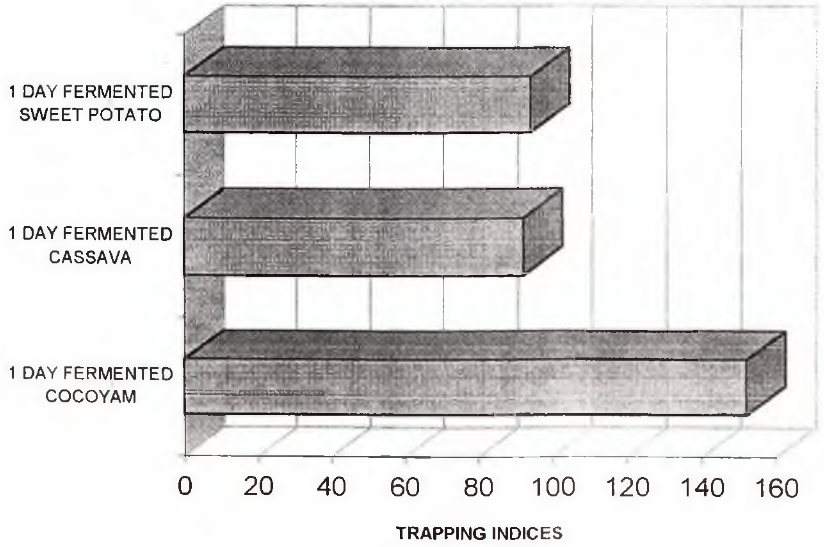
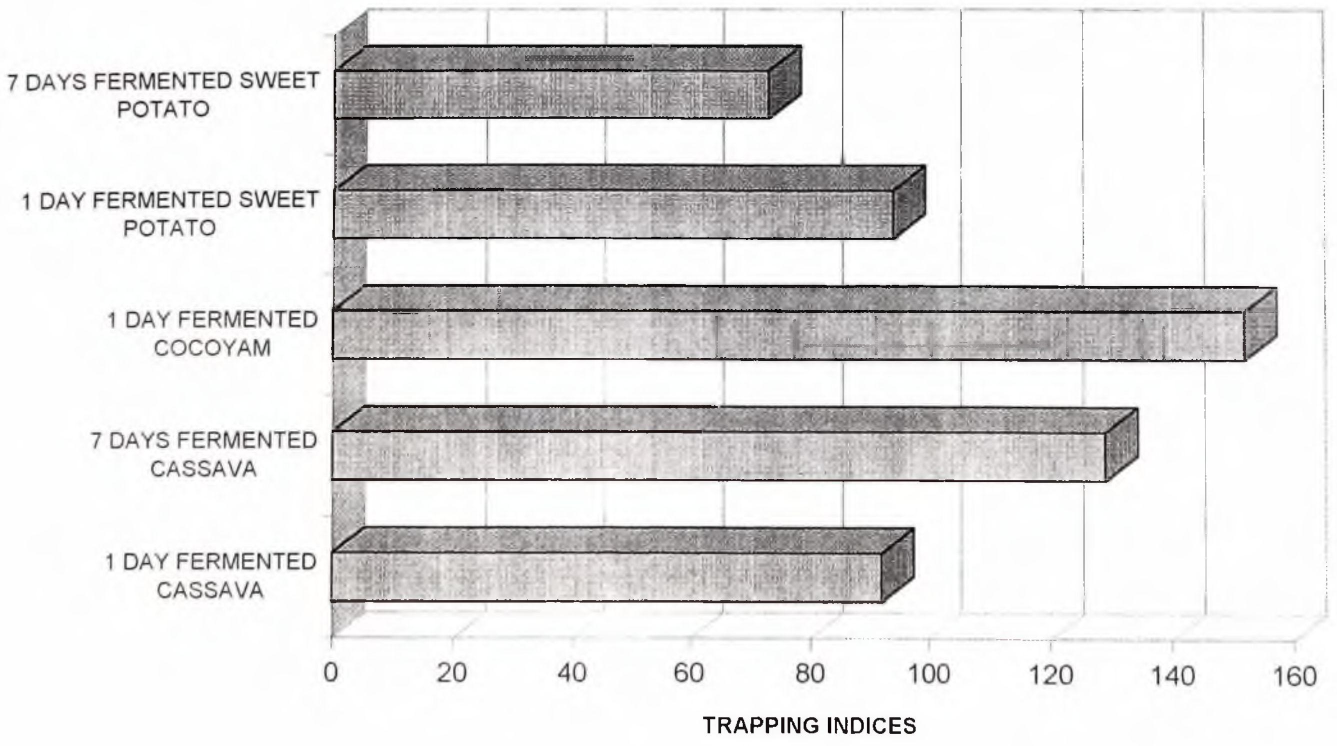


FIGURE 3*q*; A COMPARISON OF TRAPPING INDICES SHOWING THE RESPONSE OF BIOMPHALARIA SNAILS TO SOME BIOACTIVE NATURAL MATERIALS



3.4 DISCUSSION

The ability of the fermented natural bioactive materials, namely fermented products of cocoyam, cassava, and sweet potato to attract or arrest the schistosome host snails has been studied in diffusion bioassays using olfactometers (Domeh, 1998). Results of the present studies which were conducted under S.N.E. conditions have revealed a concordance with those of diffusion bioassays. Each of the natural bioactive materials tested in traps caught significantly more snails than the control traps ($p < 0.05$), and cocoyam (1 day fermented) recorded the highest trapping index for both *Bulinus truncatus* and *Biomphalaria pfeifferi* snails (Figures 3o & -3q). This suggests that cocoyam (1 day fermented) was the most efficient bioactive natural material among the others tested. This result was not unexpected, as Domeh (1998) showed that 1 day fermented cocoyam was among the top three attractants identified during his studies. Observations that were made during the present studies showed that 7 days fermented cocoyam became so soft and got disintegrated into pieces, while 1 day fermented cocoyam did not. This might possibly explain why 1 day fermented cocoyam performed more efficiently than 7 days fermented cocoyam.

To support this, it can be postulated here that the diffusion gradient that was created in the water medium, with the test material as the source, to attract the snails (Kpikpi, 1990) was disturbed in the case of the 7 days fermented cocoyam. This is because as the material got disintegrated and floated out of the traps, there was little or no diffusion gradient maintained for snails to travel on to the traps, hence the low catch of snails.

It was also observed that 7 days fermented sweet potato which recorded the second highest trapping index for *Bulinus truncatus* (Figure 3 o) did not also disintegrate during the test period. These seem to support the view that test materials are capable of establishing a more efficient diffusion gradient to attract snails than crushed forms, as suggested by Dogbey (1995).

The observations that were made of the ability of fermented bioactive natural materials to attract or arrest snails support the hypothesis put forward by Thomas (1996). He suggested that a link exists between fermented natural materials in aquatic ecosystem and the release of short-chain carboxylic acids (C_2-C_5) and other metabolites by bacteria present in the water. He explained that due to the high biochemical oxygen demand of the bacteria, they resort to anoxic, glycolytic fermentation with the release of short-chain carboxylic acids ($C_2 - C_5$) to obtain their energy.

Essentially, these short-chain carboxylic acids are potentially a valuable source of energy for detritivorous invertebrates such as snails. In addition, hydrolysed starch and maltose present in cocoyam, cassava, and sweet potato could be a valuable food source for the snails (Thomas et al, 1986).

Although all the tested materials belong to the same class of food (i.e. carbohydrates), there may be variations in the proportions in which their chemical constituents are combined. This may possibly affect their rate of fermentation in water and consequently the rate of release of carboxylic compounds together with any other chemical compounds into the aquatic medium. This may account for the differences in the performance of the different materials tested, as represented by their trapping indices (Figure 3q & Figure 3o).

The generally fluctuating trend of trapping indices (Figure 3l & Figure 3m) recorded over the test period occurred probably because of variations in the weather (i.e.

whether day or night). All the S.N.C. tests were started at a period prior to darkness (i.e. in the late afternoon). The first set of results (i.e. 12 hours) were periods of darkness. Generally, the dark periods corresponded with the troughs on the graphs, while the light periods were represented by the crests. The presence of light probably came with some amount of heat to slightly increase the temperature of the water, which might have increased the metabolic activity of the snails. The snails probably might be more active during the day than in the night. Secondly, an increase in temperature during daylight might have increased fermentation process (Thomas, 1996), leading to an increase in rate of release of short-chain carboxylic acids (C_2 – C_5) which the snails depend on for energy. This could be the reason of different trapping indices recorded for the different periods, although the same test material was used. It can therefore be suggested that, in working out a control programme using attractants or arrestants, better results could be achieved when traps are set during the day than in the night.

The bioactive materials that were tested also recorded different times of peak performance, all occurring during 'day' periods rather than 'night' periods (Figures 3l & 3m). Cocoyam had a peak performance at 12-24 hours for both snail species. The peak performance recorded for cassava were during 48 hours (for *Bulinus truncatus*) and 12-24 hours for *Biomphalaria pfeifferi*. Sweet potato recorded its peak performance at 48-72 hours for both species. These differences in peak performances might have occurred as a result of the different rates of fermentation in the different test materials. This essentially determined the rates at which the chemical factors in each of the materials will be released into the aquatic medium.

There have been several reports on differences in the chemoreception niches of snails that belong to different species (Thomas et al, 1985; Kpikpi & Thomas, 1992). Trapping indices recorded in the present studies are suggestive of the fact that the response rate of *Bulinus truncatus* to each of the bioactive materials is higher than *Biomphalaria.* Whilst the highest trapping index in the tests with *B. truncatus* was 71, the highest for *B. pfeifferi* was only 37. It can therefore be suggested that *B. truncatus* snails have better preferences for fermented cocoyam, cassava, and sweet potato than *B. pfeifferi.* These observations may be very useful in both selective snail sampling and snail control.

The test materials are recommended for field trials so that they can be combined with a toxicant to form a 'slow release system' to be used in schistosome host snails control. These materials are very common in every market in the country, and can be obtained at all seasons within the year. The only point that seems to be a limitation of their use on a large scale scale for snail trapping is the fact that these materials form a major foodstuff consumed by many people in the country. However, studies conducted by Dogbey (1995) have revealed that the quantity of the bioactive materials does not affect the trapping index. Smaller quantities can therefore be used in the traps for any snail control programme, so that consumption by humans will not be affected.

CHAPTER FOUR

A COMBINATION OF BIOACTIVE SUBSTANCES AND A TOXICANT

4.1 Introduction

One of the effective means of interrupting the life cycle of the schistosome parasite is by controlling the populations of intermediate host snails (Schiff and Evans, 1977). The use of molluscicides, which has been known and used for so many years, remains an effective means of controlling snails population in an infested water body. Unfortunately, the setbacks that accompany the method, notably among them being the indiscriminate destruction of other non-target aquatic organisms has not- changed. All available molluscicides may have adverse effect on fish and may hamper water productivity (Paperna, 1969). Again, although molluscicides have been used extensively in some endemic situations, they may be expensive and the organisation needed to apply them is complex (Pointier and McCullough, 1989). It has been suggested that the modern approach is not necessarily to indulge in the widespread application of chemical to natural waterbodies but through focal mollusciciding (Schiff and Evans, 1977). Focal mollusciciding deals with only selected sites such as the water contact sites alone. This reduces the quantity of molluscicides applied to the water body. The disadvantage of this method however is that it is not very effective if the water body has a relatively high velocity of flow (Meyer-Lassen et al, 1994). Schiff and Evans (1977) pointed out that focal control of snails in slow moving, or stagnant aquatic bodies by judicious application of molluscicides is inexpensive and may prevent major outbreaks of schistosomiasis. Focal control, using slow-release systems may be an effective means of controlling the snails with minimum hazards posed in the aquatic medium. Earlier attempts in the use of slow-release systems have not made use of any bioactive materials. Various slow-release systems that have been used were formed by incorporating molluscicidal chemicals into a rubber or elastomer matrix during manufacture (Schiff and Evans, 1977). Kpikpi (1997) pointed out that currently, much emphasis is on the search for very potent bioactive substances in terms of being attractant and arrestant with a toxicant incorporated in them to form a slow release system. It is believed that the system, formed with a potent bioactive substance will attract the snails, and the toxicant in it will kill the snails. The main objective of the present study is to combine identified bioactive substances and a molluscicidal chemical (Bayluscide) to find out whether

(i) the bioactive substances maintain their attractant and arrestant properties,

(ii) the snails that are trapped die as a result of the molluscicide. The first part of this investigation involved bioassay tests using diffusion olfactometers, whilst the second part was simulated natural environment experiments in an aquarium.

4.2. MATERIALS AND METHODS

4.2.1 **Snail breeding**

Bulinus truncatus snails were used for this experiment. Snails were collected from a parent stock and bred in separate glass tanks measuring 66 x 38 x 21 cm, and filled with tap water. These were kept inside the laboratory at a room temperature of 26±1°C and approximately 12 hours light and 12 hours darkness each day. The snails were routinely fed with lettuce. The water in each of the 10 tanks was changed weekly. As eggs were laid by the snails and hatched, the number of snails in each glass tank increased. The juvenile snails were selected and put into separate tanks for them to grow. In a period of two months a lot of snails could be obtained. Healthy adult snails were then selected and used in both the diffusion bioassay tests and the simulated natural environment experiments.

4.2.2 Preparation of test materials for bioassay tests

Pieces of sugar cane that could fit into the cylindrical chambers within the olfactometers were prepared. Sugar cane was chosen for this experiment because of its high attractant indices for both *Bulinus* and *Biomphalaria* snails (Dogbey, 1995). Secondly, sugar cane has a spongy nature which appears to have the capability of retaining chemical properties, and to release them slowly. With the aid of a sharp pointed needle, small holes were created longitudinally through the mid portion of each piece of sugar cane. Each hole measured about 2/3 of the full length of each sugar cane piece. Four different treatments were given to the test materials as follows:

(I) sugar cane pieces each with a single hole; (II) sugar cane pieces each with 3 holes; (III) sugar cane pieces each with 5 holes, and (IV) sugar cane pieces each with 7 holes.

With the aid of a micropipette of capacity, (50-200μl), 100μl of 0.6ppm that was prepared in the laboratory from dilution of a measured quantity of powdered bayluscide, was carefully poured into each hole. These were allowed to stand for a period so that the chemical will permeate the matrix of the sugar cane to mix well with chemicals of sugar cane. According to Meyer-Lassen et al (1994), 0.6ppm bayluscide concentration is capable of killing both *Bulinus* and *Biomphalaria* snails in water that is stagnant.

4.2.3 **Diffusion or Gradient olfactometers**

The olfactometers used consisted of a rectangular 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 2.0cm in depth. A perspex block consisting of 20 olfactometers was used (Plates 4a & 4b).

4.2.4 BIOASSAY EXPERIMENTS

The olfactometers were thoroughly washed with hot water and detergent and rinsed several times with tap water before and after each experiment. Tap water was poured into each chamber at the start of each experiment. The test materials and controls were placed in the cylindrical chambers, with one test material and one control oppositely placed in each central chamber. A snail was placed in the middle of each chamber. The position of each assay snail was noted at 2.5 minute intervals for 30 minutes. Snails that were found on the test side were considered positive, +, and recorded. Snails that were found on the side of the controls were scored negative, - .

In some instances the snails were found attached to the tests or controls. These were considered arrested, and were scored as (+), for tests and (-) for controls. The controls were sugar cane pieces without bayluscide. 20 controls and 20 test materials were used. In the first test the test materials had a single hole, and contained 100μl of 0.6ppm bayluscide. The experiment was performed for three more times with varying quantities of the bayluscide. After the 30 minute tests, the snails were allowed, in each case to remain in the set up to find out whether they will die after a period, since none of the snails died in the process of the tests. This was to be sure that the chemical molecules of the toxicant were also diffusing into the surrounding water. The results were analysed to determine the levels of significance of any difference in bioactivity by using paired t-test (Bailey, 1981).

Plate 4a; A perspex block of 20 olfactometers

Plate 4b; A set up for the diffusion bioassay experiments

Plate 4c; Sugar cane pieces joined together to form a grid, with holes containing bayluscide

Plate 4d; Calabashes with windows created on the sides

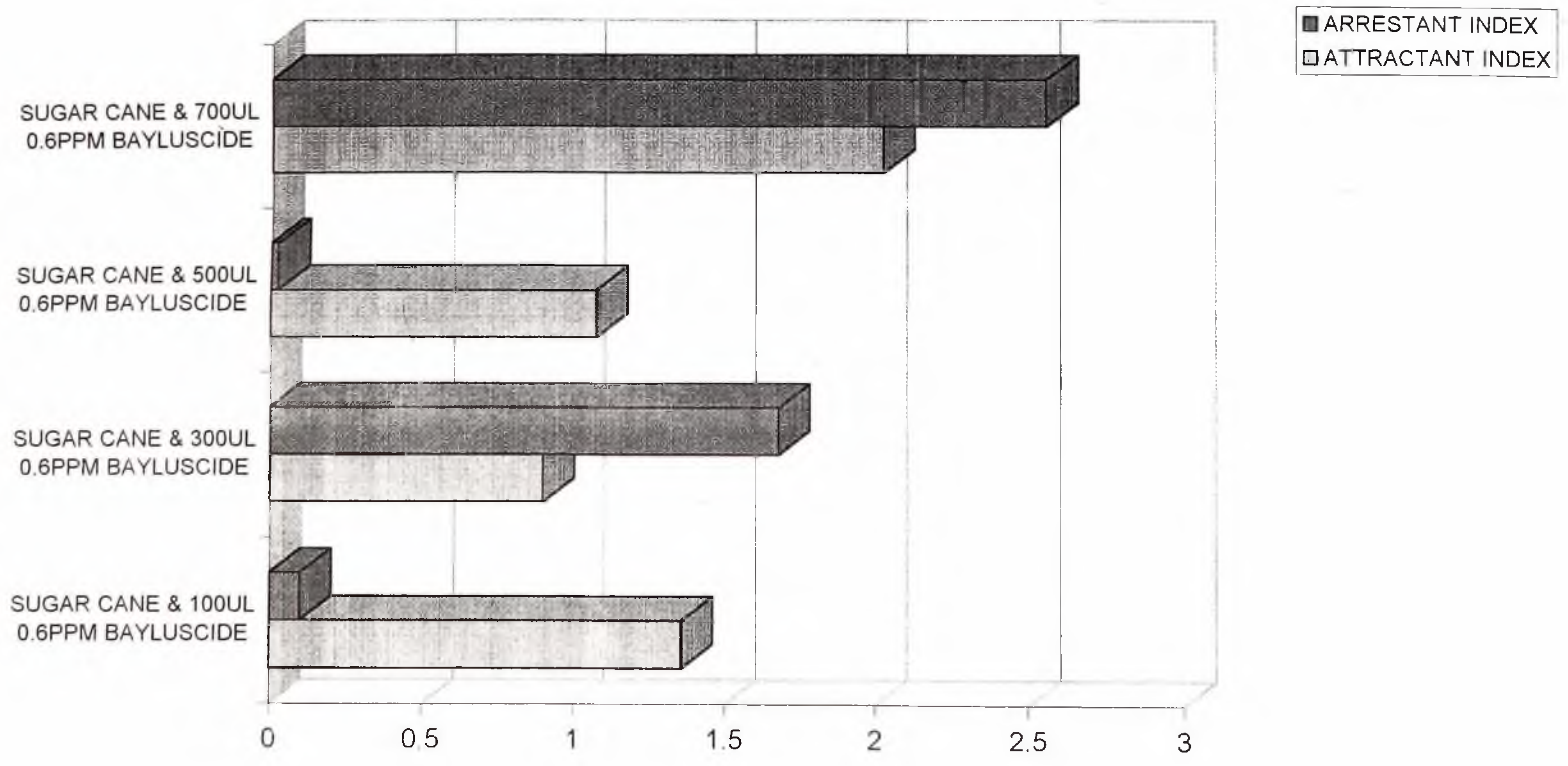
Table 4a; COMBINATION OF SUGAR CANE & TOXICANT (DIFFUSION BIOASSAYS) Statistical analysis using t-test.

Test in 20 olfactometers	MEAN ATTR. INDEX	LEVEL OF SIGNIFICANCE	MEAN ARR. INDEX	LEVEL OF SIGNIFICANCE
Sugar cane + 100μl of 0.6ppm bayl. (i.e. 1 hole per test material)	1.3579	-	0.1028	-
Sugar cane + 300μl of 0.6ppm bayl. (i.e. 3 holes per test material)	0.8992	-	1.6810	-
Sugar cane + 500μl of 0.6ppm bayl. (i.e. 5 holes per test material)	1.0771	-	0.0245	-
Sugar cane + 700μl of 0.6ppm bayl. (i.e. 7 holes per test material)	2.0269	-	2.5595	-

* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$

(ATTR. = Attractant; ARR. = Arrestant; bayl. = bayluscide)

FIGURE 4a: EFFICACY OF A COMBINATION OF AN ATTRACTANT (Sugar cane) AND A TOXICANT



4.3 RESULTS

Results of the bioassay tests using diffusion olfactometers indicated that attractant and arrestant properties of sugar cane were maintained when 0.6ppm of bayluscide, in various amounts was added. Of the four different tests which were performed, only the fourth test using the largest quantity of bayluscide (7 x 100µl) produced a significant change in the arrestant effect of the sugar cane. The first 3 tests namely, (I) sugar cane with only one hole and 100µl of the toxicant, (II) 3 holes and (3 x 100µl) of the toxicant, (III) 5 holes and (5 x 100µl) of the toxicant, produced no significant differences in attractant and arrestant indices, as can be seen from Table 4a. The fourth test also gave the highest attractant and arrestant indices (Figure 4a). It was discovered that in each of the tests, all the snails died within two hours.

4.4 DISCUSSION

The present results have made it possible to identify the extent of tolerance of the snails to attractants that have some amounts of toxicant (0.6 ppm bayluscide) combined with them. In the separate experiments involving additions of 100µl, (3 x 100µl), and (5 x 100µl), there was no significant difference ($p < 0.05$) in the time spent by the snails on the side of the test materials, as compared with the time spent on the side of 'test materials and toxicant'. This implies that addition of the toxicant factor in various quantities of 100µl, (3 x 100µl), and (5 x 100µl) per 6.495 gm of the sugar cane did not alter attractant effects of the sugar cane. However, using (7 x 100µl) of the toxicant, there was a difference in the time spent by snails attached to the test material (i.e. arrested) as compared with time spent attached (i.e. arrested) to 'test materials and toxicant' (controls). This difference was stastically significant ($p < 0.05$). This means that the arrestant effect of the sugar cane was altered when (7 x 100μl) of the toxicant was added to 6.495 gm of the sugar cane. This suggests that the quantity of toxicant can have an effect on the bioactive substance. Large quantities of the toxicant can probably produce a very significant change in attractant and arrestant properties. This may be due to the fact that with large volumes of the toxicant, its molecules may be encountered by the snails in the aquatic medium more frequently than the attractant molecules. The fact that none of the assay snails died in the process, that is within the 30 minutes period of the experiments indicates that the sucrose molecules of the sugar cane were probably diffusing out faster than that of the toxicant into the surrounding water medium to create a diffusion gradient. The snails were attracted to move up the chemical gradient using their chemoreceptors (Kpikpi, 1990). It seems very likely that within the test period (i.e. 30 minutes), not much of the toxicant molecules were encountered by the assay snails since the molecules probably had not yet diffused into the surrounding water medium. However, it was evident after 2 hours that the molecules of the toxicant had diffused into the water and the snails were killed. The slow rate of diffusion of the toxicant molecules was probably due to the position in the sugar cane test pieces at which it was introduced. The toxicant was actually introduced into the middle portion of the sugar cane test materials, and might have taken some time to pass through the matrix of sugar cane to reach the outside water environment.

4.5 Simulated Natural Environment Tests

4.5.1 PREPARATION OF TEST MATERIALS/ TRAPS FOR SIMULATED NATURAL ENVIRONMENT EXPERIMENTS.

The sugar cane grid trap, already described in the previous chapter was used. Each grid was prepared by joining 4 pieces of sugar cane that have already been split into two (Plate 4c). Each of the grids was then fixed inside a calabash trap with windows created on the sides (Plate 4d) after the toxicant had been added. Bayluscide toxicant was used in combination with the test materials. The same concentration (i.e. 0.6ppm of bayluscide) used in the olfactometer diffusion assays was used in varying quantities in separate experiments. The quantities of the bayluscide needed to be added to each sugar cane grid was calculated by simple proportion, based on the combination used in the olfactometer diffusion tests. This determined the number of holes to be created in each test material. Small holes were then created in the sugar cane pieces by means of a giant needle. By means of a micropipette 100μl was measured and carefully emptied into each hole. These test materials were then allowed to stand for a period of 30 – 60 minutes for the chemical to diffuse into the spongy nature of the sugar cane. They were then placed in the prepared calabash traps and were ready for use. In the first test, 1424.9μl of 0.6ppm bayluscide was added to 92.549gm of sugar cane (i.e. weight of each sugar cane grid). In the second test the quantity of bayluscide was increased to 4,274.5μl (i.e. 3 x amount in the first test). In the third test, 7,124.5μl of 0.6ppm bayluscide (i.e. 5 x amount in the first test) was used.

Sample of calculation of bayluscide used:
Range of weights of sugar cane pieces in olfactometer = 6.501 – 6.489gm

Average weight = 6.495gm,
this weight corresponded with 100µl of 0.6ppm bayluscide (i.e. for 1 hole).
Range of weights of sugar cane grids for S.N.E. experiments = 89.099 – 95.999gm
Average weight = 92.549gm.
Thus, quantity of bayluscide needed for 92.549gm of sugar cane = 92.549/6.495 x 100
= 1,424.9µl,

and this implies that 3-4 holes be created in each of the 4 sugar cane pieces forming the whole grid. Following similar calculations it was realised that, for the second test a total of 4,274.5µl of bayluscide will be added to the sugar cane grid, which means that 10-11 holes be created in each of the 4 pieces forming a grid. Also, in the third experiment 7,124.5µl of bayluscide was to be added to each sugar cane grid, which means that 17-18 holes be created in each of the 4 pieces.

4.5.2 THE SUMULATED NATURAL ENVIRONMENT EXPERIMENTS

10 test traps and 10 control traps were set up in the aquarium. Each trap was placed in the aquarium water, slightly submerged and suspended from a wooden bar placed at the mouth of the aquarium (Plate 4f). The control traps were similar sugar cane grid traps without the toxicant. All the test traps were arranged on one half of the aquarium whilst the control traps were also placed on another half. One week prior to the beginning of the experiments, 800 adult snails were selected and introduced into the aquarium to get them acclimatised to the aquarium conditions. They were fed routinely with lettuce leaves, but were denied of food 24 hours before each experiment. The traps were inspected every 12 hours. At each inspection, the traps were removed from the aquarium individually and the snails that were found inside the traps, as well as snails attached to the outside part of the calabashes were considered trapped. They were counted and recorded. Suspected killed snails in each trap were also counted; and to be very sure the snails were dead they were removed and placed in water to find out whether they will recover. Dead snails were also identified by change of colour of the snails or inability to bubble in 5% potassium hydroxide (Okafor, 1990). Three different sets of

experiments were performed in the aquarium separately, with varying quantities of 0.6ppm bayluscide combined with the sugar cane.

Plate 4e; Traps ready for use

Plate 4f; Traps placed in aquarium, suspended by mea[illegible] strings tied to wooden bars at mouth of the aquarium

4.6 Results

The numbers of snails trapped after every 12 hours for each of the 3 different experiments have been depicted in Figures 4b, 4c, and 4d. Table 4c shows the total number of snails trapped and recorded over a 3-day period. The amount of the toxicant was varied in the 3 different experiments to find out its lethal effect on the snails as they were attracted by the attractant. It can be observed that the test, involving the use of sugar cane (92.579gm) and 7,124.5µl of the toxicant (i.e. 0.6ppm bayluscide) recorded the highest number of snails trapped (i.e. 231 snails). Out of this, 15 snails forming 6.44% of the total snails were killed. This was followed by the test involving sugar cane (92.579gm) and 1,424µl of the toxicant. A total of 211 snails were trapped, out of which none was killed. In the third test, in which sugar cane and 4,274.5µl of the toxicant was used, a total of 196 snails was recorded as snails trapped. Out of this number, 16 snails forming 8.33% were killed. The control traps which were made up of the same quantity of sugar cane (i.e. 92.579gm) but no toxicant added, in similar traps revealed a trend of results that was similar to those of the test traps. Observations that were made revealed that none of the snails died in any of the control traps. A comparison of the number of snails trapped in test traps and those of the control traps shows that there was not much difference in the snails trapped (Figure 4f). A statistical analysis (Table 4d) suggests that with the exception of the test involving the use of 1,424µl of bayluscide, in which results after 24 hours and 60 hours gave a significant difference ($p < 0.05$), all the other tests showed no significant difference in number of snails trapped in test traps as compared with control traps.

Table 4b; PERCENTAGE OF SNAILS KILLED BY COMBINATION OF SUGAR CANE (92.579gm) & VARIOUS AMOUNTS OF 0.6ppm BAYLUSCIDE IN SIMULATED NATURAL ENVIRONMENT EXPERIMENTS

TEST	DURATION (HOURS)	T	Tk	K%	C	Ck	K%
SUGAR CANE (92.579gm) & 0.6ppm BAYLUSCIDE (1,424.9μl)	12	24	0	0	29	0	0
	24	39	0	0	24	0	0
	36	29	0	0	37	0	0
	48	35	0	0	36	0	0
	60	46	0	0	38	0	0
	72	38	0	0	45	0	0
SUGAR CANE (92.579gm) & 0.6ppm BAYLUSCIDE (4,274.5μl)	12	34	11	32.4	27	0	0
	24	19	5	26.2	23	0	0
	36	34	0	0	39	0	0
	48	29	0	0	35	0	0
	60	42	0	0	39	0	0
	72	38	0	0	38	0	0
SUGAR CANE (92.579gm) & 0.6ppm BAYLUSCIDE (7,124.5μl)	12	50	14	28.0	50	0	0
	24	40	1	2.50	37	0	0
	36	25	0	0	37	0	0
	48	21	0	0	28	0	0
	60	45	0	0	49	0	0
	72	52	0	0	62	0	0

T = Total number of snails trapped in test traps; Tk = Total number of snails found killed in test traps; K% = percentage of snails killed; C = Total number of snails trapped in control traps; Ck = Total number of snails found killed in control traps.

Table 4c; PERCENTAGE OF SNAILS KILLED BY COMBINATION OF SUGAR CANE & VARIOUS AMOUNTS OF BAYLUSCIDE IN 3 DAYS OF SIMULATED NATURAL ENVIRONMENT EXPERIMENTS.

DURATION	TEST/TREATMENT	T	Tk	K%	C	Ck	K%	T.I.
3 DAYS	Sugar cane (92.579gm) & 1,424µl of 0.6ppm bayluscide	211	0	0	209	0	0	2
3 DAYS	Sugar cane (95.579gm) & 4,274µl of 0.6ppm bayluscide	196	16	8.33	201	0	0	5
3 DAYS	Sugar cane (92.579gm) & 7,124.5µl of 0.6ppm bayluscide	231	15	6.44	263	0	0	32

T = Total number of snails trapped in test traps; Tk = Total number of snails found killed in test traps; K% = percentage of snails killed; C = Total number of snails trapped in control traps; Ck = Total number of snails found killed in control traps;

T.I. = Trapping index.

* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$

Table 4d; NUMBER OF SNAILS TRAPPED IN A SIMULATED NATURAL ENVIRONMENT EXPERIMENTS INVOLVING A COMBINATION OF AN ATTRACTANT & A TOXICANT.

TEST	DURATION (HOURS)	TRAPPING INDEX	LEVEL OF SIGNIFICANCE
(1) Sugar cane (92.549gm) & 1,424µl of 0.6ppm of bayluscide	12	-5	-
	24	15	*
	36	-8	-
	48	1	-
	60	12	*
	72	-7	-
(2) Sugar cane (92.549gm) & 4,274.5µl of 0.6ppm of bayluscide	12	7	-
	24	-4	-
	36	-5	-
	48	-6	-
	60	3	-
	72	0	-
(3) Sugar cane (92.549gm) & 7,124.5µl) of 0.6ppm bayluscide	12	0	-
	24	3	-
	36	-12	-
	48	-7	-
	60	-4	-
	72	-10	-

* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$

FIGURE 4b; EFFICACY OF COMBINATION OF 92.549gm SUGAR CANE & 1424.9ul OF 0.6 ppm BAYLUSCIDE IN S.N.E. EXPERIMENT

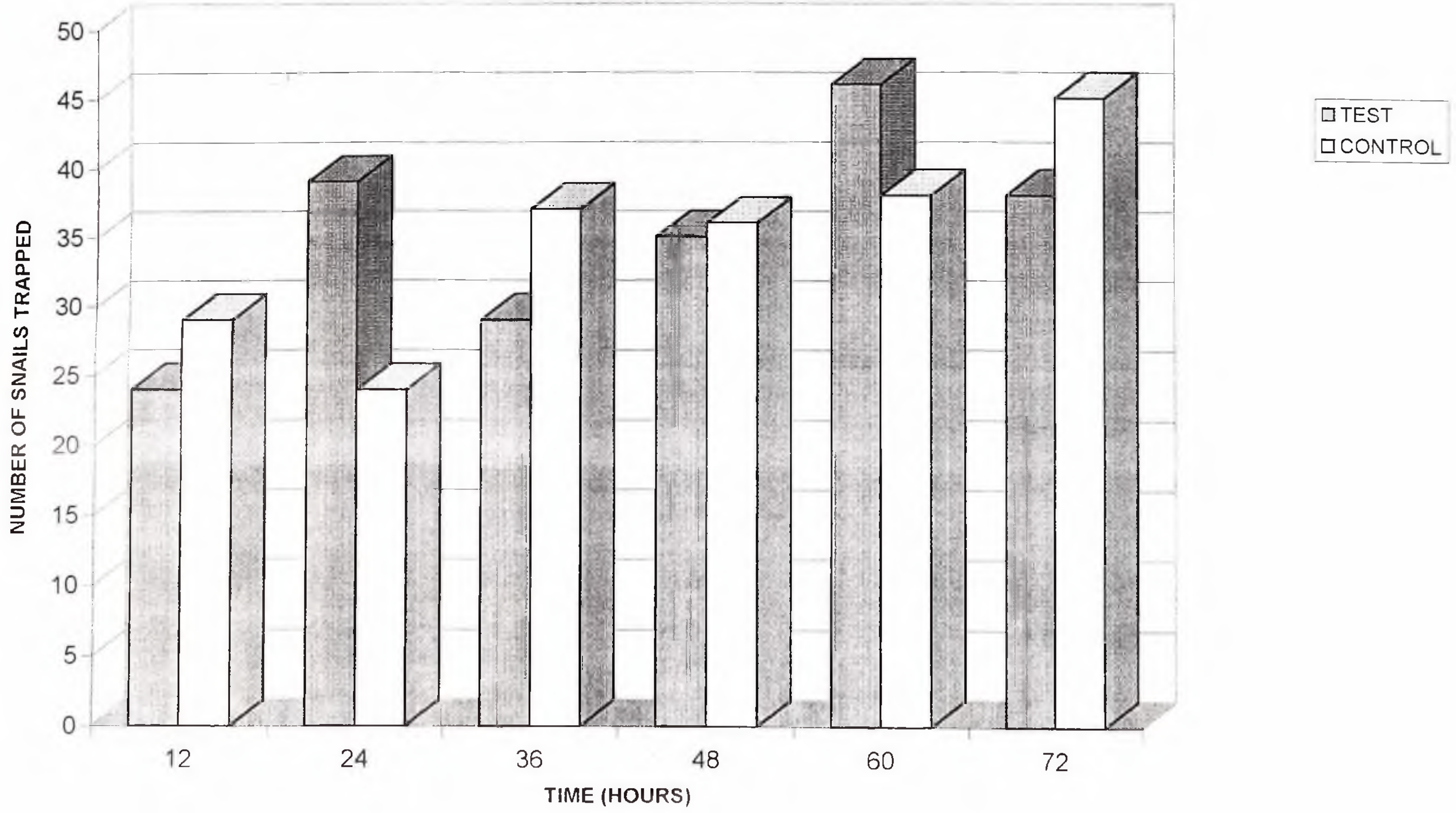


FIGURE 4c; EFFICACY OF COMBINATION OF 92.549gm SUGAR CANE & 4,274.5ul BAYLUSCIDE IN S.N.E. EXPERIMENT

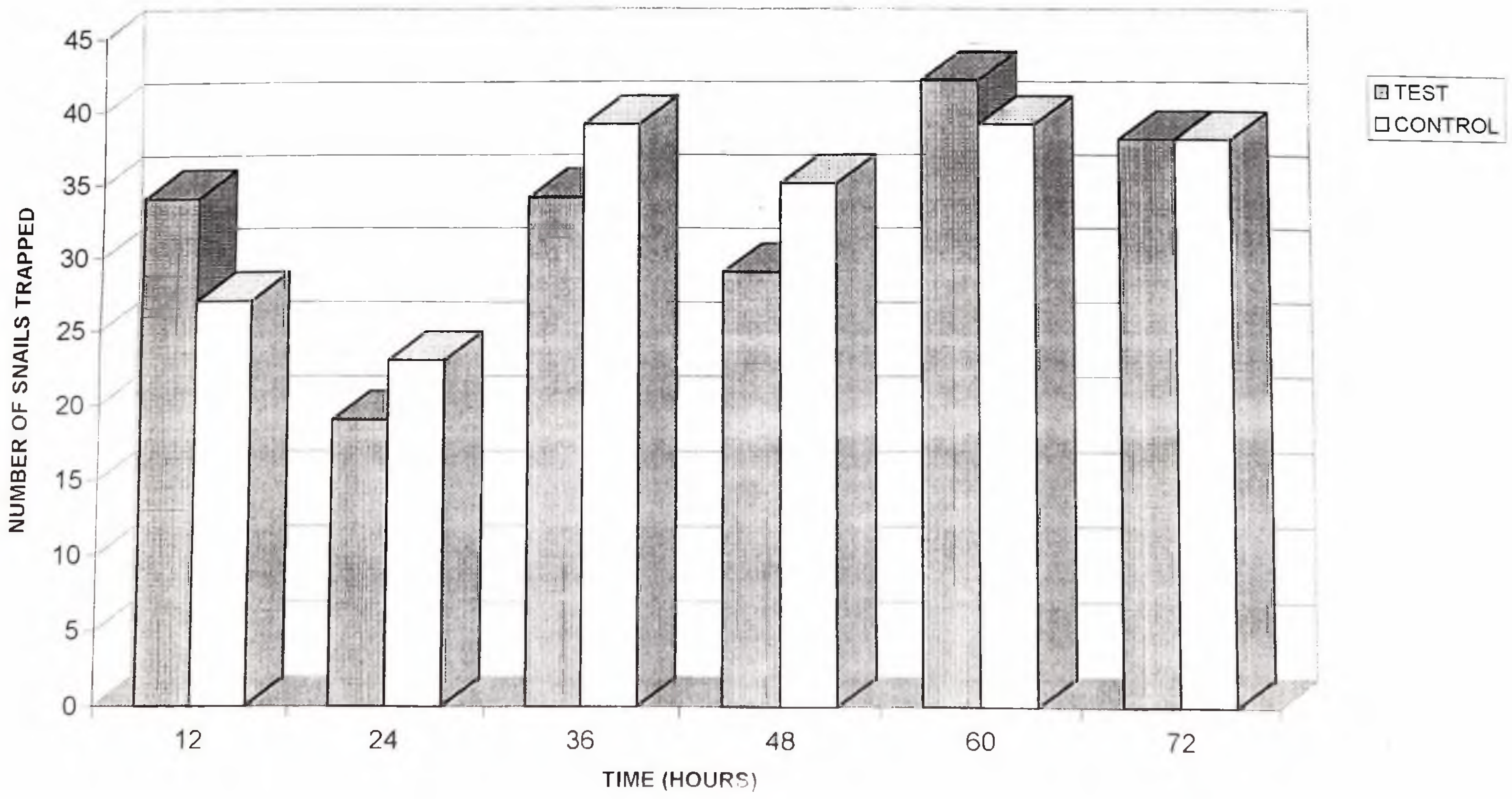
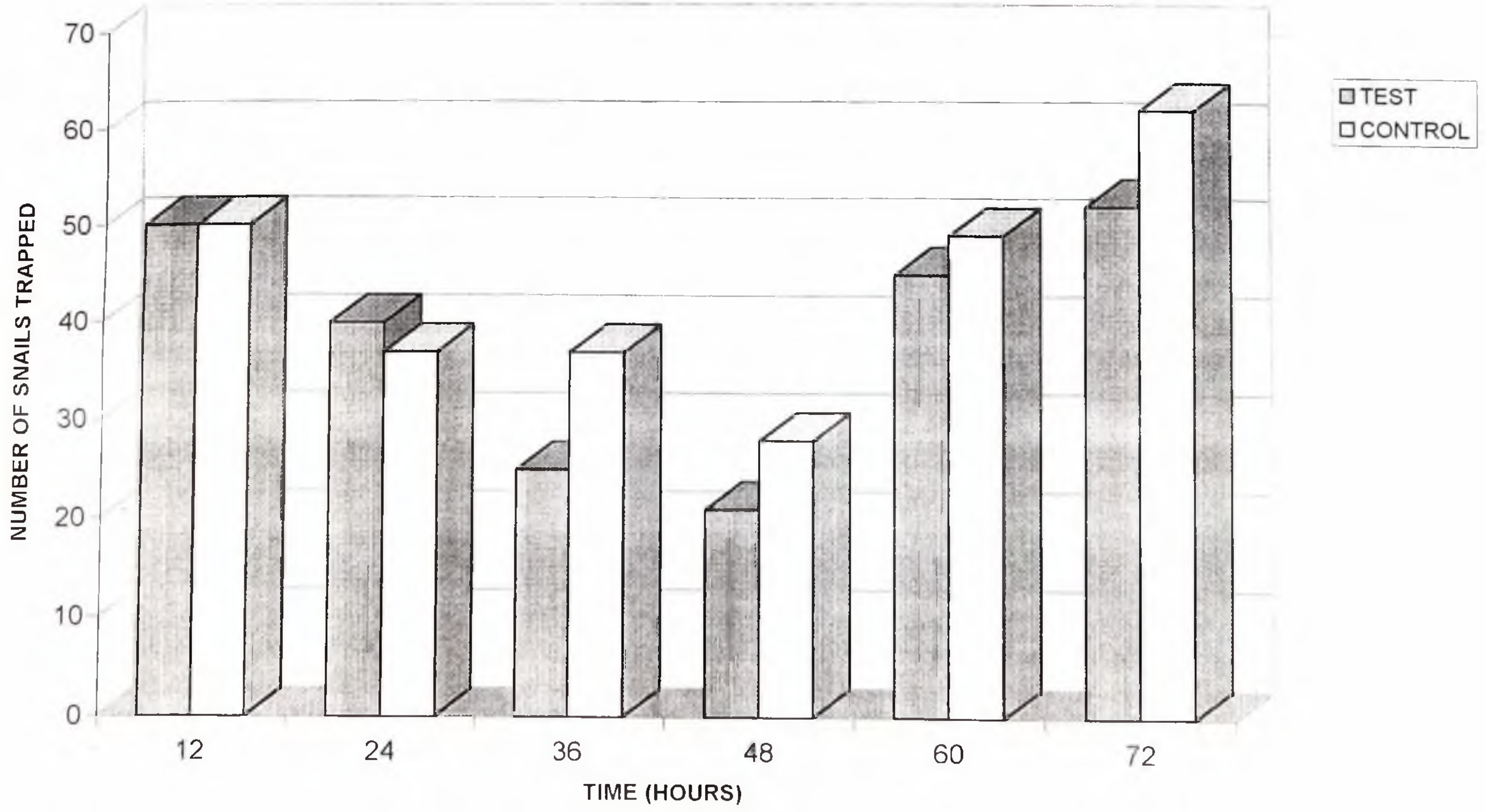


FIGURE 4d; EFFICACY OF COMBINATION OF 92.549gm SUGAR CANE & 7,124.5ul BAYLUSCIDE IN S.N.E. EXPERIMENT



4.7 Discussion

The results obtained from the simulated natural enviroment experiments indicate that a combination of some limited amount of 0.6ppm bayluscide and sugar cane was capable of attracting the schistosome host snails. This conforms with results obtained from the diffusion bioassay experiments using olfactometers. From Figure 4f it could be seen that there was not much difference in snails attracted by test traps as compared with the controls. A statistical analysis, shown in Table 4d indicates that there was no significant difference in almost all the tests. The importance of this observation is that the potency of sugar cane as an attractant or arrestant still persists although some amount of toxicant had been added. The potency of sugar cane to attract snails in simulated natural environment was first studied by Dogbey (1995).

With regards to the killing effect of the attractant-toxicant combination it was realised that none of the snails died in the first test in which 1,424.9μl of 0.6ppm bayluscide was used. This was probably because the quantity of the toxicant was so small that it had minimal effect in the aquarium. In experiments 2 and 3, the quantity of toxicant was increased to 4,274.5μl and 7,124.5μl respectively and it was observed that a number of snails out of those trapped, that is, 8.33% in experiment 2 and 6.4% in experiment 3 (Table 4c) were killed.

The trapping system was designed such that it formed an enclosure with only few small windows at the sides (Plate 4e) so that the snails trapped could be killed by the toxicant. The toxicant's inability to kill all or a larger number of the snails trapped may be due to a dilution effect of the toxicant by the water. Although the toxicant was introduced into very small holes created inside the sugar cane pieces it might be possible for some of the water to diffuse into the sugar cane and effect a dilution before the snails got into contact with the toxicant. This same reason probably explains why the snails were killed only during 12 hours and 24 hours after setting the traps. After the 24 hours the aquarium water might have diluted the toxicant beyond its lethal effect and thus its inability to kill the snails that were trapped.

Although the percentage of snails killed was small (Figure 4e), it gives a strong indication that such a system is workable.

CHAPTER FIVE

A FIELD EVALUATION OF THE MOST EFFECTIVE TRAPPING UNIT & ATTRACTANT-TOXICANT COMBINATION

5.1 Introduction

The indiscriminate destruction of non-target organisms, chemical pollution of water bodies and high cost of molluscicides have been considered as major problems associated with its use to control the schistosome host snails. These have prompted many scientists to research into other alternative methods of controlling the snails. Essentially, the aim of these methods has been that a method that employs the discharge of little amounts of the molluscicide, that is in controlled manner can be a useful alternative. In view of this, Cardarelli et al (1977) investigated the possibilities of using controlled release technology to increase the efficiency of molluscicide applications.

To aid in target specificity the use of attractants, arrestants and phagostimulants in combination with snail toxicants has been suggested by Thomas et al (1980). Thomas (1995) also studied the possibility of using microcapsules, formed from attractants, arrestants and phagostimulants in combination with snail toxicants to kill schistosome host snails.

Already, studies on chemoreception niches of various schistosome host snails have revealed a lot of chemical compounds that are capable of attracting, arresting, or to act as phagostimulants to these snails (Thomas et al, 1980, 1985, 1986, & 1989; Thomas & Assefa, 1979; Kpikpi & Thomas, 1993). Through a series of studies conducted by Dogbey (1995), Kpikpi & Ansa (1995), and Domeh (1998) some naturally occurring bioactive substances have also been identified.

Dogbey (1995) discovered that sugar cane, pawpaw, sweet potato, and cassava which were tested in the Weija lake near Accra, Ghana acted as potential attractant and arrestant to *Biomphalaria pfeifferi* and *B. truncatus*. This revealed concordance with results of laboratory experiments.

Earlier on Kpikpi (1990) had also discovered similarly that maltose acted as a potential attractant, arrestant and phagostimulant to *B. globosus* in the field in Lake Kpong – Ghana. This result was also in agreement with results of laboratory experiments.

In the present study, similar work conducted under simulated natural conditions in the laboratory revealed that cocoyam, cassava, and sweet potato (all in their raw fermented states) as well as sugar cane and the peels acted as attractant and arrestant to both adults and juveniles of *Bulinus truncatus* and *Biomphalaria pfeifferi* (Chapter 3). Furthermore, experiments conducted under similar conditions showed that when sugar cane was combined with a snail toxicant the attractant property of sugar cane was maintained when limited amounts of the toxicant were used. There were also preliminary studies for attractant-toxicant combination in diffusion bioassays using olfactometers (Chapter 4).

To ascertain whether the fermented bioactive substances, as well as the sugar cane-toxicant combination would exert the same effects on the schistosome host snails in their natural environments, plans were made to evaluate their efficacy. This was done in the Weija lake near Accra, Ghana. The present chapter describes the field studies.

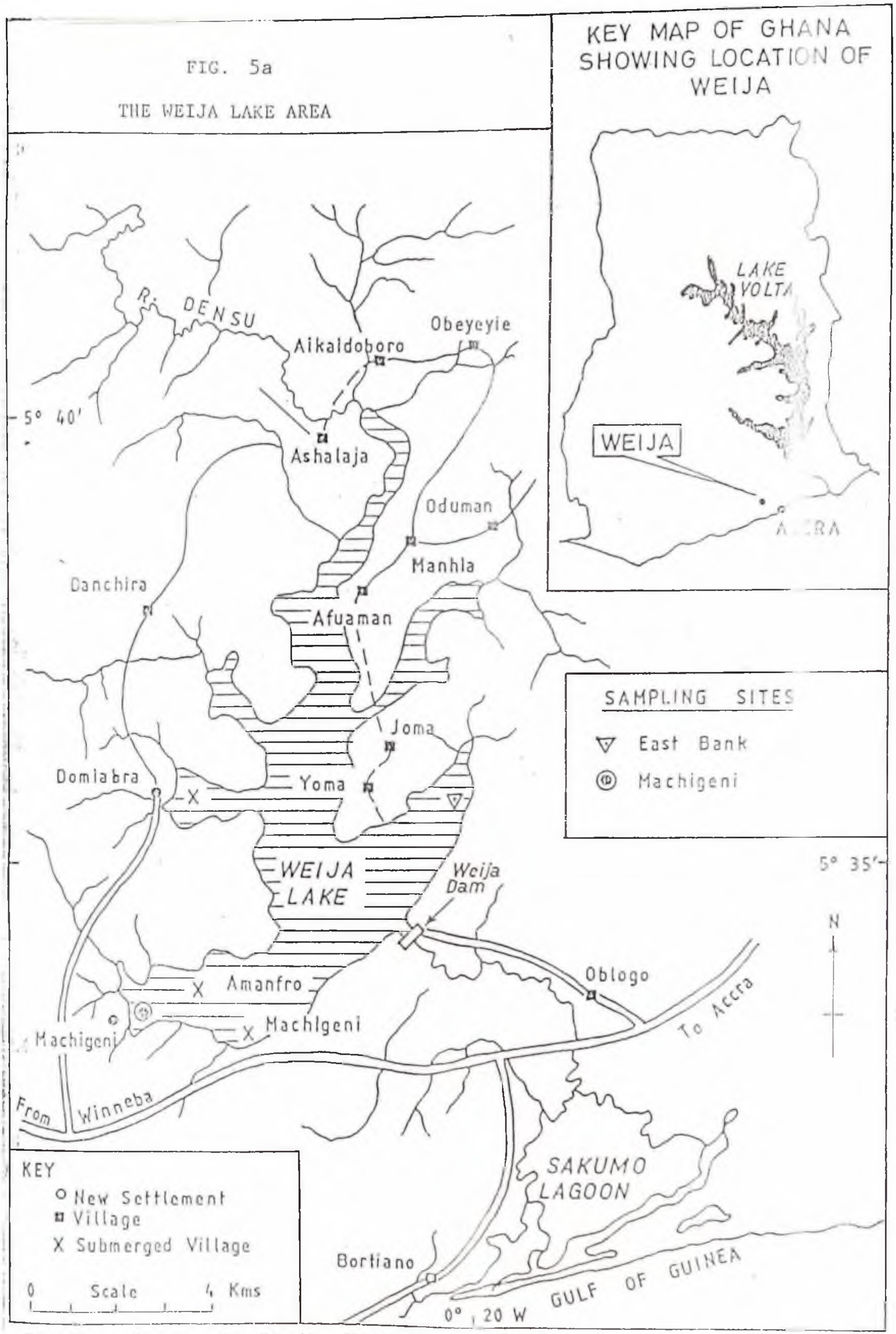
5.2 The study areas

Weija lake located 17 km of Accra is situated on Latitude 5° 30', Longitude 0° 20' (Figure 5a). The lake was created by construction of a dam on river Densu. Work on the construction started in 1973 and was completed in 1978 (deGraft Johnson, 1977). The first dam which was constructed was destroyed and washed away. The reservoir lies within 0° 20′ N and 5° 45 N. The lake covers approximately 3361.5 hectares of land at maximum water level, with an estimated storage capacity of 116.04 x $10^6 m^3$ (Dissan and Abban, 1979). Shoreline of the lake is 48 km. The normal surface elevation is estimated at 14.37m with a maximum of 15.24m (Nukunya et al, 1979, as cited by Zuta, 1994). The reservoir provides water to western Accra, supports an irrigation facility and is also an important fishery. The Weija lake area is low lying with undulating topography and isolated ridges (Plate 5e). The area is lacking in riverine forest but has a scanty natural tree population made up mainly of Borassus palm (deGraft-Johnson, 1977). It principally consists of a dense thicket interspersed with small patches of grass (Boateng, 1970; Rose Innes, 1977). Climatic conditions are tropical with temperatures averaging 27°C. Rainfall at this area is moderate and seasonal averaging 65mm anually. The rainfall has its peak in June, with dry periods during December through March. The riparian population there are of different ethnic backgrounds, these include Ga-Adangbe, Ewe, Akan, and Northeners. These people were mostly peasant farmers, freshwater fishermen and traders. Just about a short distance from the lake (less than 1 km) can be found some squatter communities in which some of the fishermen live. The people lack sanitary facilities and pipe-borne water supply. There is one major contact site for the villages where activities such as swimming, bathing, washing, docking of canoes, trading, and domestic water collection take place. The people who are involved in these activities are of all ages and of both sexes. As a result of the non availability of toilet facilities the people defaecate in the bushes that are very close to the water contact site; the result is that any time it rains the faecal matter is washed into the lake water by run-off water. The host snails encountered at the site included *B. truncatus* and *B. pfeifferi*. The presence of these snails coupled with the activities at the contact sites enhance the transmission prevalence of schistosomiasis in communities at Weija. Zuta (1994) reported that the prevalence rates of *S. haematobium* generally decreased with increasing distance from the Weija lake. Prevalence rate of *S. haematobium* was significantly higher in the squatter communities which were closer to the lake, as compared to the townships.

The site was chosen for the present studies partly because of the history of schistosomiasis in the surrounding communities, as well as presence of the snail species, *B. truncatus* and *B. pfeifferi* in the water body. The choice of trap location was influenced by the availability of aquatic vegetation, predominantly among which were *Ceratophyllum demersum, Pistia, Echinochloa pyramidalis* and *Ipomoea aquata*, all found in the ecotone zone of the water. The snails have a close association with some of these plants (Paperna, 1969; Odei, 1983).

FIG. 5a

THE WEIJA LAKE AREA



Source : from de Graft - Johnson (1977)

5.3.1.2 EFFICACY OF BIOACTIVE SUBSTANCES

The test materials, 1 day fermented cocoyam, and 7 days fermented sweet potato were prepared as described in Chapter 3. They were tied individually to macrophytes along the banks of the lake. These traps were inspected every 12 hours for snails trapped. They were identified, counted and recorded.

5.3.1.3 EFFICACY OF ATTRACTANT-TOXICANT COMBINATION

The test materials were prepared as described in Chapter 4, and tested with the best trapping unit. The trapping units were tied to the macrophytes by means of strings attached to the traps to ensure that they were only slightly submerged. During inspection of the traps after every 12 hours the traps were carefully removed to identify any dead snails, and then the total numbers of snails were also counted and recorded.

5.4 Results

5.4.1 THE EFFICACY OF BIOACTIVE SUBSTANCES

The results show that significantly more snails were caught in test traps as compared with control traps in the area with a dense population of snails ($p < 0.05$) (Figures 5c & 5e), Tables 5a(ii) & 5b(ii). Results obtained from the experiment conducted in the less dense populated area also showed that significantly more snails were caught in test traps as compared with control traps ($p < 0.05$) for some periods only, whilst during the other periods although more snails were caught in the test traps, the difference was not statistically significant ($p < 0.05$) (Figures 5b & 5d), Tables 5a (i) and 5b(i).

5.4.2 EFFICACY OF ATTRACTANT-TOXICANT COMBINATION

From Table 5e, (Figure 5f), it can be observed that the numbers of snails caught in test traps were not very different from those caught with control traps. Statistical analysis indicated that the difference was not significant ($p < 0.05$) (Table 5f). The test trapping unit, which contained the toxicant was capable of killing snails for only 24 hours, that is, after 12 hours when 34.39% of the snails trapped were killed, and 24 hours when 23.07% of snails trapped were killed (Table 5e), (Figure 5g). Beyond this period (24 hours) none of the snails died in the test traps. In the control traps none of the snails caught were found dead.

Table 5a(i) FIELD EVALUATION OF 1 DAY FERMENTED COCOYAM -AMONG LESS MACROPHYTES/ LESS DENSE POPULATED AREA.

Duration	Number of snails trapped (test)			Number of snails trapped (control)			Trp.
(hours)	BUL	BIOM	TOTAL	BUL	BIOM	TOTAL	Index
12	6	8	14	4	3	7	7
24	13	10	23	2	4	6	17**
36	13	13	26	12	7	19	7
48	14	8	22	4	4	8	14*
60	15	10	25	2	8	10	15**
72	18	3	21	3	12	15	6

(ii) FIELD EVALUATION OF 1 DAY FERMENTED COCOYAM -AMONG MANY MACROPHYTES/ DENSE POPULATION AREA

Duration	Number of snails trapped (test)			Number of snails trapped (control)			Trp.
(hours)	BUL	BIOM	TOTAL	BUL	BIOM	TOTAL	Index
12	30	25	55	13	12	25	30***
24	35	21	56	15	8	23	33***
36	14	23	37	5	7	12	25**
48	22	10	32	12	13	25	7
60	16	21	38	11	5	18	20**
72	44	43	87	21	16	37	50***

BUL = *Bulinus truncatus*; BIOM = *Biomphalaria pfeifferi*; Trp. = Trapping

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

Table 5b (i) FIELD EVALUATION OF 7 DAYS FERMENTED SWEET POTATO - AMONG LESS MACROPHYTES/ LESS DENSE POPULATION AREA

Duration	Number of snails trapped (test)			Number of snails trapped (control)			Trp.
(hours)	BUL	BIOM	TOTAL	BUL	BIOM	TOTAL	Index
12	7	5	12	0	3	3	9*
24	8	8	16	1	3	4	12**
36	2	2	4	0	1	1	3
48	3	2	5	0	1	1	4
60	2	1	3	1	1	2	1
72	3	3	6	1	0	1	5*

(ii) FIELD EVALUATION OF 7 DAYS FERMENTED SWEET POTATO -AMONG MANY MACROPHYTES/ DENSE POPULATED AREA

Duration	Number of snails trapped (test)			Number of snails trapped (control)			Trp.
(hours)	BUL	BIOM	TOTAL	BUL	BIOM	TOTAL	Index
12	4	32	36	0	2	2	34***
24	6	17	23	2	5	7	16**
36	13	27	50	3	3	6	44***
48	11	26	37	1	2	3	34***
60	5	18	23	3	6	9	14**
72	7	17	24	4	4	8	16**

BUL = *Bulinus truncatus*; BIOM. = *Biomphalaria pfeifferi*; Trp = Trapping

* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

Table 5c; TABLE SHOWING SOME PARAMETERS MEASURED DURING PRELIMINARY SURVEY

Quadrat	Avge water Depth/cm	PH	Conductivity (ms/cm)	Avge Temp.(°C)	Avge Oxy. Conc. (mg/l)	Total number of snails counted	
						BUL	BIOM
1	30.2	6.1	0.3421	28.7	2.3	6	9
2	28.6	5.8	0.4105	29.2	3.4	4	5
3	22.5	5.8	0.4212	30.1	3.8	4	3
4	24.0	5.9	0.3853	30.3	3.9	10	10
5	24.2	6.0	0.3287	30.1	4.1	6	13

Avge = average; temp. = temperature; oxy. = oxygen; conc. = concentration;

BUL = *Bulinus truncatus*; BIOM= *Biomphalaria pfeifferi*.

Quadrats 1, 4, and 5 were water contact sites.

Table 5d; TOTAL NUMBER OF SNAILS TRAPPED AT THE TWO CONTRASTING MICRO-ENVIRONMENTS BY USE OF THE TWO BIOACTIVE SUBSTANCES.

EXPERIMENT	TOTAL NUMBER OF SNAILS TRAPPED IN 3 DAYS					
	TEST			CONTROL		
	BUL	BIOM	TOTAL	BUL	BIOM	TOTAL
EFFICACY OF 1 DAY FERMENTED COCOYAM (IN LESS POPULATED AREA)	79	52	131	27	38	65
EFFICACY OF 1 DAY FERMENTED COCOYAM (IN DENSE POPULATION AREA)	161	143	304	77	61	138
EFFICACY OF 7 DAYS FERMENTED SWEET POTATO (LESS DENSE POPULATION AREA)	25	21	46	3	9	12
EFFICACY OF 7 DAYS) FERMENTED SWEET POTATO (IN DENSE POPULATION AREA)	46	137	183	13	22	35

BUL = *Bulinus truncatus*; BIOM = *Biomphalaria pfeifferi*.

Table 5e; EFFICACY OF SUGAR CANE-BAYLUSCIDE COMBINATION AS AN ATTRACTANT/ ARRESTANT KILLER

Dur (hrs)	NUMBER OF SNAILS TRAPPED						NUMBER OF SNAILS KILLED						PERCENTAGE OF SNAILS KILLED (%)	
	Test			Control			Test			Control			Test	Control
	Bu	Bi	**T**	Bu	Bi	**T**	Bu	Bi	**T**	Bu	Bi	**T**		
12	16	25	**41**	30	14	**44**	6	8	**14**	0	0	**0**	34.39	0.00
24	16	10	**26**	15	6	**21**	4	2	**6**	0	0	**0**	23.07	0.00
36	21	7	**28**	15	10	**25**	0	0	**0**	0	0	**0**	0.00	0.00
48	15	4	**19**	6	14	**20**	0	0	**0**	0	0	**0**	0.00	0.00
60	17	6	**23**	6	12	**18**	0	0	**0**	0	0	**0**	0.00	0.00
72	12	7	**19**	14	4	**18**	0	0	**0**	0	0	**0**	0.00	0.00

Bu = Bulinus truncatus; Bi = Biomphalaria pfeifferi; **T** = total of both snails;
Dur = duration

Table 5f; Statistical analysis to determine whether combination of toxicant has a significant effect on attractant/ arrestant potency of sugar cane.

Duration (hours)	Number of snails in test traps	Number of snails in control traps	Trapping Index
12	41	44	-3
24	26	21	5
36	28	25	3
48	19	20	-1
60	23	18	5
72	19	18	1

* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$

FIGURE 5b; EFFICACY OF 1 DAY FERMENTED COCOYAM (IN LESS DENSE POPULATION AREA)

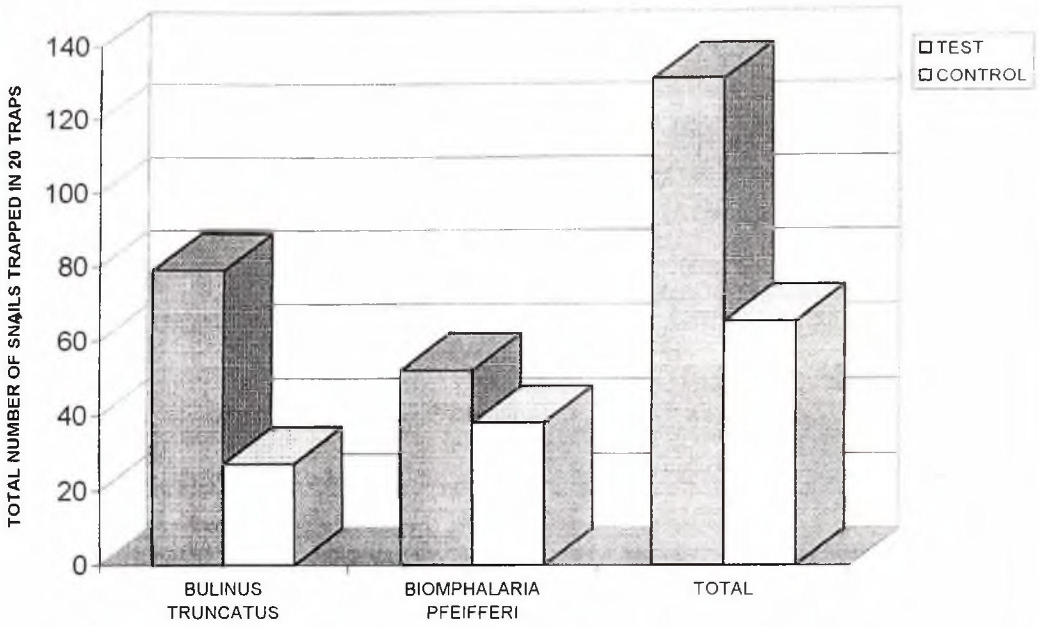


FIGURE 5c; EFFICACY OF 1 DAY FERMENTED COCOYAM (IN DENSE POPULATION AREA)

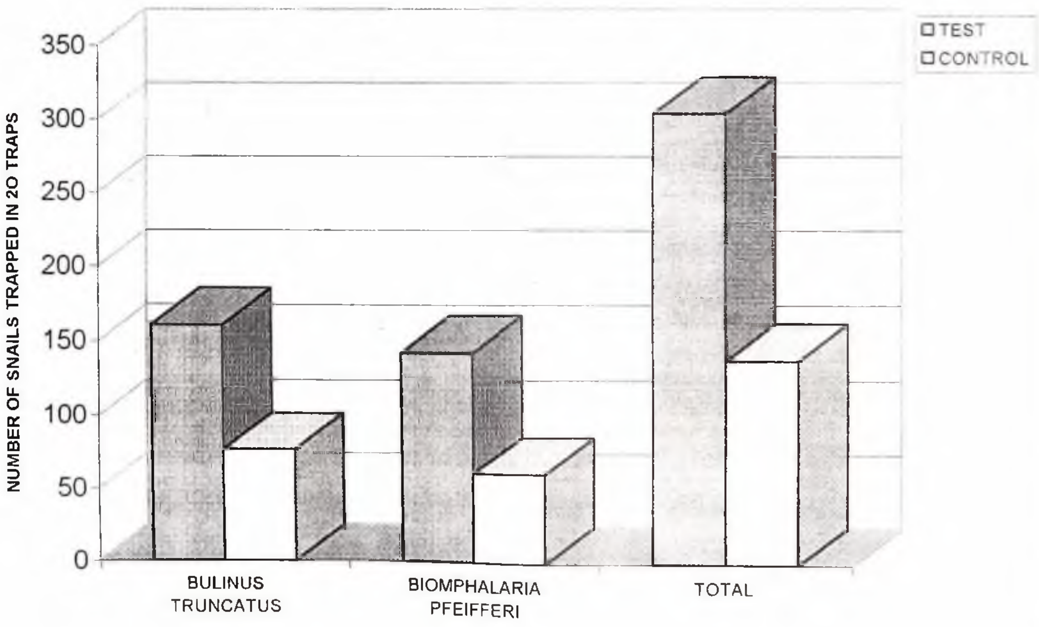


FIGURE 5d; EFFICACY OF 7 DAYS FERMENTED SWEET POTATO (IN LESS DENSE POPULATION AREA)

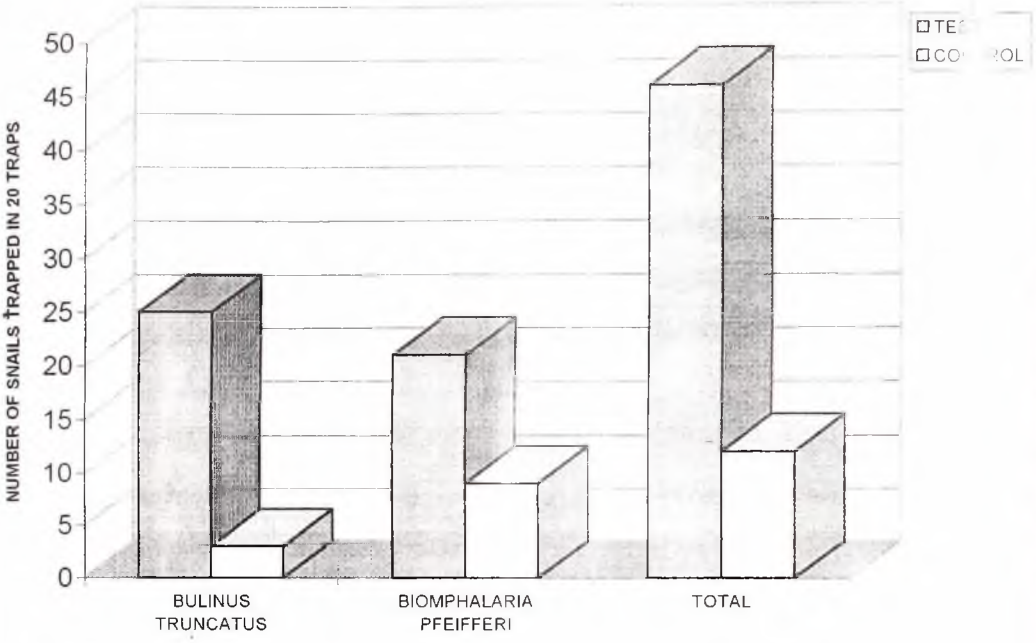


FIGURE 5e; EFFICACY OF 7 DAYS FERMENTED SWEET POTATO (IN A DENSE POPULATION AREA)

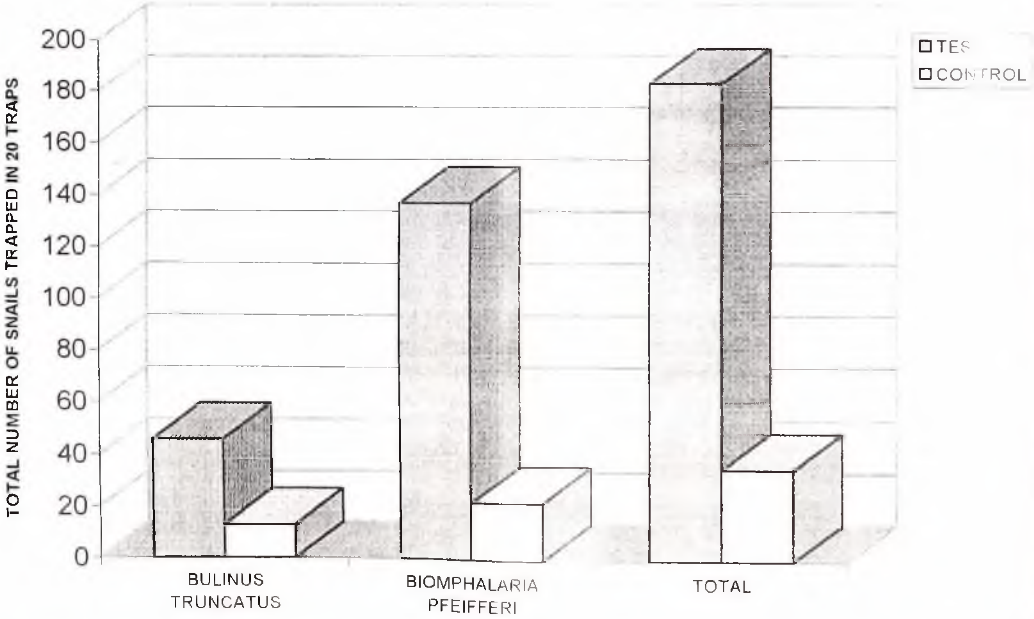


FIGURE 5f; A COMBINATION OF SUGAR CANE & 0.6 ppm BAYLUSCIDE

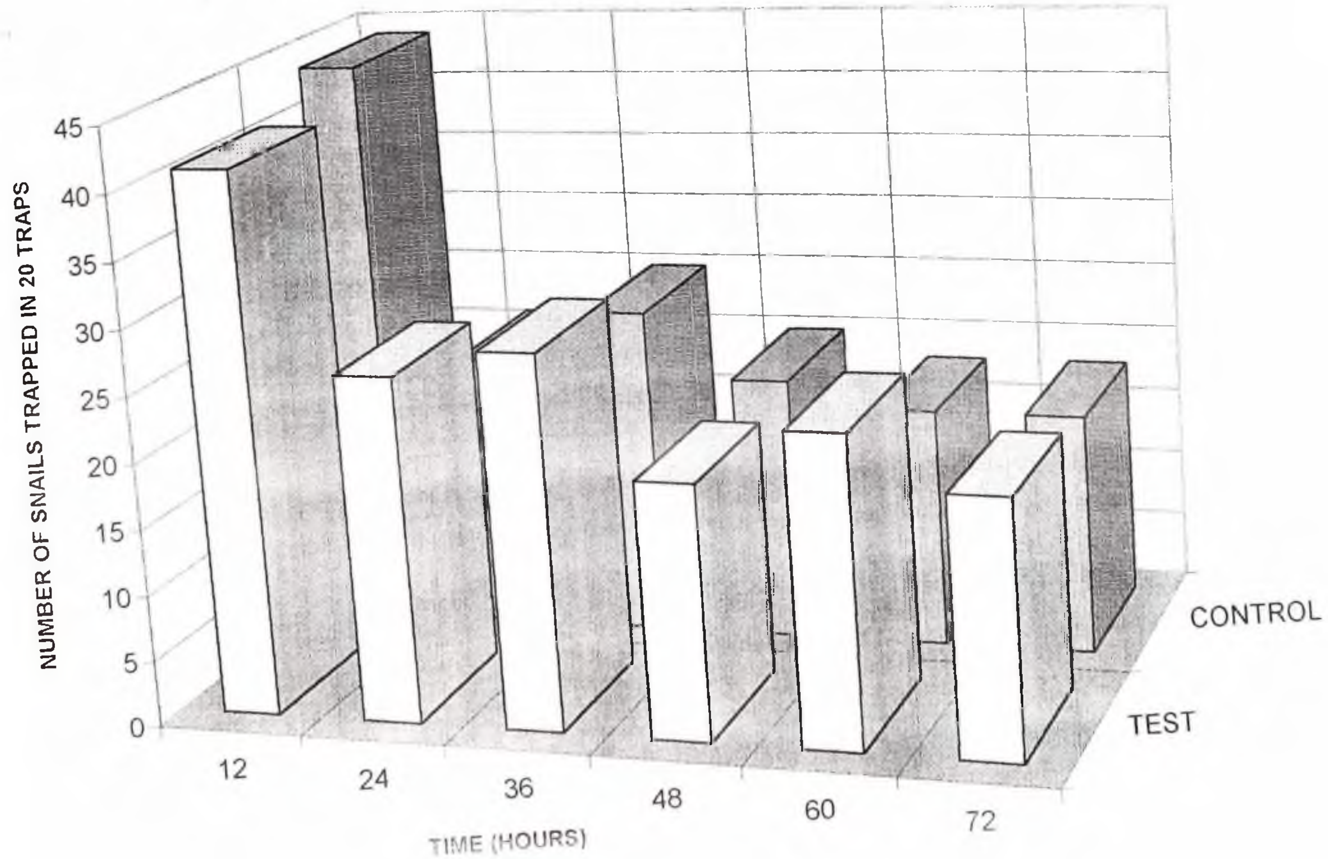
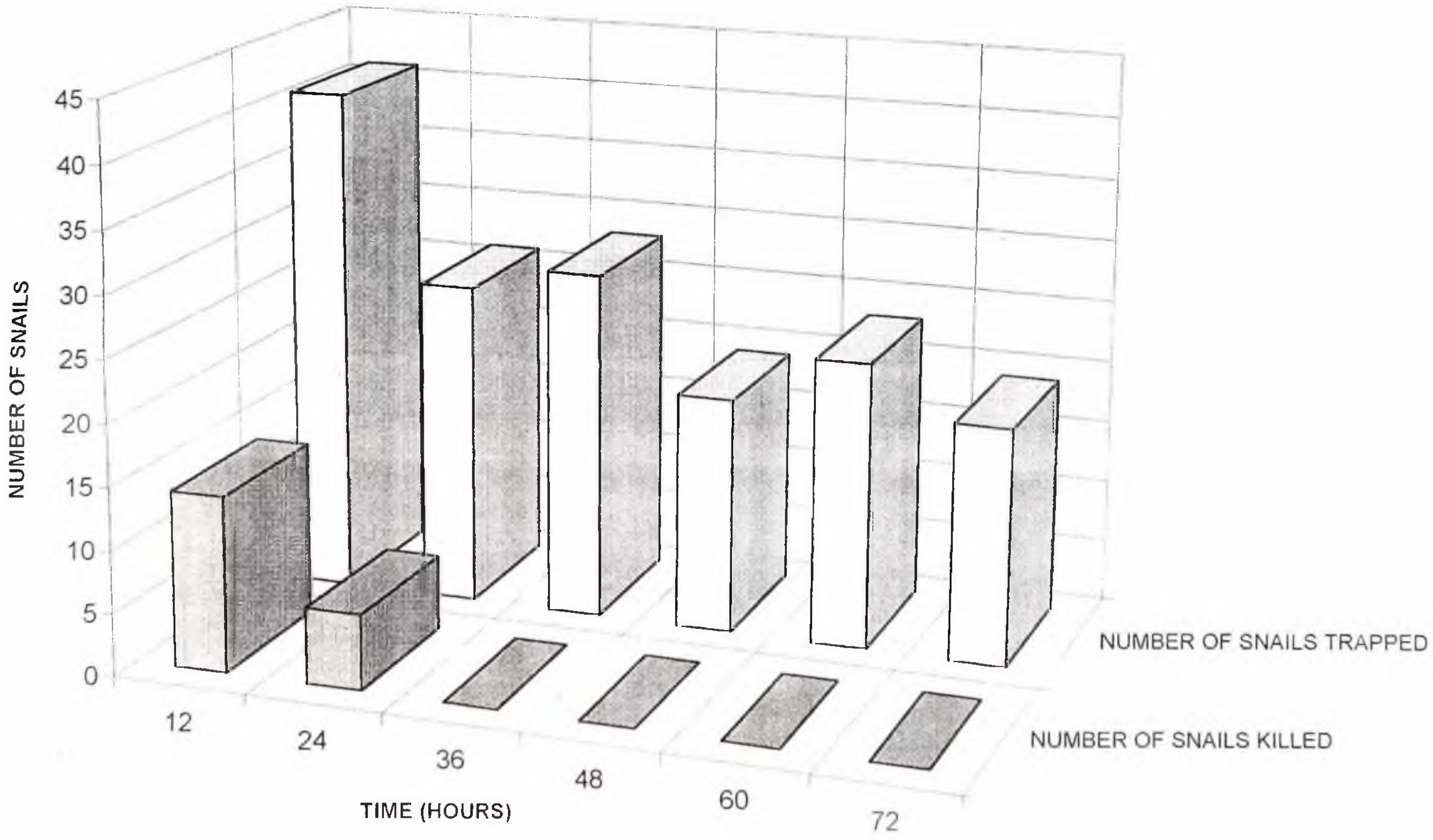


FIGURE 5g; KILLING EFFECT OF SUGAR CANE- BAYLUSCIDE COMBINATION AT WEIJA



(i) Canoes for fishing docked at a water contact site

(ii) A market scene at a water contact site

(iii) Fish traders waiting to buy fish

Plate 5a; Some of the activities that take place at the water contact sites at Weija lake

Plates 5b & 5c; A section of the shore of Weija lake showing some of the traps

Plate 5d; Student setting snail traps at Weija lake

Plate 5e; A section showing the east bank of the Weija lake

5.5 DISCUSSION

5.5.1 THE EFFICACY OF BIOACTIVE SUBSTANCES

The present results show that significantly more snails were caught in test traps than in control traps, when both fermented raw cocoyam and sweet potato were tested in the Weija lake. These results revealed a concordance with results obtained in simulated natural environment experiments using the same test materials and trap design (Chapter 3), and results of diffusion bioassay experiments (Domeh, 1998). Similar observations were made by Dogbey (1995) when he tested the efficacy of some bioactive materials under S.N.E. and also in the field.

Bioactive substances that were used created a diffusion gradient around them with the substance as the source when placed in the aquatic medium, and the snails were attracted along this gradient and subsequently trapped (Kpikpi, 1990). Response to chemical substances in general by the schistosome host snails has been extensively studied (Thomas & Assefa, 1979; Thomas et al, 1985; Thomas et al, 1983; Kpikpi & Thomas, 1992). The present results showed that the test traps were effective in both dense population area, and less dense population area of the snails. For each of the two bioactive substances tested the test traps caught more snails than the control traps. This was in agreement with results obtained in the simulated natural environment experiments. However, whilst the values of trapping indices for the tests in the dense population area were almost all statistically significant ($p < 0.01$), about half of the trapping indices recorded for the area of less dense population were not significant ($p > 0.05$) (Table 5a & 5b). This seems to suggest that the traps performed better in a dense population area than in a less dense population area.

Again, results from the studies indicated that there was no remarkable change in the attractant potency of the bioactive materials over the 3-day period. This was in agreement with results of S.N.E. experiments. In the test involving the use of 1 day fermented cocoyam (conducted in dense population area of snails) the largest number of snails trapped was recorded even after 72 hours (3 days) (Table 5a, ii).

5.5.2 THE EFFICACY OF ATTRACTANT-TOXICANT COMBINATION

In the second aspect of the present studies in which sugar cane was combined with a toxicant to assess its efficacy in attracting snails as well as killing them, results show that there was no statistically significant difference ($p > 0.05$) in number of snails caught in test traps as compared with control traps. These results are in agreement with those of simulated natural environment experiments using the same bioactive materials and trap design (Chapter 4). This might probably be due to the strong attraction of sugar cane for the snails. This seemed to be strong enough to supersede negative effects of bayluscide (0.6 ppm). The lake water might have been able to reach into the tiny spaces in the sugar cane where the chemical was poured and might have diluted its concentration with time, such that its potency to kill snails had reduced. This might explain why only a small percentage of the snails that were trapped were killed (12 hour and 24 hour periods only) with none killed in the subsequent periods.

The use of calabash for the trap design was very suitable in terms of its ability to hold the test materials in the aquatic medium and to release the chemical slowly (partly through the windows created on the sides) into its immediate environment. Secondly, it has a wide aperture to allow snails to enter, as well as a rigid framework such that snails can be trapped inside it.

Weija lake was considered suitable and chosen for the present studies because of the history of schistosomiasis in the surrounding communities as well as the availability of schistosome host snails in the water, which was found to be true during the preliminary study. The sites chosen for traps location were influenced by the availability of aquatic vegetation, notably *Ceratophyllum demersum* with which the snails associate most (Paperna, 1969; Odei, 1983; Madsen et al, 1987).

Preliminary studies involving snails sampling revealed that the snails population at the water contact sites was higher than the non-contact sites (Table 5c). Water contact sites are mainly sites where people wash clothes or utensils, collect water for domestic purposes, bathe or swim. In a study carried out on snails survey in the Niger basin, Madsen et al (1987) observed a close link between water contact sites and occurrence of the schistosome host snails. They remarked that human water contact activities create favourable biotopes for the snails, for example by increasing the food resources of the habitat. This important observation at the water contact sites should be kept in mind when setting up a snail control programme.

Results of the present studies indicate that it is possible to remove schistosome host snails selectively by using traps with bioactive materials as baits. When sugar cane was combined with 0.6 ppm bayluscide, it was discovered that the quantity of the toxicant at the given concentration did not have much effect on the attractant /arrestant potency, but only few snails were killed for a limited period of 24 hours. It may be very useful to remove the traps after every 24 hours to add some more of the toxicant. This means a daily inspection of traps and application of more toxicant. The advantage in using the described trapping unit is that it is easily affordable locally, and its application, that is the setting of traps along the banks of the lake is already a common practice by the fishermen and the people living in the communities around infested water bodies.

Sweet potato and cocoyam are foodstuffs that are very common in Ghana. The use of the traps can therefore be patronised easily.

CHAPTER SIX

GENERAL DISCUSSION

6.0 Summary of findings

The present results show that calabash which can be easily obtained in some villages in Ghana, at a low cost can be used to design a trapping system to catch schistosome host snails using bioactive materials as baits. Kpikpi (1990) designed a similar trap using bamboo which was also used by Dogbey (1995) in both simulated natural environment experiments and also in field tests on the Weija lake. From these studies, it was shown that cocoyam, cassava, and sweet potato, all in their raw fermented states attracted more snails than their controls. These naturally occurring materials have therefore been documented as bioactive materials or known to contain bioactive factors for these snails It was also found that limited quantities (in the range of 1,424μl – 7,124.5μl) of 0.6ppm bayluscide did not reduce the attractant effects of sugar cane; on the contrary snails were still attracted and killed in the traps over a 24-hour period.

In the present chapter an attempt will be made to summarise the findings of the overall studies and to point out how this will contribute to solving the problem associated with the extensive application of toxicants in water bodies to control the snails.

6.1 Summary of the results on trap design

The main objective of this test was to design an effective trapping unit to trap the schistosome host snail. Sugar cane was selected for this test because of its outstanding potency as an attractant/arrestant material (Dogbey, 1995). The traps were as follows:

(i) single units of sugar cane trap, (ii) sugar cane peels mat trap, (iii) sugar cane grid trap-with calabash. Results obtained from simulated natural environment experiments involving each of these traps revealed a significant difference ($p < 0.05$) between tests and controls, with regard to the number of snails trapped. The highest total number of snails trapped over the 3 days period of test (308 snails) was when the sugar cane grid trap (with calabash) was used as shown in Table 6.1. The design of the calabash trap was such that it somehow formed an enclosure with few small windows created on the sides. This design might have contributed to the trapping efficiency of the unit, since snails that were attracted or arrested could be prevented to some extent from moving away easily. In other traps there were no such enclosures to prevent the snails from moving away easily. That might have accounted for the observed results as shown in the table. Secondly, the diffusion gradient created by the sugar cane in the water medium (Kpikpi, 1990) could have dispersed more easily in the traps without enclosures than the one in which the sugar cane was partially enclosed as was done with the calabash trap. Essentially, it was along the diffusion gradient that the snails were attracted.

Table 6.1 Total number of snails trapped over 3 days in different trap designs in simulated natural environment experiments & their trapping indices.

Trap types	A	B	C	D
Total number of snails trapped in 3 days	154	145	154	308
Trapping Index	66**	84*	96*	135***

* = P < 0.05; ** = P < 0.01; *** = P < 0.001

A = single units of sugar cane trap

B = sugar cane peels mat trap

C = sugar cane grid trap (with poles)

D = sugar cane grid trap (with calabash).

6.2 Summary of the results on test of bioactive substances under simulated natural environment.

The results from the simulated natural environment experiments revealed that all the test materials, raw cocoyam, cassava, and sweet potato (fermented for 1 day and 7 days) when used in the calabash traps caught more snails than the control traps. The difference was found to be statistically significant ($P < 0.05$) for each of the materials. This revealed a concordance with diffusion bioassay studies conducted by Domeh (1998) using the above mentioned test materials.

It was discovered however that, in experiments where cassava or cocoyam (fermented for 7 days) was used the number of snails trapped reduced after 48 hours. After 48 hours it was observed that the test materials had become so soft in water that they easily got disintegrated and fell into the aquarium water in bits. Since each of these bits was capable of attracting snails, the snails somehow eventually got scattered along different diffusion gradients set up by the scattered bits of bioactive material. This might have accounted for the low numbers of snails caught in the test traps after 48 hours. Results showed that sweet potato (7 days fermented) did not become as soft as cassava or cocoyam fermented for 7 days did. As a result of this, more snails were still attracted /arrested and caught by the test traps after 48 hours. In increasing order of effectiveness of these bioactive materials to attract or arrest the snails, cassava (7 days fermented) < sweet potato (1 day fermented) < cassava (1 day fermented) < sweet potato (7 days fermented) < cocoyam (1 day fermented).

In general, more *Bulinus truncatus* snails were trapped than *Biomphalaria pfeifferi*. This difference was probably due to the differences in their chemoreception niches, as already reported in some previous studies (Thomas et al, 1979; Thomas & Assefa, 1978; Kpikpi, 1991; and Kpikpi & Thomas, 1993). The two different species of snails had different response rates to the bioactive materials.

6.3 Summary of the results on combination of attractant (sugar cane) and toxicant (bayluscide)

Results of the first part of these studies which involved diffusion bioassay tests in olfactometers revealed that the schistosome host snails could still be attracted by sugar cane when limited amounts of 0.6 ppm bayluscide was combined. There was no significant difference in attractant and arrestant indices ($p > 0.05$) for sugar cane-bayluscide combination used as test, as compared with sugar cane alone used as control for almost all the four different experiments conducted. It was however observed that as the quantity of bayluscide used to combine with the sugar cane increased to 700µl, there was a difference in attractant/ arrestant index. This was statistically significant ($p < 0.05$). This suggested that the quantity of the toxicant combined to the sugar cane to achieve success in attracting the snails could be critical. However, the fact that none of the snails died within the 40 minutes test period until 2 hours later after the test when left in the olfactometers showed that the sugar cane molecules probably diffused into the surrounding water medium faster than the molecules of the toxicant was introduced.

Similar results were obtained when the same studies were conducted under simulated natural environment experiments. This formed the second part of the studies. In the S.N.E. experiments the test and control materials were placed in the calabash traps. There was no significant difference in number of snails caught ($p > 0.05$) in test traps as compared with those caught in control traps. This suggested that the attractant/ arrestant potency of sugar cane did not change when limited amounts of 0.6 ppm bayluscide was added. With regard to the killing effect of the sugar cane- bayluscide combination, it was discovered that proportions of snails killed out of the number trapped were not very high (Table 6.2) and occurred only during 12 and 24 hour periods.-Results show that in all, 8.33% of the snails trapped were killed in the second experiment when 4,274.5μl of the bayluscide was combined with the sugar cane. Likewise, 6.44% of the snails trapped were killed in the third experiment when 7,124.5μl of the bayluscide was used.

None of the snails died in experiment 1, when 1,424.9μl of the bayluscide was combined with the sugar cane. These observations suggest that the quantity of bayluscide to be used effectively could be critical. This was in agreement with results of the diffusion bioassay experiments. The low percentages of snails killed could be attributed to the dilution of the toxicant that might have occurred in the aquarium water. For the same reason it could be that the concentration of the bayluscide after 24 hours became so low that it could not kill the trapped snails.

Removal of the test traps after every 24 hours to add some more bayluscide might be highly recommendable.

Table 6.2 Percentage of snails killed in S.N.E. experiments involving the use of sugar cane-bayluscide combination.

Experiment	Total number of snails trapped (test)	Number of snails killed (test)	Percentage killed (%) (test)	Total number of snails trapped (control)	Number of snails killed (control)	Percentage killed (%) (control)
92.579 gm sugar cane & 1,424.9µl of 0.6ppm bayluscide	211	0	0	209	0	0
92.579 gm sugar cane & 4,274.5µl of 0.6ppm bayluscide	196	16	8.33	201	0	0
92.579 gm sugar cane & 7,124.5µl of 0.6ppm bayluscide	231	15	6.44	263	0	0

6.4 Summary of the results on field evaluation

The field work which was conducted at Weija lake near Accra, produced results that were in agreement with results obtained at the laboratory. The best trapping design identified previously in S.N.E. experiments in the laboratory together with the two top bioactive materials, also identified in a similar manner in the laboratory were tested on the field. The choice of the sites for the situation of the test traps was influenced by the presence of aquatic weeds, such as *Ceratophyllum demersum* with which the snails are known to be closely associated along the shoreline. The tests were conducted in two contrasting micro-environments, namely 'a dense population area' and 'a less dense population area', determined from a preliminary survey. Results obtained from the evaluation of the bioactive materials namely, raw cocoyam (1 day fermented) and raw sweet potato (7 days fermented) showed that more snails were caught in the test traps than in the control traps. The difference was statistically significant ($p < 0.05$). This conformed with results obtained in S.N.E. experiments at the laboratory. Similar results were obtained by Dogbey (1995) when he tested some bioactive materials in the same lake using a different trapping unit designed by Kpikpi (1990). Results in the less dense population area showed that although all the test traps caught more snails than the controls, during some few traps inspection periods (i.e. 12-hour, 36-hour, & 72-hour periods for fermented cocoyam, and 36-hour, 48-hour, & 60-hour periods for fermented sweet potato) the difference was found not to be statistically significant ($p > 0.05$).

With regard to the test involving a combination of sugar cane and 0.6 ppm bayluscide, results again revealed a concordance with what was obtained in S.N.E. experiments. There was no significant difference ($p > 0.05$) in snails caught in test traps as compared with those of control traps. This suggested that the quantity of bayluscide added did not adversely affect the attractant potency of the sugar cane. It was also observed that, 34.39% of the snails trapped after 12 hours were killed by the toxicant, whilst 23.07% were killed after the next 12 hours (i.e. 24 hours). None of the snails died in the subsequent traps inspection periods (Table 6.3). This again suggested that the unit was capable of killing snails trapped only over a 12 to 24 hour period. The lake water probably diluted the concentration of the toxicant to some extent that it could no longer kill the snails. To restore the killing effect of the attractant-toxicant combination after 24 hours, it would be very necessary that after 24 hours the traps are removed and more bayluscide added.

Table 6.3 Percentage of snails killed in sugar cane-bayluscide combination experiments

Time (hours)	Number of snails caught in test traps	Number of snails killed in test traps	Percentage of snails killed (%)
12	41	14	34.39
24	26	6	23.07
36	28	0	0
48	19	0	0
60	23	0	0
72	19	0	0

6.5 Conclusion

According to the results obtained from the present studies, it can be concluded that cassava, cocoyam, and sweet potato, all in their raw fermented states were capable of attracting the schistosome host snails, *B. truncatus* and *B. pfeifferi*, when tested in simulated natural environment experiments. Cocoyam (1 day fermented) and sweet potato (7 days fermented) however emerged as the top two attractants. Results from field experiments conducted at Weija, on the lake showed that these top two attractants were effective in attracting the snails. Both cocoyam and sweet potato are food materials that are common in Ghana, and can easily be obtained in almost all-the market centres. They can be obtained at all times within the year to be used for snail trapping. A combination of sugar cane and some limited amounts of 0.6 ppm bayluscide and used in the calabash trap could still attract snails which were trapped and killed.

Although the percentages of snails killed were comparatively small the highly selective manner in which they were killed is of considerable interest. One major objective of this study was to find ways of killing schistosome host snails without killing non-target organisms. The present findings show that this is now possible. Moreover, the pollution of the aquatic body could be reduced significantly if this method or improved versions are adopted rather than blanket molluscicIding regimes. The fact that the potency of the attractant-toxicant combination could last for only 24 hours means that a daily inspection of the traps for some fresh bayluscide to be added would be necessary.

Since the trapping system makes use of only little amounts of bayluscide one can conclude that the use of this method to control the snails will not only be at an affordable cost but will be environmentally friendly.

6.6 Recommendations

In the light of the findings made in the present studies the following points are recommended:

1. Since traps setting, normally for fish and other aquatic organisms are already a common practise by the fishermen and the other inhabitants of communities around such water bodies, the use of the schistosome host snail traps can easily be integrated into the work pattern of the people. They however must be educated to be aware of the need to control the snails so that the idea will be accepted and practiced willingly by them. This will essentially reduce the high cost of labour-normally associated with applications of snail toxicants to control snails.
2. Calabashes, together with the bioactive materials can be obtained in almost any market in Ghana at an affordable cost; so this trapping unit can easily be constructed locally at anytime since the components are always available. Care must be taken so that the calabashes will not crack so that they can be reused for a long period. The quantities of the bioactive materials used in the traps generally are small and will not affect human consumption. Again since very small amounts of bayluscide needs to be used, the cost of using this method is less expensive and will cause little or virtually no harm to other aquatic organisms.
3. It will be necessary to combine the toxicant with other bioactive materials that have been already identified to ascertain their efficacy with regard to ability to attract and kill.

4. There will also be the need to conduct a regular chemical analysis of the water at the sites where the traps are positioned to find out whether these traps will at any point in time cause any pollution in the water.

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