INNOVATIVE CONTROL METHODS FOR *AMBLYOMMA VARIEGATUM* (FABRICIUS, 1794), USING ENTOMOPATHOGENIC FUNGI, *BEAUVERIA BASSIANA* AND *METARHIZIUM ANISOPLIAE* IN TRAPS BAITED WITH THE ATTRACTION-AGGREGATION-ATTACHMENT PHEROMONE

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DECLARATION BY CANDIDATE

This thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

To

My dear parents Orangi Oseko and Peris Nyaboke

My loving husband Dr. Charles Maranga and my son George

I dedicate this thesis

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ABSTRACT

Studies were commenced to investgate the potential use of the fungi, *Beauveria bassiana* and *Metarhizium anisopliae* and the attraction-aggregation-attachment pheromone (AAAP) for the control of *Amblyomma variegatum*. The objectives of the study were threefold: to determine the range of perception of the optimal dose of the pheromone with and without carbondioxide in the field; to investigate if there were any synergistic or additive effects of *B. bassiana* and *M. anisopliae* on *A. variegatum;* and to develop and test a device which could be used for pheromone delivery and infection of ticks by the fungi.

The number of ticks that responded to AAAP in the laboratory decreased with decreasing doses of AAAP. The relation of the tick response to decreasing doses of AAAP was given by: $r = 0.62$, $r^2 = 0.39$;, $r = 0.13$, $r^2 = 0.018$ and $r = 0.613$, $r^2 = 0.376$ for males, females and nymphs respectively. For increasing doses of AAAP, tick responses were given by the relation $r = 0.81$, $r^2 = 0.066$ and $r = 0.58$, $r^2 = 0.337$ for males and females respectively. The nymphs showed a weak response to both decreasing and increasing doses of AAAP. When AAAP alone was used, a dose of 6.6mg attracted the highest number of adult ticks in the field from a distance of 4m but when 500g of CO_2 was incorporated, more ticks were attracted to the AAAP and the range of attraction was increased to 5m. Upto 90% of the released ticks within a radius of 4m were attracted to the combination of AAAP and CO_2 within 3hrs while only 23% were attracted with AAAP alone within the same period. Reducing the weight of CO_2 from 500g to 50g reduced the number of ticks atttracted to AAAP and the number of ticks even reduced further when the weght of CO_2 was reduced to 5g. These differences

were significantly different at the 5% level. High soil temperature ranges, 31-52°C were found to reduce the attraction of ticks to the AAAP.

The oil formulations of the separate and the mixture of fungi of a concentration of $1x10^8$ spores/ml, caused higher tick mortalities (sometimes as high as 100%) in the laboratory while that of the water formulation had less than 25% even at higher fungal concentrations.

Tick mortalities were higher during the wet season (92%) compared to the dry season when the highest mortality recorded was only 30% and these results were significantly different at the 5% level. The mixed fungi caused significantly higher tick mortalities compared to the separate fungi. Tick mortality increased with increasing fungal concentrations and the highest mortality was obtained with a concentration of $1x10^{11}$ spores/ml in the field, while in the laboratory 100% tick mortality was obtained with a fungal concentration of $1x10^8$ spores/ml and above.

In the experiments to study any potential detrimental effects of AAAP to the fungi, the number of ticks killed due to fungal infection decreased when the amount of AAAP added to the fungi was increased but the differences were not significant at the 5% level. Tick mortality due to infection by lateral transfer of fungi occurred with the ratio of 1:1 for exposed: non-exposed combination producing the highest tick mortality. There were significant differences between mortalities caused in long term exposure (24hrs) and short term exposure (30 minutes) with higher mortalities being observed in long term exposure. Tick mortalities took place irrespective of sex combinations. However, the overall efficiency of tick mortality due to fungal infection by lateral transfer was relatively low.

The tick trap that was divised showed 79% efficiency in mortality for ticks that were incubated in the laboratory and 66.3% for ticks that were left in the field after exposure to the mixed fungi and these results were significantly different at the 5% level.

This technology could be improved and can be transferred to small scale farms where it can be incorporated with other Integrated Tick Management (ITM) packages to help reduce *A. variegatum* populations in the field and also reduce losses due to heartwater, poor quality of animal products and improve the general economic gains for the farmers.

CHAPTER ONE

1.1 GENERAL INTRODUCTION AND LITERATURE REVIEW

1.2 Background information

Ticks are an important constraint on the livestock industry in the tropics and subtropics, mainly because of the diseases they transmit and the losses they cause to animal productivity.Ticks transmit fatal diseases such as babesiosis (by *Boophilus* spp.), theileriosis (by *Rhipicephalus spp.*, *Hyalomma spp.* and *Amblyomma spp.*) and cowdriosis or heartwater by *Amblyomma spp.*. They also cause significant reductions in liveweight gains, milk yield, calf production, hide and udder damage as a result of their feeding. Loss of blood leads to anaemia which makes hosts more susceptible to other diseases. Riek (1957) noted severe anaemia in cattle infested with *Boophilus microplus* that was roughly propotional to the level of infestaion. Damage due to *B. microplus* infestation in Australia amounted to US$ 60 million in 1972-1973 season (FAO, 1977). Ticks affect approximately 800 million cattle and a similar number of sheep throughout the world (Sutherst *et al.*, 1982). Out of the 200 million cattle in Africa, 90% are infested with ticks, 70% from simultaeous infestation by several species (FAO, 1976). In East Africa, there are 30 million cattle most of which are kept in areas where tickborne diseases are endemic (FAO, 1976). Mortalities from East Coast fever (ECF) alone, which is transmitted by *R. appendiculatus* have been estimated as 30-50% among calves and upto 90% for adults which have not been previously exposed to the disease (FAO, 1976). About 37% of the nearly 5.7 million cattle in Zimbabwe are in the regions of the country endemic for heartwater. The total annual costs for control of all tickborne diseases and their vectors in these regions were estimated at Z$ 56.8 million (US$ 8.7

million) in 1992. These costs include reduced productivity of milk, meat, manure and disease control costs and on the application of acaricides to control the vector ticks (Laker *et al.*, 1992). According to Norval (1990), losses in cattle directly attributed to ticks are estimated at 4.4gm per *Rhipicephalus appendiculatus* female and 10gm per *Amblyomma hebraeum* female. Diseases due to *Amblyomma variegatum*, have reduced the number of cattle from 5000 to 500 in less than 10 years in the Nevis (Tickler, 1996).

Pastoralists depend heavily on their herds for food supply, social standing and commercial purposes while peasant farmers rely on their livestock for milk, ploughing and as a source of cash income. Losses due to ticks therefore,are a major drawback to their developement (Norval *et al.*, 1992).

1.3 Ticks

Ticks are in the phylum Arthropoda,having jointed legs and a chitinous exoskeleton as principal characters. They differ from insects by having chelicerae as the primary mouthpart structure, (subphylum Chelicerata) while insects have mandibles (subphylum Mandibulata). They are in class Arachnida with scorpions , spiders, harvestmen and mites in the order Acarina. There are two well defined families of ticks, Ixodidae, the hard ticks (genera: *Amblyomma ,Rhipicephalus, Haemaphisalis, Dermacentor, Hyalomma, Boophilus* and *Aponoma*) and Argasidae, the soft ticks (genera:*Argas, Ornithodoros*) (Walker, 1959). Ixodid ticks may be divided into three groups depending on their life cycle. They are one-host ticks if they complete their life cycle on one host e.g *Boophilus decoloratus*; two-host ticks if two hosts are required e.g *Rhipicephalus evertsi evertsi*;and three-host ticks if they require three different hosts

to complete their life cycles e.g *Rhipicephalus appendiculatus* and *Amblyomma variegatum* (Walker, 1959). The ticks' life cycle is made up of four stages: the egg, six-legged larva, eight-legged nymph and adult (Walker, 1959). These include those of the genus *Rhipicephalus*, the most important being *R. appendiculatus*. This species is primarily a parasite of cattle but can also infest sheep and goats and to a lesser extent, wild animals (Yeoman and Walker, 1967). It is a vector of *Theileria parva* which causes East Coast fever, *Theileria Parva lawrencei,* the causitive agent of Corridor disease and *Theileria mutans* causing benign theileriosis in cattle (Wellcome, 1980). *Rhipicephalus appendiculatus* is mainly found in central and East Africa ranging from the Transvaal in the south to southern Sudan (Ebl and Anastos, 1966). It does not occur in West Africa (Hoogstraal, 1956).The other important species transmitting disease in cattle is *Boophilus decoloratus,* which is a vector of *Babesia begimina* and *Anaplasma marginale* which cause Red water and anaplasmosis respectively. *Boophilus decoloratus* species favours wet areas from sea-level upto 8000-9000 ft. East Coast fever, babesiosis or redwater and anaplasmosis are among the mojar diseases causing mortality of livestock in East and central Africa (Wellcome, 1980).

1.3.1 *Amblyomma variegatum*

Amblyomma variegatum (Fabricius, 1794), is one of the most important ticks in Africa and is well distributed in cattle rearing regions. It is a widely distributed species in Africa, occurring across the continent from Senegal through West Africa into the Central African Republic, southern Sudan, Ethiopia and to the extreme north-western tip of Somalia. It is prevalent in most of eastern Africa (Kenya, Uganda, Tanzania,

Rwanda and Burundi), as well as Malawi, Zambia, Zaire and eastern Angola, Namibia, north eastern Botswana, Zimbabwe and Mozambique. It is absent from the desert areas of the horn of Africa (Norval *et al.*, 1992). It is a commonly occurring tick within its distributional ranges. In livestock, the species is normally exceeded only by *R. appendiculatus* and in areas free from *R. appendiculatus*, it may be the most abundant species (MacLeod and Colbo, 1976; Kaiser *et al.*, 1982; Pegram *et al.*, 1986).This tick has been spreading to new areas and islands over the last 20 years (Tickler, 1996).

Amblyomma variegatum is one of the major parasitic ticks of cattle in Africa and cattle are the most important domestic hosts for all stages of its life cycle. Cattle alone can maintain populations of this species (MacLeod and Colbo, 1976). Sheep, goats and other domestic animals also become parasitised but to a lesser extent (Norval *et al.*,1992). Wild hosts parasitised by adults of *A. variegatum* are mainly medium herbivores the most important being the buffalo (Norval *et al.*, 1992). Adults and nymphs attach in highest numbers in the ventral surface of the host including the lower dewlap, brisket, abdomen, axillae and genitalia (Hoogstraal, 1956; Yeoman and Walker, 1967 and MacLeod *et al.*, 1977). Nymphs also attach on the legs, especially around the hooves (Walker, 1974; Yeoman and Walker, 1967). Larvae are widely distributed over the body (Kaiser *et al.*, 1982). Adults attach in clusters (Yeoman and Walker, 1967) as a result of a male produced aggregation-attachment pheromone (Norval and Rechav, 1979). Unfed nymphs and adults shelter in well protected areas on or near the soil and only emerge in response to stimulation by carbon dioxide (Norval *et al.*, 1987; Pegram and Banda, 1990).

All engorged stages detach from the hosts around mid-day (Barre, 1989) and

engorged instars will mostly detach under the shades of trees where hosts rest during the heat of the day. Adults are most abundant on hosts prior to and during the wet season while larvae and nymphs may be abundant during the dry season (Norval *et al.*, 1992). The species passes through one generation each year but there may be more than one generation when there is more than one rainy season (Hoogstraal, 1956).

Amblyomma variegatum transmits *Cowdria ruminantium* which causes heartwater in cattle, sheep and goats.Heartwater is characterized by high mortality to susceptible animals in enzootic areas and has long been considered one of the most important livestock diseases of Africa (Mare,1984) and it can also be fatal to wild ruminants (Oberem and Bezuidenhout,1987). It has also been reported to be involved in the transmission of theileriosis in Africa (Walker and Olwage, 1987). It is a vector of *Theileria mutans* (Uilenberg *et al.*, 1974) and *Theileria velifera* (Uilenberg and Schreuder, 1976), both of which cause begnin theileriosis in cattle and buffaloes. Both heartwater and theileriosis are killer diseases of livestock in Africa.

1.4 Tick control

1.4.1 Acaricides

The conventional tick control method is by application of an aqueous suspension of acaricides by dipping or spraying. This method is fast and effective but the dip tanks used are costly to construct and the large volumes of water required is a problem in dry areas or during the dry season. The acaricides are expensive and sometimes, especially in the third world countries there is lack of adequate cattle which makes the dip tank operation economically unviable. There is also the problem of poor dip tank

management and social upheavals which make the method less dependable. In Zimbabwe, heavy losses of cattle have been reported to have occurred following the disruption of dipping in tribal areas as a result of war (Norval, 1979). The mechanical spraying equipment is prone to mechanical failure and blockage making this control method much less dependable. The method is time consuming as animals have to travel long distances and worsen health conditions of weak and young animals. The mechanical spray race is fast and the acaricide is freshly prepared but it is wasteful. On the other hand, hand spraying is affordable to most small scale farmers, it requires very few animals and does not provide complete wetting resulting in poor tick control (FAO,1984). In dipping and spraying, the animals must be herded and driven to or through the treatment area. These techniques are labour intensive and stressful to the livestock. There is a high potential for spillage posing environmental hazards to the surrounding area as well as health hazards to the workers.

The other way of applying acaricides is by using systemic acaricides which are chemicals of low mammalian toxicity administered by mouth, injection or implantation. This is an innovative method which eliminates or bypasses some of the problems incurred when chemicals are applied to the host surface since they attack ticks by way of bloodmeal. Nolan *et al.* (1981) found ivermectin to be one such systemic acaricide which is extremely effective against ticks by injection route, implant or rumen bolous. This method is not generally acceptable especially for food providing animals because of the toxic residues that can concentrate and remain in the animal tissues for long periods (Norval *et al.*1994b). Slow release devices such as acaricide impregnated ear tags have been shown to be effective in controlling the ear tick

R. appendiculatus for up to six months (Young *et al.*, 1985a). However, these devices are not commercially available in Africa and there is a risk of skin infection or irritation when the device is attached to the animals body. None of the above procedures is suitable for use with wildlife such as deers or other large herbivores (Norval *et al.*, 1994b).

Self-medication is a more recent technique which may be used to apply acaricides. In this method an animal is attracted to a device that offers a bait (food) and on contact is sprayed or coated with pesticide. This method minimises the amount of pesticide dispersed to the host and consequently into the environment. A well known example of a self medicating device is the Duncan applicator and, more recently, the Norval applicator (Norval *et al.*, 1994b).

The most serious problem with the chemical acaricides is the escalating costs , which is a major drain on the foreign exchange reserves in African countries where annual importation costs have been estimated as US$ 6-10 million for Zambia (Pegram *et al.*, 1988). In addition ticks have also developed resistance to these acaricides (Roulston *et al.*, 1981). Modern pesticides are highly effective for removing susceptible ticks but they can impose strong selection pressure for the development of resistance (Waller and Pritchard, 1986). Selection by one type of a chemical can hasten the development of resistance against another previously effective compounds (Waller and Pritchard, 1986). They are also great contributors to environmental pollution and have probable residual contamination of meat and milk. There is also a difficulty experienced by veterinary services and farmers in sustaining an infrastructure to apply them effectively on a long term basis. Pesticide disposal may create problems

particularly when large volumes of liquids are involved. Proper and legal disposal is becoming more difficult and of greater public concern. Acaricides have therefore to a large extent failed to control ticks and for this reason alternative tick control strategies have been sought. These strategies may be incorporated into an integrated tick management (ITM) system which is now thought to be the most effective way of controlling livestock ticks. The advantages of an integrated tick management programme is that it is adaptable to local conditions, economically affordable, sustainable and makes use of none or very little amount of chemical acaricides.

1.4.2 Alternative tick control strategies

1.4.2.1 Farm management practices

The restriction of livestock movement has been used in the control of ticks in many African countries for a long time. Tick infested animals are not allowed to be moved from one place to another thus minimising the distribution of ticks. This is unpopular with farmers and enforcement seems to break down during periods of civil unrest and war when ticks and tickborne diseases can spread rapidly and cause high mortality in cattle (Norval, 1979; Lawrence *et al.*, 1980).

Habitat modification is the manipulation of the environment to make the survival of the non-parasitic phase of ticks difficult. It may involve the removal of ground cover by burning or overgrazing. *Amblyomma* species are less affected by overgrazing and can occur in low numbers in overgrazed traditional areas (Norval, 1983). This is an unsuitable method since it leads to pasture deterioration and soil erosion. Reducing the stocking rate may reduce chances of the tick larvae finding a host but it increases the

grass cover, and therefore, favours tick survival (Sutherst *et al.*, 1978).

Pasture spelling is the temporary destocking of pastures (Wilkinson, 1957) and can be effective in controlling ticks by denying hosts to free living larvae. If it involves rotational grazing, major costs are incurred due to fencing, water supply and labour since the land has to be divided into paddocks (Sutherst *et al.*, 1979).

Changing the type of grasses can also affect tick survival. Tick killing plants such as the mollases grass (*Melinis minutiflora*) and gamba grass (*Andropogon spp.*), have been shown to reduce tick survival with subsequent low infestation levels on cattle but the effect is small and low (Thompson *et al.*, 1978). Tropical legumes of the genus *Stylosanthes* have been reported to be tick killing and two South American species, *S. scabra* and *S. viscosa* which produce sticky secretions that immobilise larvae of *B. microplus* and poison them within 24hrs by a vapour emanating from these secretions have been reported (Sutherst *et al.*, 1982). Similar results have been reported with *A. variegatum* (Zimmerman *et al.*, 1984), where the tick larvae were poisoned by an unidentified vapour from the secretions. However, tick larvae tend to avoid stems of these plants making them less effective as a tick control strategy.

1.4.2.2 Host resistance and immunization

The production of antibodies in response to tick infestation has been reported in a number of cases. Trager (1939a) transferred passive immunity to *Dermacentor variabilis* larvae by injecting immune serum into naive guinea pigs at the time of infestation and observed that the number of ticks engorging was reduced to 50% . Studies carried out on rabbit by Bowessidjaou *et al.*, (1977), suggested that there was a

toxic effect on *Ixodes ricinus* ticks.

Ovine and caprine resistance to tick infestation has also been documented. Maranga (1983) reported that goats acquire resistance to *R. appendiculatus* after successive tick infestation. The resistant goats rejected ticks during the attachment phase and significantly impaired the ability of ticks to take a normal blood meal and to produce their full potential yield of eggs. He has also reported the induction of resistance through immunization in susceptible goats using crude mid-gut homogenate (Maranga, 1988). Similarly, Wishitemi (1983), observed that the percentage of *R. appendiculatus* ticks engorging and their engorgement weights on Red maasai sheep were reduced significantly with repeated tick infestation.

The use of tick resistant cattle has been advocated in Australia for many years (Wharton *et al.*, 1973). Improved tick control following the use of tick resistant cattle has been demonstrated in different breeds of cattle (Riek, 1962; Seifert, 1971; Wharton *et al.*, 1970). Galun (1975) suggested a novel alternative attack based on immunizing the host to produce specific antibodies against the ticks' own hormones or against target antibody within the tick. However, the enzymes or tissues of ticks which could act as antigens must be identified and this is a very taxing and expensive venture. Most ticks in the natural environment feed on previosly exposed hosts and are likely to have adapted in some way to host immunity. Strategies adopted by the ticks to thwart, subvert or co-exist with the damaging host protective immune mechanisms are unknwon and could be diverse. An understanding of the humoral and cellular immunity mechanisms is essential for the tick control and this should be done for each host-parasite system.

The search for tick antigens that could be used for tick vaccine has been intensified (Essuman *et al* 1991,1992). Willadsen *et al.* (1989) and Rand *et al.* (1989) reported a genetically engineered tick vaccine against the 1-host tick *Boophilus microplus*. However studies are underway to develop a vaccine against ticks (deCastro and Newson, 1993) which might help to depress tick numbers in addition to short term protection of vaccinated animals against the ticks and tickborne disease transmission. Development of an anti-tick vaccine based on the salivery gland antigens could result in reduced transmission of tick-borne diseases (Nyindo *et al.* 1996).

1.4.2.3 Genetic control

Genetic control is another dimension in the ITM system that could be utilised. The release of sterile males has been effective with parasites of veterinary importance such as the screw-worm fly, *Cochliomyia hominivorax*. Sterilization in ticks has been achieved by irradiation as has been demonstrated in *Hyalomma anatolicum excavatum* (Beuthner, 1975; Srivestava and Sharma, 1976), *Amblyomma hebraeum* (Spickett, 1978) and *R. appendiculatus* (Purnell *et al.*, 1972; Beuthner, 1975). It is however difficult to breed ticks in very large numbers which is a prerequisite in this technique. Ticks are also less mobile so their dispersion is not wide.The vector should mate only once for this method to be effective but ticks mate more than once with different mates.

Hybrid sterile technique could also be employed whereby closely related species are crossed to produce sterile offspring. Graham *et al.* (1972) and Thompson

et al. (1981), showed that interspecific crosses between *B. annulatus* and *B. microplus* produced F1 progeny in which all males were sterile but females were fertile when back crossed to the male parent species. The sterile males were reported to mate with twice as many females as did the normal males and they also survived longer on the host indicating that they could successfully be used for biological control (Davey *et al.*, 1983). However no further follow up of this work has been done.

1.4.2.4 Anti-tick botanicals

Another component of ITM programme is the use of natural products to replace the more expensive chemical acaricides. The use of plant extracts can be traced to as far back as 4,000 B.C, when the Egyptians used ceder oil in embalming their dead (Maitai *et al.*, 1983). For a long time,plants have been a source of bioactive compounds that are used to control insects.

Plants of the family *Meliaceae* have been shown to have lethal effects on mosquitoes (Mwangi and Rembold, 1987,). A number of plant extracts have been reported to be capable of causing tick mortality. *Derris spp.* root extracts have been demonstrated to be lethal to all stages of ticks at concentrations as low as 1 part of extract in 100,000 parts of water. The difficulties with this extract is that areas of the animal with low hair density do not retain high concentrations of *Derris* particles (Arthur, 1962) and this leads to quick reinfestation of ticks. Aqueous solutions of pyrethrum (*Chrysanthemum cinerariaefolium)* flower heads have a rapid knock-down of feeding ticks but is more toxic to newly attached ticks and disintegrates

quickly under field conditions (Robinson, 1944). However, Simpkin and Galun (1983) have demonstrated that the microencapsulation of the natural pyrethrum significantly increased the persistence of the repellancy and toxicity of the natural pyrethrum towards tsetse and ticks. This could greatly enhance the use of natural pyrethrum in tick control. Furthermore, synthetic pyrethrum products which are resistant to atmospheric factors and light have been developed (Elliot, 1983; Elliot *et al.*, 1978). In Rwanda, Puyvelde *et al* (1985) have reported the presence of acaricidal activity in petroleum ether extracts from *Solanum dasypllum* and *Neorautanenia mitis* on female *R. appendiculatus*. Kaaya *et al* (1995) have reported an oily extract from a plant, *Margaritaria discoidea* (Euphorbiaceae) with strong acaricidal activity against *R. appendiculatus* and *A. variegatum*. Dipeolu and Ndungu (1991), reported that a ground mixture of tobacco leaves and a mineral called " magadi soda" which they named " kupetaba ", prevented completion of feeding, suppressed oviposition and hatchability of eggs in *R. appendiculatus* and also caused high mortality in this species. A shrub, *Gynandropsis gynandra* has been reported to be capable of killing and repelling all stages of *R. appendiculatus* and *A. variegatum*.

1.4.2.5 Biological control

One of the most important components of ITM practices, is biological control. This involves predation, parasitism and pathogenicity.

1.4.2.5.1 Predation

Predation of ticks occurs during both the parasitic and non-parasitic phases.

Various predators have been reported including the fire ant *Solenopsis geminata* which has been reported to have preyed on 63% of gravid females of *B. microplus* (Buttler *et al.*, 1979). Others include *S. invicta* (Oliver *et al.*, 1979), *Iridomyrimex detectus*, *Pheidole megacephala* and *Aphaenogaster longiceps* which have also been reported to prey on *B. microplus* (Wilkinson, 1970a).The spider, *Phidippus rimator* (Ault and Elliot , 1979) is reported as a predator of an argasid tick in California. Reporting on predation of ticks by two spiders *Lycosa godeffroyi* and *P. rimator*, Wilkinson (1970a) concluded that spiders were second to ants in predation.

Predation by lizards involving *Gerrhosaurus flavigularis* and *Mabuyu quinquetaniata* on *A. hebraeum* in Zimbabwe has been reported (Norval, 1976). Rodents have also been reported to prey on ticks (Maywald, 1987). Short and Norval (1982) reported predation by the shrew *Crocidura hirta* on *R. appediculatus*. Hunter and Bishopp (1911) found domestic chicken preying on ticks that had dropped from cattle. Similar results have been reported by Mwangi (1990).

Predation of ticks by the red-billed oxpecker, *Buphagus erythrorhynchus* and the yellow -billed oxpecker, *B. africanus* has been reported (Moreau, 1933; Van Someren, 1951; Stutterheim, 1976). The cattle egret, *Ardeola ibis* has also been found feeding on ticks (Rothschild and Clay, 1952; Mckilligan, 1984). The pee-wee bird *Grallina cyaleuca* and the starling *Sturnus vulgaris* are on- host predators (Legg, 1930).

1.4.2.5.2 Parasitoids

The first tick parasitoid reported was *Ixodiphagus texanus* (Howard, 1907)

which was collected from the nymphal stages of *Haemaphysalis leporispalustris, D. variabilis* and *Ixodes dentatus ticks* (Howard, 1907; Smith and Cole, 1943). Howard (1908) identified and described a new hymenopteran parasitoid from *Rhipicephalus sanguineus* and named it *Hunterallus hookeri*. It has since been found parasitizing many species of ticks. Mwangi (1990) reported a hymenopteran parasitoid with features similar to both *H. hookeri* and *I. texanus* in the nymphs of *A. variegatum* from the Maasai Mara, Kenya. Other tick parasitoids include *Hunterellus thelerae* (Fielder, 1953), *H. sagarenis* (Geevarghese, 1977) a parasitoid of *A. variegatum* from the trans- mara district in Kenya (Mwangi, 1990). Although the genus *Hunterellus* has been obtained from five tick genera , there are only two available records of it in *A. variegatum spp.* (Mwangi *et al.*, 1991). *Amblyomma* ticks are not suitable hosts of *I. texanus* (Bowman *et al.*, 1986), the most cosmopolitan of the known parasitoids (Philip, 1954) and suitability studies of ticks and species of ticks for infestation with *H. hookeri* are not well documented (Mwangi *et al.*, 1991). Trials to control ticks using the parasitoid, *H. hookeri* have proved fruitless as they are known to be successful at first, with proven reduction in tick numbers, but later the ticks increase in number (Mwangi, *et al.*, 1991).

1.4.2.5.3 Pathogens

Natural enemies of ticks such as viruses, rickettsiae, bacteria and fungi are potential components of an ITM package. These enemies or their metabolic products may be used to kill the target pest or vector (Calberg, 1986). Sidorov and Shcherbovok (1973), reported viral infection in various tick species. Megaw (1978)

reported virus-like particles pathogenic to the salivary glands of *B. microplus*. *Rickettsia prowazeki* has been used to artificially infect females of *Dermacentor marginales* and *D. albipictus* (Rehacek, 1965). Infected females died prematurely and egg production was reduced. Another rickettsia, *Wolbachia persicus* when innoculated into the gut of *Ornithodoros moubata* caused damaging effects and ticks died after a few weeks (Weyer, 1973).

Several bacteria have been found to be pathogenic to insects of veterinary importance. *Klebsiella pneumoniae*, *Pseudomonas mirabilis* and *Staphylococcus spp*. have been isolated from dead ticks in a laboratory colony of *Boophilus decoloratus* (Hendry and Rechav, 1981). However,bacteria as pathogens of ticks have not been studied fully (Hendry and Rechav, 1981), probably due to difficulties in potential application and fear that some bacteria are not specific to ticks and might be pathogenic to man and domestic animals (Mwangi *et al.*, 1991).

Fungi are the most versatile entomopathogens, some of which have toxins and the potential for quick damage (Wright *et al.*, 1982). Many of them have a wide range of infectivity and infect different ages and stages of their hosts and are virulent (Ferron, 1978). The variety of species and strains provide screening possibilities. In nature transmission is almost entirely by environmental contamination and they usually cause natural epizootics that devastate insect populations (Burges and Hussey, 1971). They can inhabit water (Chapman *et al.*, 1972), soil (Gottwald and Tedders, 1984), aerial and plant surfaces (Pickfold and Reigert, 1964). Hosts with both sucking as well as chewing mouthparts are susceptible. They are able to infect through the insect/vector integument and this gives them an advantage over most of

the other natural enemies. However, they require high relative humidity at several stages in their life cycle (Ferron, 1978).

Samsinakova *et al.*(1974) isolated 17 species of fungi from *D. marginales, D. reticulatus* and *I. ricinus* from the field. Fungi mostly isolated included *Aspergillus parasiticus, Beauveria bassiana, Beauveria tenella, Cephalosporium coccorum* and *Paecilomyces fumoroseus. Beauveria bassiana* and *Metarhizium anisopliae* have been widely used for biological control of agricultural pests (Ferron, 1981; Anderson *et al.*, 1988). These two species and *Hirsutella spp.* have been used to artificially infect tsetse (Poiner *et al.*, 1977). Krylova (1977) used toxins of *B. bassiana, M. anisopliae* and *P. fumosoroseus* against the soft tick *Argas persicus*, but only found 10% mortality. Kaaya (1989) found *B. bassiana* and *M. anisopliae* to be highly pathogenic to adult tsetse. Mwangi (1990) also isolated fungi belonging to the genera *Aspergilus, Fasurium,* and *Mucor* from *R. appendiculatus*. There is no extensive research yet of laboratory trials on ticks using spores or mycelia of *B. bassiana* and *M. anisopliae* on fed and unfed stages of *R. appendiculatus* except the work of Mwangi (1990) who reported that 10 spores/ml concentration of *B. bassiana* killed 70% of adult *R. appendiculatus* and a similar concentration of *M. anisopliae* killed only 30%. Similar studies have been carried out on *R. appendiculatus* and *A. variegatum* where both *B. bassiana* and *M. anisopliae* induced approximately 30% mortality in adult *R. appendiculatus* feeding on rabbits while *M. anisopliae* induced a mortality of 37% in adult *A. variegatum* (Kaaya *et al.*,1996). Both fungal species induced reductions in engorgement weights, fecundity and egg hatchability in adult *A. variegatum. Metarhizium anisopliae* reduced

fecundity by 94% in *A. variegatum* and egg hatchability to 0%, while 11% of the females laid no eggs with infections of *B. bassiana*. Both the fungi induced high mortalities ranging from 76-85%, reduction in fecundity of 85-99% and reduction in egg hatchability of 94-100% on
R. appendiculatus in ticks feeding on cattle in the field (Kaaya *et al.*, 1996).

Among the pathogens found to infect ticks, perhaps fungi could be the most suitable biocontrol agents because they have the ability to penetrate the tick cuticle, are self propagating, do not pose health hazards to man and his livestock and work effectively under natural conditions. However, the safety of these fungi to non-target organisms needs to be investigated. Their mode of application could be made innovatively more effective if pheromones are incorporated in traps as attractants. The attraction-aggregation- attachment pheromone (AAAP) found in *A. variegatum* could be a suitable canditate for these studies.

1.4.2.6 PHEROMONES

Pheromones are natural chemicals released by an animal and influence the behaviour of other individuals of the same species (Karlson and Luscher, 1959). Three categories of tick pheromones have been identified; sex pheromones, produced by fed females of hard ticks (Metastriata) and are attractive to males (Berger *et al.* 1971, Sonenshine *et al.*,1974,1976,Wood *et al.*,1975); assembly pheromones induce clustering of ticks in natural habitats (Leahy *et al.*,1973.1975 and Treverrow,1977) and the attraction-aggregation-attachment pheromones which attract ticks to the site of feeding individuals and inducing them to aggregate and

attach around the selected site (Rechav *et al.*, 1976).

The attraction-aggregation-attachment pheromone (AAAP) is produced by feeding males of *Amblyomma spp*. They induce unfed conspecific female and male ticks to migrate, aggregate and attach around the emitting source. The first reported evidence of this phenomenon was shown by Lounsbury (1899), who described mate seeking behaviour by unfed *A. hebraeum* and concluded that it was guided by some signal- mediated process. Gladney (1971), described a similar type of behaviour by *A. maculatum* males which he regarded as evidence of a male pheromone attractive to unfed females. With *A. hebraeum*, both unfed females and males introduced to a male infested bovine or rabbit migrated to the feeding sites and attached in aggregations around the feeding males. Similar responses were elicited with extracts of fed males obtained with diethyl ether washings of the fed males. Fed males or fed male extracts attracted both sexes but were more attractive to females (Rechav *et al.*, 1977b). Nymphs of this species were also attracted to the pheromone (Rechav *et al.*, 1976). Males of *Amblyomma gemma, A. hebraeum, A. maculatum, A. lepidum* and *A. variegatum* begin to emit aggregation-attachment pheromone after several days of feeding on the host. Males of *A. variegatum* and *A. hebraeum* become attractive only after about three to five days of feeding respectively (Norval and Rechav, 1979, Schoni, 1987, Schoni *et al.*, 1984).This pheromone attracts conspecific females and males to the feeding males, and in *A. hebraeum* it also attracts nymphs (Gladney *et al.*, 1974a,b, Norval and Rechav 1979, Schoni, 1987). Nymphs of *A. variegatum* unlike those of *A. hebraeum* were not attracted to the pheromone (Rechav *et al.*, 1976). Assembly of males and females around feeding males reached the peak after

the males had fed for 5 days (Norval and Rechav, 1979). Steers infested with male *A. hebraeum* (Norval *et al.*, 1989a) or artificial sources of pheromone for *A. hebraeum* and *A. variegatum*, proved to be attractive over several metres downwind (Hess and de Castro, 1986). The attraction-aggregation-attachment pheromone (AAAP) of six day fed male of *A. variegatum* is composed of ortho-nitrophenol, methyl salicylate and nonanoic acid in the ratio of 2:1:8, one tick producing approximately 11 micrograms per tick (Schoni,1987, Schoni *et al.*,1984). Ortho-nitrophenol is a long range attractant which induces incomplete aggregation ,MS induces mounting and clasping behaviour leading to lasting aggregation of the ticks, while NA is probably the solvent for ortho-nitrophenol and methyl salicylate (Schoni *et al.*,1984;Diehl *et al.*1991). These workers also reported that the AAAP components, ortho-nitrophenol and methyl salicylate in male *A. variegatum* ticks appeared after three days of feeding on the host and reached high values after about six days. In *A. variegatum* and *A. hebraeum*, AAAP is known to be responsible for host location and selection (Norval *et al* .,1989a,b; Yunker *et al.*,1990) whereby host location is facilitated by initial stimulation of unfed ticks by carbon dioxide (Norval *et al.*,1987,1988b), attracting unfed ticks to those areas of the host that are groomed least effectively and bringing the sexes together and so facilitating mating (Norval,1974; Rechav *et al.*,1977a)

Unknown pheromones believed to be produced by the foveal glands of *Rhipicephalus evertsi evertsi* males attract unfed females to form pre-parasitic and pre-copulatory assemblages (Gothe and Neitz, 1985).

Pheromone-acaricide mixtures could be used in the control of ticks (Rechav

and Whitehead,1978; Norval *et al.*,1991b),or to attract the ticks to young animals to expose them to heartwater while they are protected by an age related resistance to the disease (Norval *et al.*,1989b). Exposure to heartwater is particularly important in young cattle, which gradually lose their resistance to the disease beyond the age of one month (Neitz and Alexander,1941). Immunity to heartwater in cattle is thought to be transient unless reinforced by repeated exposure (Uilenberg ,1983). Therefore, the chances of young calves acquiring immunity to heartwater might be increased by treating them with AAAP or infesting them with male ticks, thus making them attractive to infected unfed nymphs and adults. The situation of reducing bont tick numbers to below threshold levels for economic damage, yet maintaining sufficient tick numbers on animals for maintening natural immunity in areas where heartwater is present along with populations of wild reservoirs is desirable (Norval *et al.*,1994b). Norval *et al.*(1991a), have also reported that if the aim of a control strategy is also to maintain enzootic stability for heartwater, transmission of the disease by ticks attaching to and feeding on the hosts for several days may actually be beneficial and if this is the case, the use of myco-acaricides may be most suitable since they take time to kill the ticks unlike the chemical acaricides which kill ticks quikly.

The attraction-aggregation-attachment pheromones known only for *Amblyomma spp.* have been used in experimental tick control programmes (Rechav and Whitehead, 1978). Gladney *et al* (1974a), Rechav and Whitehead (1978, 1981) and Sonenshine *et al.* (1979) applied AAAP baited acaricides to single locations on bovine hosts and ticks were attracted to these sites and were killed when they

attached. Norval *et al.* (1991b) reported similar findings with *A. hebraeum*. The use of pheromone-acaricide impregnated decoys has been well documented. Norval *et al.*(1994a) have described a decoy impregnated with AAAP and used it to attract hungry males, females and nymphs of *A. hebraeum* to a location where an acaricide could kill them. A large scale field test using pheromone-acaricide impregnated plastic tail-tag decoys demonstrated excellent efficacy of these devices for control of the bont tick *A. hebraeum* on cattle in Zimbabwe (Norval *et al.*,1996).These workers also found the use of pheromone acaricide impregnated tail tags to be advantageous over conventional control methods for several reasons. They are inexpensive and easy to manufacture, they preferentially target the bont tick, making them more target specific than conventional methods, are environmentally sound, require no water for application which is a major advantage in drought-prone areas, require no specific training for application, require no special facilities or mechanical apparatus for operation, have a long-lasting effect of upto three months and reduce the need for expensive toxic chemicals which in poor countries is a major advantage since their purchase greatly drains reserves of foreign currency.

Pheromone analogs that are more economical and more stable and therefore of more practical value in pest management may be synthesised (Carlson and MacLaughlin, 1982) and used in the field.

1.5 Justification for the study

Amblyomma variegatum is one of the most economically important ticks of cattle in Africa. It transmits heartwater, which is a killer disease of livestock in the African continent. The rapidly rising costs of acaricides, the ever growing problem of tick resistance to the conventional acaricides, their environmental pollution as well as their residual contamination of meat and milk, have stimulated research into new alternative methods of tick control. A number of these methods are employed in ITM programmes for best results. One of such methods is the recent introduction of myco-acaricides and from the foregoing review, it is clear that this is a promising method for tick control. However, the efficiency cost effectiveness and environmental safety of this method needs to be enhanced and hence this proposed study.

Most of the tick control effort has been directed on the parasitic stages on their mammalian hosts. However, the African 3-host ticks spend 95-97% of their time in the environment/vegetation (Punyua, 1992). It is therefore important to develop effective and environmentally friendly methods of controlling the free living non-parasitic tick stages in their environment. The purpose of this study was to explore the use of the attraction-aggregation-attachment pheromone to attract *A. variegatum* to mycopathogen traps placed off-host as means of controlling the tick.

1.5.1 Overall objective

To investigate control potentials of the fungi *Beauveria bassiana* and *Metarhizium anisopliae* in devices baited with the attraction-aggregation-attachment pheromone on *A. variegatum.*

1.5. 2 Specific objectives

1. To determine the range of perception of *A. variegatum* to the optimal dose of the AAAP combined with carbon dioxide in the field.

2. To investigate if there is any synergistic or additive effects of *B. bassiana and M .anisopliae* on *A. variegatum.*

3. To develop and test a device which could be used for pheromone delivery and infection of attracted ticks.

CHAPTER TWO

2.1 GENERAL MATERIALS AND METHODS

2.2 Ticks

Unfed nymphs and adult ticks of *A. variegatum* were obtained from the ICIPE tick rearing unit from a colony that has been maintained since 1978 and whose origin was from a dog in the Langata area (Thuo,personal communication). The ticks were reared on adult naive Newzealand white rabbits. Ticks of between one and three months post moulting age were used in the experiments (Adult male and female of *A. variegatum* are shown on Plate 1).

2.3 Rabbits

Naive adult Newzealand white rabbits were obtained from the Animal Rearing and Quarantine Unit (ARQU)at the ICIPE. They were kept in rabbit cages and fed on commercial pellets obtained from Unga feeds Kenya limited and clean piped water.

2.4 Fungi

Isolates of *B. bassiana* and *M. anisopliae* were obtained from cultures that have been maintained in the department of Molecular Biology and Biotechnology at the ICIPE by preserving them in mineral oil and liquid Nitrogen. The *Beauveria bassiana* isolate was originally obtained from the banana weevil, *Cosmopolites*

sordidus (German), in Nairobi while *M. anisopliae* was isolated from the migratory locust, *Locusta migratoria* in Madagascar (Kaaya et al., 1993). Both fungi are frequently passaged on *A. variegatum* ticks to maintain their virulence.

2.4.1 Culturing fungi

The fungi were cultured on solid Sabouraud dextrose agar (SDA) which was supplied by Mast laboratories limited, Mersyside, U.K. The medium was prepared by dissolving 62gm of SDA in 1 litre of sterile water in a conical flask. This was boiled in a heater stirrer until it completely dissolved. It was then autoclaved at 121 degrees, 15 pounds per inch squared for 20 minutes and was left in a sterile hood to cool to (42-45) ^{o}C after which it was poured into sterile petri-dishes. These plates were then left in the hood overnight to harden and culturing was carried out in the sterile hood the following morning. In culturing, spores were scooped from a culture of three to four weeks old using a sterile metal loop and were gently spread on the surface of the medium. The loop was sterilized by heating it red hot over a hot flame after every spread. The plates were then covered tightly using laboratory parafilm, labelled with a marker pen and incubated at room temperature for 2-3 weeks after which spores were harvested.

Plate 1. Photograph showing adult female and male of *A. variegatum*

2.4.2 Harvesting fungi

The fungi were harvested using 0.01% Triton X-100 (Kaaya,1989). A little of the triton was added to the fungi growing in a petri dish just to cover the surface completely. A copper loop sterilised by heating it red hot and cooled in sterile water was used to lightly scrap the surface of the fungi to loosen the spores. The spores were then transferred to a sterile beaker and this was done with several petri-dishes until enough spores were collected. A magnet was then placed in the universal bottles and the spores were thoroughly mixed using a magnetic mixer. The spores were then transferred into sterile universal bottles containing glass beads and mixed on a vortex mixer. The spores were then transferred into centrifuge tubes in equal amounts and centrifuged in a megacentrifuge at 2000 revolutions for 20 minutes. The supernatant in the tubes were decanted, sterile water added and the mixture stirred using a vortex mixer and centrifuged as above. This procedure was repeated three times to wash off the triton. The final rinse was suspended in sterile water and used to infect the ticks immediately using the required formulations.

2.4.3 Quantification of fungi

The above suspension was diluted 100 times by adding 1ml of the suspension to 99mls of sterile water. By using a 1ml syringe, two drops of the dilution were placed on a Neuber chamber for observation and counting of the spores under a compound microscope at a magnification of 40/0.65. In the Neuber chamber used, 1 medium square has 25 small squares and all spores in the 25 small squares were counted. The volume of the chamber was calculated to allow the

determination of spore concentration from the relation:

Volume of chamber = Depth of chamber x Area of squares

The volume of 1 medium square was calculated from the volume of the chamber and converted from milimetres cubed to mililitres cubed. This volume of the medium square contained the spores counted. To get the spore concentration in an equal volume of the stock concentration, the number of spores was multiplied by the dilution factor. From this concentration and volume, the concentration of spores in 1ml of the stock solution was calculated. Serial dilution was done to work out the various concentrations used.

2.4.4 Passaging fungi through ticks

Ticks were infected with fungi by allowing them to wade for about 5 seconds in a petri dish containing the fungi and kept in tetra paks in the field. They were left for three weeks after which the dead ones were removed and taken to the laboratory where they were surface sterilised as follows: the ticks were washed in 10% *jik* and rinsed in 70% alcohol at least twice and dried on sterile filter paper. Each tick was rubbed individually in aluminium foil and an opening left at the top. They were then placed in a petri dish containing sterile filter paper. A small amount of sterile water was then added to provide humidity and the petri dish was then carefully sealed using laboratory parafilm. The ticks were then incubated at 28°C for two weeks for the fungi to grow. After the fungi had sporulated, cultures were made by slightly touching the media with the infected tick and the spores spread on its surface using a sterile metal loop and incubated at room temperature for 2-3 weeks.

The clean culture so obtained was then subcultured on more plates for the multiplication of spores.

2.5 Preparation of the pheromone stock solution

The synthetic AAAP components used were supplied by Sigma-Aldrich chemical company limited of the Oldbrick yard New road Gillingham,Dorset SP8 4JL,England (UK). The components included; ortho-nitrophenol methyl salicylate and nonanoic acid.

The pheromone was prepared by mixing the components at a ratio of 2:1:8 respectively as follows: First, 200mg of ortho-nitrophenol was weighed using a balance after which it was placed in a clean dry universal bottle.Then 85 ul an equivalent of 100mg of methyl salicylate was added to the ortho-nitrophenol followed by 883 ul of nonanoic acid an equivalent of 800mg of nonanoic acid. The volumes of methyl salicylate and nonanoic acid were calculated from the relation: Volume = Mass / Density. This mixture was then dissolved in 1ml of hexane which was used as the solvent and this formed the stock solution of the pheromone. One microlitre of this solution contained 0.2mg of ortho-nitrophenol, 0.1mg of methyl salicylate and 0.8mg of nonanoic acid.

CHAPTER THREE

3.1 RESPONSE OF *AMBLYOMMA VARIEGATUM* TO VARYING DOSES OF SYNTHETIC AAAP IN THE LABORATORY.

3.1.1 Introduction

Adult males of the tropical bont tick *Amblyomma variegatum*, produce aggregation-attachment pheromone after feeding on a host for approximately 4-6 days (Norval and Rechav, 1979; Norval *et al.*, 1989a,1989b). The synthetic aggregation-attachment pheromone has been identified to consist of ortho-nitrophenol, methyl salicylate and pelargonic(= nonanoic) acid at a ratio of 2:1:8 respectively (Schoni *et al.*, 1984).

Studies carried out have demonstrated strong responses to either ortho-nitrophenol alone, methyl salicylate alone or both compounds in combination with other components

(Schoni *et al.*,1984; Hess and deCastro, 1986; Norval *et al.*, 1991a; 1991b). The work of Hess and deCastro (1986) also demonstrated that the synthetic attraction-aggregation-attachment pheromone of *A. variegatum* was attractive to the ticks off the host in the ticks' natural environment. However, the effect of varying the doses of the pheromone on the response of the tick has not been investigated. The purpose of this study was to evaluate the response of ticks to varying pheromone doses in the laboratory.

3.1.2 Materials and methods

The T-tube olfactometer method which presents alternative choices to ticks was used. Tubes used for adult tick bioassays measured (4x4)cm with an inner diameter of 1.5cm (Fig. 1) while those for nymphs measured (2.5x2.5)cm with an inner diameter of 1cm (Fig.2). A small filter paper carrying the test material was placed in one arm of the T-tube and a blank having hexane in the other arm. An unfed nymph was introduced into the base of the tube and allowed to climb up the tube and to select one of the two arms. The number of unfed nymphal ticks choosing the test site as well as the control site were recorded. Six replications of this bioassay were undertaken and this was repeated with unfed adult male and female ticks with twelve replicates.

Serial dilutions of the stock solution of AAAP were made (Table 1) and response of the ticks to each dilution was tested by introducing the adult ticks or nymphs at the base of the T-tube and letting them choose between the AAAP and the hexane control. One tick or nymph was investigated at a time. Responses of the male and female ticks were recorded separately. The experiment was repeated with double, triple, quadrible etc. doses of the stock AAAP. The adult ticks and nymphs were then subjected to various concentrations, one at a time and each treatment was triplicated. The filter paper carrying the AAAP as well as the hexane control were discarded and the T-tubes washed in detergent (*OMO*) and then rinsed in hexane every time a treatment was carried out. The T-tubes were then dried in the oven at 100° C for 30 minutes and left to cool before using them again. All the experiments were carried out in a hood that sucked out air so as to prevent accumulation of the AAAP in the working area.

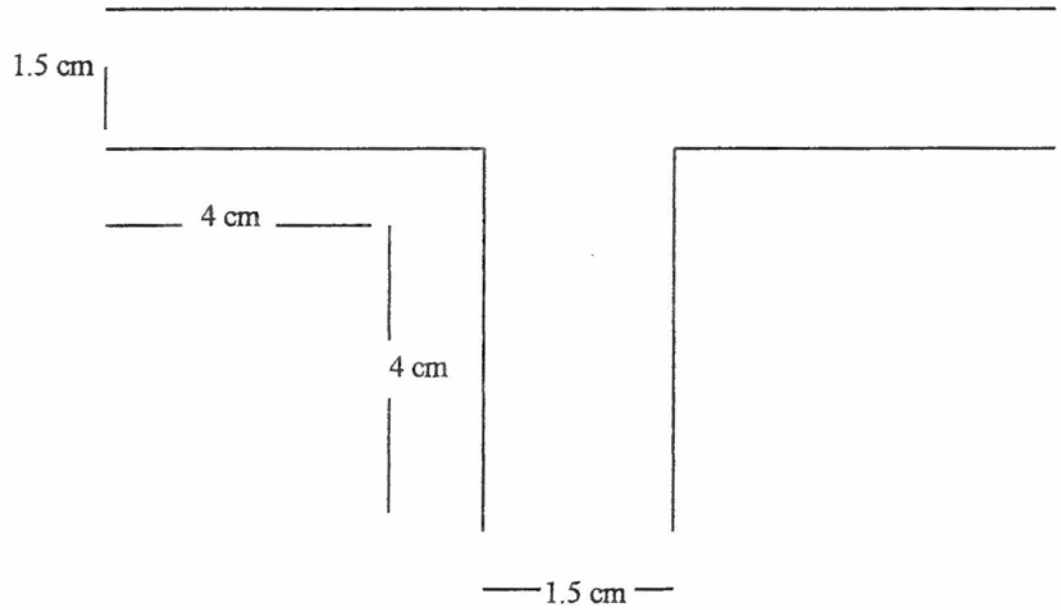

Fig. 1. T-tube olfactometer used for adult tick bioassay

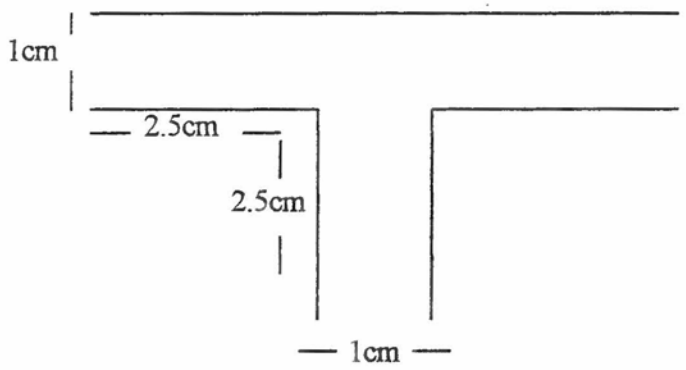

Fig. 2. T-tube olfactometer used for nymphal bioassay

3.1.3 Data analysis

Tick response to various doses of AAAP were recorded, entered on lotus 1-2-3 and Analysis of Variance(ANOVA) carried out on Statistical Analysis Systems (SAS, 1988) package. Mean separation was carried out using Student-Newman-Keuls test (SNK). All the values were subjected to Square root transformation ($\sqrt{(\log_{10}(x+0.5)+1)}$ prior to analysis.

3.1.4 Results

The ticks that were exposed to the synthetic AAAP in the tube followed a particular behavioural pattern. First, the tick raised its fore palps after a few seconds of exposure (Plate 2) and then walked up to the junction of the tube where it halted before raising its forelegs as it moved into either arms (Plates 5 and 6).

The ANOVA results on the attraction of *A. variegatum* to the decreasing concentration of AAAP are presented on Appendix 1. Interactions between; tick type (male, female or nymph) and treatment, dose and type, were not significant ($P>0.05$), and so was the treatment(AAAP or Hexane, the control). However, the doses of AAAP and the tick type were highly significant ($P<0.001$). Mean separations are shown on Table 1.

The percentage number of ticks attracted to AAAP decreased when the dose was decreased by a factor of 10 (Fig.3). The males showed a steady dercease in response to decreasing doses of AAAP the proportion responding being given by the equation: $Y=67.37 \times \exp^{(0.23X)}+-41.7$, $r = 0.62$, $r^2 = 0.39$) while the females had a higher response to decreasing doses of AAAP ($Y= -24.07 \times \exp^{(0.072X)} + 42.94$, $r=0.13$,

r^2= 0.018) compared to the males. The nymphs showed very little response changes upto the dose of $1.1x10^{-8}$ mg when the response dropped sharply

($Y= -0.001 \times \exp^{(-1.23X)} +16.65$, r = 0.613, r^2=0.376)

Appendix 2 shows the ANOVA results on the attraction of *A. variegatum* to the increasing doses of AAAP. Variations within doses, types and treatments were significant (P<0.05, P<0.01 and P<0.001, respectively) and so was the interraction between types and treatments (P<0.001). However, the interactions between dose and types, doses and treatment, dose, type and treatment were not significant (P>0.05). Mean separations are shown on Table 2.

Both the males and the females had a sharp decrease in response to increasing doses of AAAP starting with the dose of 1.1mg which attracted the highest number of adult ticks. The number of ticks responding to the pheromone was given by the relation: ($Y = 485.44 \exp^{(-1.553X)} + -65.54$, r = 0.81, $r^2 = 0.0656$ and $Y= 113.65 \exp^{(-0.444x)} + -33.971$, r = 0.58, $r^2 = 0.337$ for males and females respectively), while the nymphs showed negative response with all the doses of AAAP used (Fig. 4). There were no significant differences at the 5% level between males, females and nymphs in their attraction to AAAP doses above 1.1mg and no significant differences were observed between the ticks' response to AAAP and the hexane (control) for these doses except for that of nymphs at the dose of 2.2mg(Table 2). The ticks were at first attracted to the AAAP from the bottom of the T-tube but once they were near the source of the AAAP, they were repelled by high doses of the AAAP and they went out of the tube through the hexane (control) side (Plate 5) or went back to the bottom of the tube (Plate 6) or even walked out on the side of the tube (Plates 7 and 8).

Table 1. Mean percentage number (±SE) of *A. variegatum* ticks responding to decreasing doses of AAAP in a choice olfactometer

Dose(mg)	Type	Number to AAAP	Number to hexane	% response
1.1	male	36.11±2.78 abcd	13.89±2.78 bcd	44.33
	female	27.78±2.78 abcd	13.89±2.78 bcd	33.20
	nymph	22.22±14.70 bcd	27.78±5.5 abcd	-11.33
$1.1x10^{-1}$	male	19.44±7.35bcd	19.44±7.35bcd	0.00
	female	19.44±7.35bcd	16.67±0.00bcd	7.62
	nymph	50.00±9.62ab	27.78±5.56abcd	28.48
$1.1x10^{-2}$	male	25.00±4.81bcd	16.67±4.81bcd	20.00
	female	22.22±5.55bcd	16.67±4.81bcd	14.35
	nymph	44.44±11.11abc	16.67±0.00bcd	45.50
$1.1x10^{-3}$	male	8.33±44.81cd	22.22±13.89bcd	-45.50
	female	11.11±2.78bcd	5.56±5.55cd	33.00
	nymph	38.89±11.11abcd	44.44±14.70abc	-6.80
$1.1x10^{-4}$	male	13.89±5.55bcd	5.56±2.78cd	42.74
	female	2.78±2.78cd	8.83+4.81cd	-50.38
	nymph	44.44±5.55abc	27.78±14.70abcd	23.04
$1.1x10^{-5}$	male	8.33±4.81cd	11.11±5.55bcd	-14.16
	female	2.78±2.78cd	0.00±0.00d	100.00
	nymph	38.89±11.11abcd	22.22±11.11bcd	30.03
$1.1x10^{-6}$	male	2.78±2.78cd	11.11±2.78bcd	-60.20
	female	8.33±4.81cd	8.33±4.81cd	0.00
	nymph	22.22±11.11bcd	61.11±14.70a	-46.70
$1.1x10^{-7}$	male	2.78±2.78cd	11.11±2.78bcd	-60.20
	female	2.78±2.78cd	0.00±0.00d	100.00
	nymph	50.00±9.62ab	16.67±9.62bcd	50.00
$1.1x10^{-8}$	male	8.33±4.81cd	11.11±2.78bcd	-14.20
	female	5.56±2.78cd	2.78±2.78cd	34.00
	nymph	44.44±5.55abc	33.33±9.62abcd	14.30

table 2. contd.....

$1.1x10^{-9}$	male	5.56±2.78cd	8.33±0.00cd	-19.80
	female	2.78±2.78cd	2.78±2.78cd	0.00
	nymph	27.78±5.55cd	38.89±5.55abcd	-66.00

Means (+SE) within the same column followed by different letters are significantly different at the 5% level based on the Student's-Newman-Keuls (SNK) test.

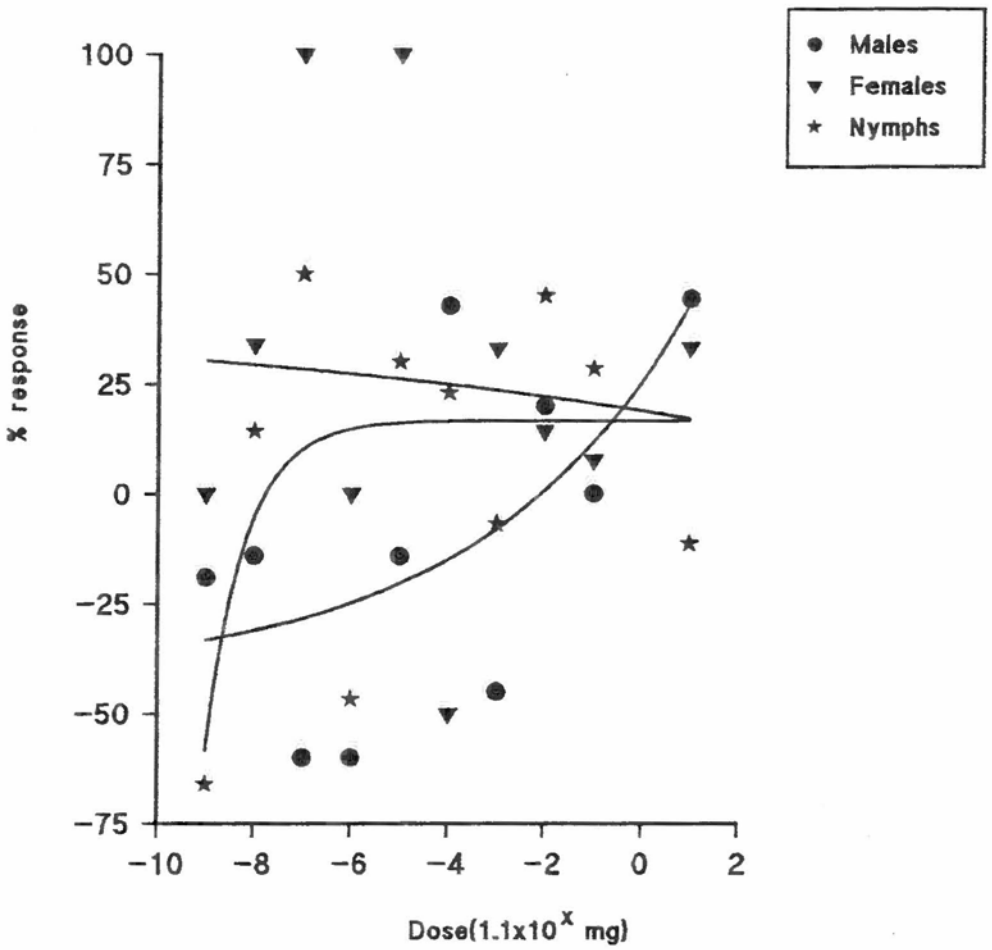

Fig. 3 Mean percentage response of males, females and nymphs of *A.variegatum* to decreasing doses of AAAP

Table 2. Mean percentage number (±SE) of *A. variegatum* ticks responding to increasing doses of AAAP in a choice olfactometer.

AAAP Dose (mg)	Type	Number to AAAP	Number to hexane	% response
1.1	male	36.11±2.78abcd	13.89±2.788bcd	44.33
	female	7.78±2.78abcd	13.89±2.78bcd	33.20
	nymph	2.22±14.70bcd	27.78±5.55abcd	-11.33
2.2	male	5.56±2.78b	22.22±7.35b	-59.88
	female	8.33 0.00b	5.56 2.78b	19.76
	nymph	11.11±11.11b	77.78±54.71a	-13.35
3.3	male	5.56±2.78b	33.33±4.81b	-71.31
	female	5.56±2.78b	11.11±2.78b	-39.52
	nymph	0.00±0.00b	16.67±16.67b	-100.00
4.4	male	5.56±2.78b	19.44±2.78b	-55.33
	female	5.56±2.78b	2.78±2.78b	34.00
	nymph	0.00±0.00b	16.67±0.00b	-100.00
5.5	male	5.56±2.78b	8.33±0.00b	-19.76
	female	11.11±5.55b	19.44±10.02b	-27.32
	nymph	0.00±0.00b	27.78±11.11b	-100.00
6.6	male	5.56±2.78b	25.00±4.81b	-63.49
	female	2.78±2.78b	11.11±2.78b	-75.19
	nymph	0.00±0.00b	22.22±5.55b	-100.00
7.7	male	8.33±0.00b	19.44±5.55b	-39.99
	female	2.78±2.78b	8.33±4.81b	-50.38
	nymph	0.00±0.00b	16.67±9.62b	-100.00
8.8	male	2.78±2.78b	19.44±2.78b	-75.19
	female	8.33±8.33b	8.33±0.00b	0.00
	nymph	0.00±0.00b	33.33±9.62b	-100.00

table 4. contd.....

9.9	male	0.00±0.00b	11.11±2.78b	-100.00
	female	2.78±2.78b	2.78±.2.78b	0.00
	nymph	0.00±0.00b	16.67±9.62b	-100.00
11	male	0.00±0.00b	25.00±4.81b	-100.00
	female	5.56±2.78b	19.44±7.35b	-55.33
	nymph	0.00±0.00b	16.67±9.62b	-100.00

Means within the same column followed by different letters are significantly different at the 5% level based on the SNK test.

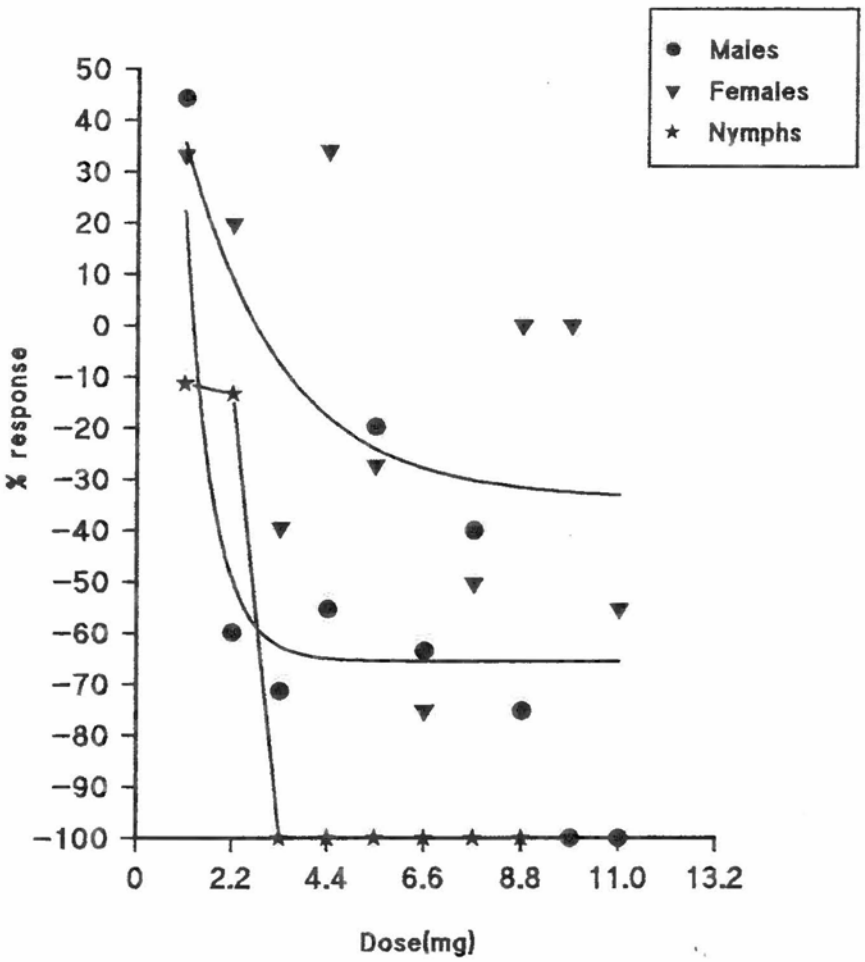

Fig. 4 Mean percentage response of males, females and nymphs of *A.variegatum* to increasing doses of AAAP

Plate 2. Tick showing raised fore legs

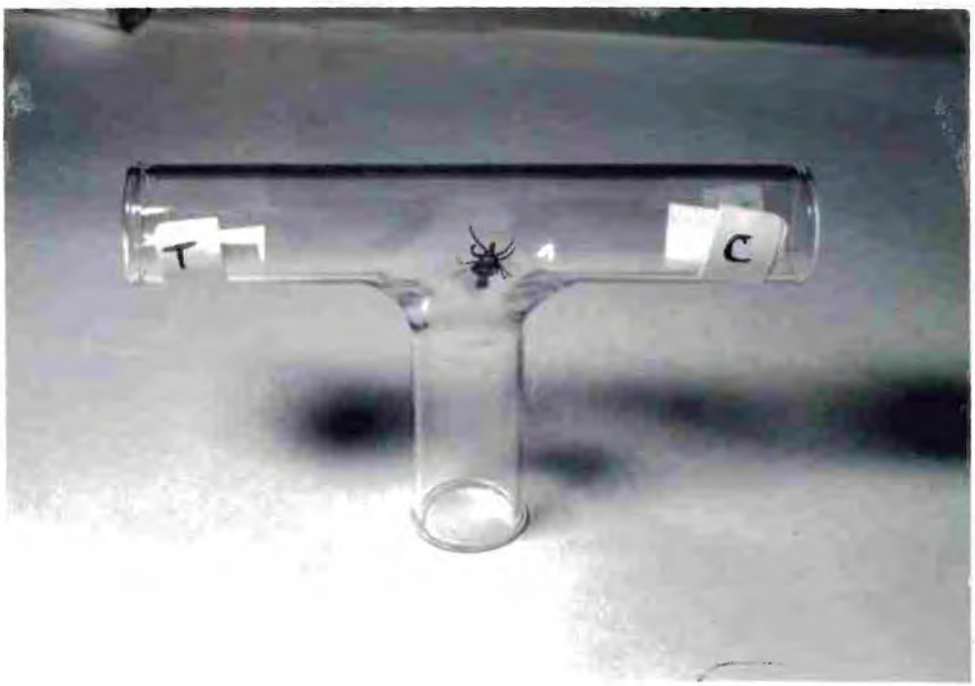

Plate 3. Tick making a choice at the junction of the T-tube olfactometer

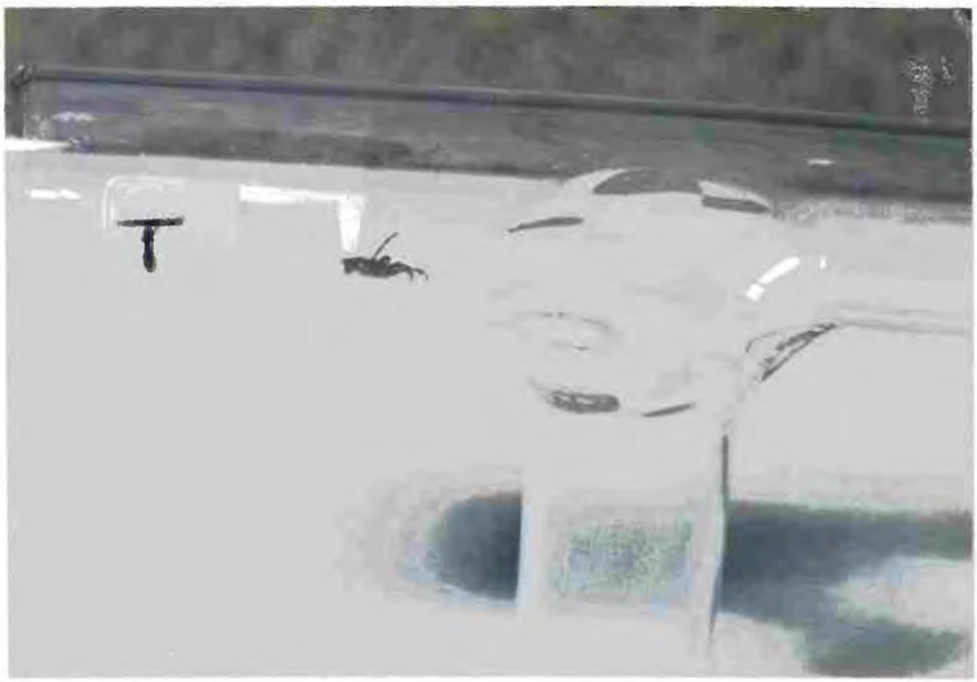

Plate 4. Tick attracted to the AAAP

Plate 5. Tick moving out of the T-tube through the control side

Plate 6. Tick walking back to the bottom of the T-tube olfactometer

Plate 7. Tick walking out of the T-tube olfactometer

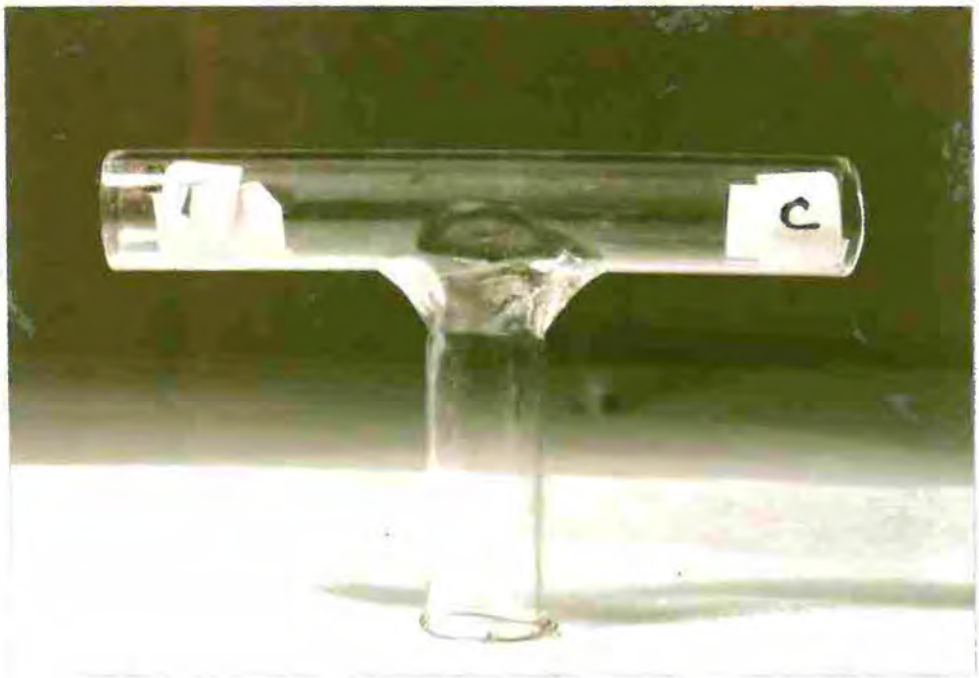

Plate 8. Tick walking out of the T-tube olfactometer avoiding the AAAP

3.1.5 Discussion

Among all the doses that were experimented upon, the 1.1mg dose of AAAP was observed to be the most attractive to the ticks and reducing this AAAP dose reduced the number of ticks attracted. A large number of ticks made no movement to the junction of the T-tube when the pheromone dose was below the 1.1mg. Some of the ticks were observed moving upto the junction of the T-tube and then back to the bottom of the tube where they settled. This behaviour was observed in both the male and female ticks.

The nymphs were not attracted to the AAAP since the number moving to the AAAP was more or less to that moving to the hexane (control).

Increasing the pheromone dose from 1.1mg had a negative effect on the response of the adult ticks to AAAP since most of the ticks that arrived at the junction of the T-tube were observed to move to the control side. When some of the ticks came into contact with high doses of the AAAP, they quickly turned back and ran very fast either through the control side or they went back to the bottom of the T-tube. Ticks that went back to the bottom of the tube made a second and even a third attempt and either went back to the bottom of the tube where they settled, or moved to the control side while those that moved to the AAAP evaded it by walking upside down (Plate 8) or on the side of the T-tube (Plate 7) and finally out of the tube. This behaviour was common with male ticks and with doses of between 6.6-7.7mg of AAAP. At higher doses, no males made any attempt on the AAAP whereby all the males that moved went out through the control side. However with these doses, a few females moved to the AAAP. Females therefore, seemed to be more attracted to very high concentrations

of AAAP compared to the males.

Since increasing the dose of the AAAP reduced the number of ticks attracted, it may be concluded that this is an adaptation for the ticks to balance the need to seek suitable hosts for feeding, thus avoiding over concentration in one host to avoid mass destruction in the event of predation or other calamity.

The results of this study showed that *A. variegatum* adult ticks were attracted to AAAP when a certain dose of the pheromone was reached. In this experiment, this concentration was found to be above $1.1x10^{-3}$ mg of AAAP. This was in agreement with the findings of Schoni *et al.* (1984) who carried out *in vitro* bioassays using the four-choice chamber test and reported that the activity threshold of the synthetic pheromone was found to be below a 10^{-2} dilution of the stock solution. The nymphs of *A. variegatum* were not attracted to the pheromone at any concentration. Similar findings have been reported by Rechav *et al.*(1976). However, Norval *et al.* (1992) reported that *A. variegatum* nymphs were attracted in the field situation from a distance of 10m when AAAP was combined with CO_2. The findings of this study suggest that *A. variegatum* nymphs have other cues for host location other than AAAP. Laboratory experiments carried out on rabbits to investigate these findings further, showed that the nymphs did not attach or aggregate near the pheromone emitting adult males but instead attached randomly.

It was observed that raising the AAAP dose above 1.1mg reduced attraction of ticks to AAAP and even caused repulsion in adult ticks when the AAAP doses were very high. The female ticks showed tolerance to very high doses compared to the males. Similar findings were reported by Barre *et al* .(1997), in the Guadeloupe, that

low concentrations of synthetic pheromone containing a one male equivaqlent were more active on males than females and the latter responded in a much greater proportion to higher concentrations of the pheromone.

This is perhaps because males usually attach and aggregate first before the females attach as a result of the AAAP produced by the males. Females generally encounter and are adapted to higher levels of the pheromone.

CHAPTER FOUR

4.1 THE OPTIMAL ACTIVE DOSE, RANGE OF PHEROMONAL PERCEPTION AND EFFECT OF SOIL TEMPERATURE ON AAAP PERCEPTION BY A. *VARIEGATUM* IN THE FIELD.

4.1.1 Introduction

It has been reported that the synthetic aggregation-attachment pheromone of *A. variegatum* was attractive to the female tick off the host in the ticks' natural environment from a distance of upto 1m, using the 1.1mg of the aggregation-attachment pheromone (Schoni *et al.*, 1984; Hess and deCastro 1986). A closely related species, *Amblyomma hebraeum*, has been shown to be attracted by its attraction-aggregation-attachment pheromone from a distance of up to 11m in the field (Norval *et al.*, 1989a).

The aim of this study was to investigate the optimal dose of the pheromone that attracts ticks from the longest distance possible in the field.

4.1.2 Materials and methods

4.1.2.1 Ticks

Two to three months old unfed adult males and females of *Amblyomma variegatum* from the ICIPE tick colony were used. The clean ticks were counted in batches of 30 consisting of 15 males and 15 females. Each batch was then placed in a vial which had a diameter of 3mm and a height of 8mm whose opening was covered

with cotton wool. The ticks were then marked using the artist's paint (Rowney Georgian oil colour,made in England, London HA 35RH). Males were marked on the lower quarter of the scutum while females were marked on the lower quarter of the dorsal side. The paint was in tubes which consisted of various colours and each colour was prepared separately by placing equal amounts of paint and linseed oil in a petridish and then mixing them using the painter's brush until the two were harmonious.Using the painter's brush, different colours were taken each at a time and the ticks that were previously counted and placed in tubes were then marked and returned into the tubes and covered with cotton wool. The marking was done a day before the experiments commenced.

4.1.2.2 The Site

The experiments were carried out at the ICIPE's Mbita Point field station (Latitute; 0°25'S and 0° 30'S and Longitude; 34° 10E and 34° 15'E) situated in Suba District,Kenya. Mbita Point field station has an altitude of 1240m with annual rainfall of 1150mm and temperatures ranging between 21.1°C and 28.3°C. Two paddocks, measuring about two acres which had previously been ploughed and planted with Rhodes grass, were used (Plate 9). By the time the experiments commenced, the grass was about 50cm tall. The experiments were carried out in 14 separate plots each of which was prepared by measuring a circular plot of radius measuring 6m.The grass within this circular plot was cut to a height of about 5cm . Starting from the centre, the plot was then marked using wooden pegs at intervals of 1m in a straight line all around the plot at 45° interval between the lines. A small circle of 10cm radius was made at

the centre of the plot and all the grass removed leaving bare soil (Plate 10). Each plot had a barrier of 5m uncleared grass surrounding it.

Plate 9. Photograph showing the two acre plot used for the field experiments

Plate 10. Photograph showing one of the cleared small plots

4.1.2. 3 Source of pheromone

The stock synthetic attraction-aggregation-attachment-pheromone (AAAP) was prepared by mixing 0.2mg of ortho-nitrophenol, 0.1mg of methyl salicylate and 0.8mg nonanoic acid (Schoni *et al.*, 1984) in 1ml of paraffin oil. This mixture was placed on whatman's filter paper fixed to the flat side of the petri- dish both with a diameter of 9mm using laboratory parafilm and this formed the source of the pheromone. The other sources were prepared by increasing this initial dose by a factor of 2 and controls were prepared using paraffin oil.

4.1.2.4 Tick release

On the experimental day, the targets consisting of various concentrations of AAAP were each placed at the different centres of the different circular plots and a control consisting of pure paraffin oil alone similarly set at 0645hrs in the morning. At 0700hrs, ticks marked with different colours were released from premarked distances and equal numbers of both male and female adult ticks were released from three different angles. All the ticks were released downwind from the centre of the plots where the source was placed. The direction of the wind was monitored using a thin thread attached to a 1m long wooden stick that was placed at the centre of the plot. The movement of the ticks was watched from a downwind position. Ticks that came to the target were collected and recorded noting down their colour and sex in each of the plots. Soil temperatures were recorded using a soil thermometer whose bulb was placed 60mm below the ground at hourly intervals from 0700hrs to 1800hrs in each experimental day. The experiment was repeated three times with each replicate being

observed for two days using a fresh source.

4.1.3 Data analysis

Data on tick attraction to various doses of AAAP from various distances in the field were recorded, entered on lotus 1-2-3 and Analysis of variance (ANOVA) carried out on the Stastical Analysis Systems (SAS). Data on the effect of temperature on tick respone to AAAP was also similarly treated. Mean separation was carried out using SNK. All the values were subjected to Square root transformation ($\sqrt{\log_{10}(x+0.5)+1}$) prior to analysis.

4.1.4 Results

The ANOVA results on the attraction of *A. variegatum* to AAAP from various distances are presented on Appendix 3. The interactions between temperature and concentration as well as distance and concentration were significant ($P<0.05$ and $P<0.01$, respectively), while temperature, concentration and distance showed a high significant effect on the response of ticks to AAAP ($P<0.001$).

A dose of 6.6mg of AAAP was observed to attract the highest number of ticks from all the distances at which ticks were released (Fig. 5). For doses lower and higher than 6.6mg, the number of ticks attracted decreased with increasing distance from the source but were much less compared to those attracted to the dose of 6.6mg. All the doses that were experimented on in the field situation showed significant differences at the 5% level when compared with the parafin oil control (Table 3). The ticks were attracted to AAAP dose of 6.6mg from distances of upto 4m and the number of ticks

attracted to the AAAP decreased with increasing distance from the source except that of 4m which was higher than those attracted from 3m. This could be attributed to observers error. Table 4 shows tick response to various doses of AAAP within different soil temperature ranges. There were significant differences in tick attraction between high temperatures 31-52°C and low temperatures 22-40°C and 23-37°C but no significant differences between the latter. The number of ticks attracted to different doses of AAAP were not significantly different when the temperature range was high 31-52°C but there were significant differences when the soil temperature ranges were low (22-40°C and 23-37°C), between the 6.6mg dose and all the others (Table 4). The soil temperature therefore, had an effect on the attraction of ticks to AAAP, with less response occurring at high temperature ranges.

Fig. 6 also shows the response of ticks to various doses of AAAP with different temperature ranges.

Table 3. Mean percentage number (±SE) of *A. variegatum* ticks attracted to varying doses of AAAP from various distances

	Distance(m)				
Dose(mg)	1	2	3	4	5
1.1	51.11±9.04a	3.33±1.92b	8.89±2.94a	0.00±0.00ab	0.00±0.00a
2.2	15.55±7.78b	1.11±1.11b	2.22±1.11b	1.11±1.11b	0 .00±0.00a
4.4	5.55±2.94b	1.11±1.11b	3.33±1.92b	1.11±1.11ab	0.00±0.00a
6.6	64.44±32.27a	33.33±16.78a	15.56±8.01a	22.22±11.28a	0.00±0.00a
8.8	23.33±11.70b	11.11±4.01b	2.22±1.11b	0.00±0.00ab	0 .00±0.00a
11	44.44±15.68a	5.56±1.11b	14.44±6.76a	0.00±0.00ab	0.00±0.00a
Paraffin oil	0.00±0.00b	0.00±0.00b	0.00±0.00b	0.00±0.00b	0.00±0.00a

Means within the same column followed by different letters are significantly different at the 5% level based on the SNK test.

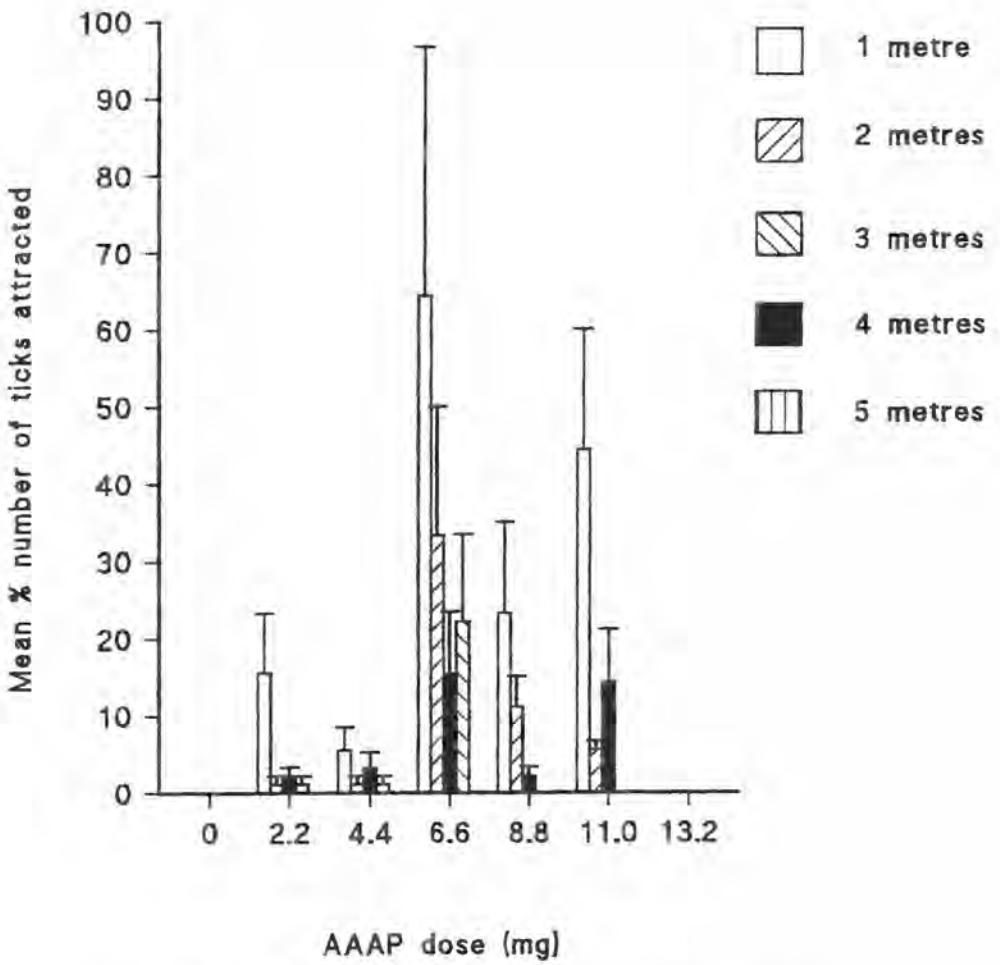

Fig. 5 Attraction of *A. variegatum* from various distances using different doses of AAAP

Table 4. Mean percentage number (±SE) of *A. variegatum* ticks attracted to varying doses of AAAP within varying temperature ranges

AAAP dose	Temperature range (°C)		
(mg)	31-52	22-40	23-37
1.1	1.10±0.70a	15.00±11.79b	15.56±12.40b
2.2	0.56±0.56a	4.44±3.82b	5.00±3.73b
4.4	0.00±0.00a	3.89±1.59b	1.67±1.14b
6.6	0.00±0.00a	35.00±14.44a	32.78±15.31a
8.8	1.11±0.70a	8.33±5.43b	8.89±6.18b
11	3.33±2.11a	16.11±10.34b	12.78±9.04b
Paraffin oil	0.00±0.00a	0.00±0.00b	0.00±0.00b

Means within the same row followed by different letters are significantly different at the 5% level based on the SNK test.

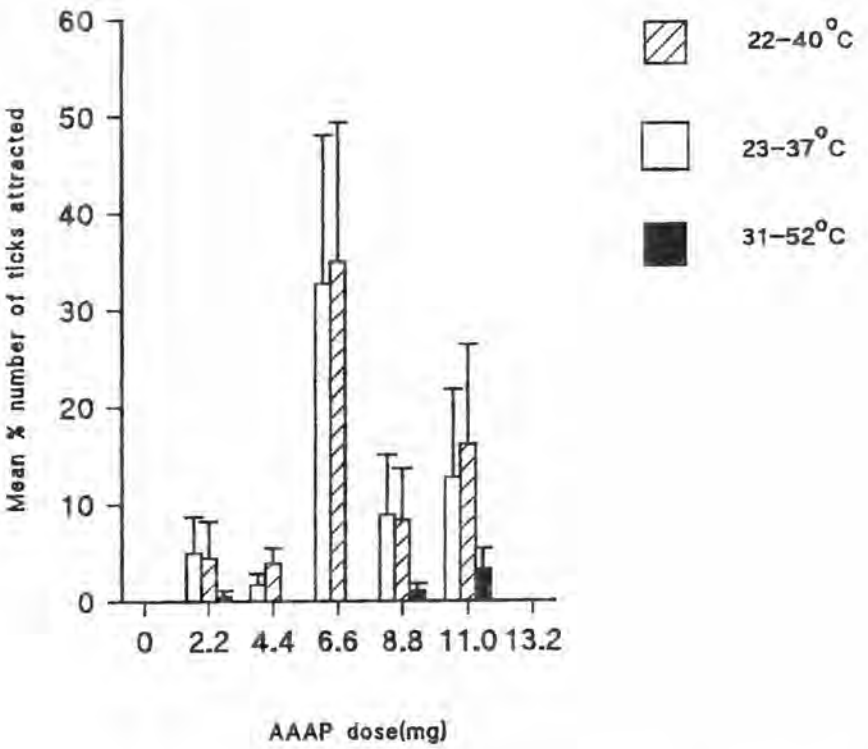

Fig. 6 Effect of AAAP dose on *A.variegatum* attraction with varying soil temperature ranges

4.1.5 Discussion

In this experiment, it was observed that the longest distance from which ticks were significantly attracted by the synthetic pheromone alone was a radius of 4 metres. Hess and deCastro (1986) documented that *A. variegatum* females were attracted to the synthetic pheromone in the field from a distance of 1metre. Bare *et al.* (1997) recorded 8 metres using AAAP and CO_2 traps with the Carribean strain. The findings of this study are in agreement with them and have also confirmed that males of *A. variegatum* are also attracted to the synthetic pheromone.

In the Guadeloupe, Bare *et al.*(1997), reported that there was a gradient of reactivity depending on the distance from the source of the pheromone with the least number of ticks being attracted from the furthest distance from the source. Similar findings were obtained in this experiment with AAAP dose of 6.6mg attracting the highest number of ticks within a radius of 5m from the source of the pheromone. However, unlike the Guadeloupean strain which was only attracted to the AAAP in the presence of CO_2 (Barre *et al.* 1997), the Kenyan strain was attracted by AAAP in the absence of CO_2 although in small numbers.

From the findings of this study, it was evident that soil temperatures play an important role in the response of ticks to the AAAP. Extreme temperatures reduced the tick movement and most ticks sought hiding places below grass roots, cracks in the ground or under the litter. Similar observations were made by Norval *et al.* (1989a) with *A. hebraeum* which were not attracted when soil temperatures were above 35°C. Barre *et al.*(1997) reported that there was a significant reduction in the number of *A. variegatum* captured from August to October in Guadeloupe, when the soil

temperature was greater than 30°C and this is in line with the findings of this study.

CHAPTER FIVE

5.1 EFFECT OF CARBON DIOXIDE ON THE PERFORMANCE OF AAAP AS AN ATTRACTANT OF *A. VARIEGATUM*

5.1.1 Introduction

Norval *et al* (1989a) demonstrated that adults of *A. hebraeum* were activated and attracted to a pheromone source in the presence of CO_2. Unfed adults of *A. variegatum*, shelter in protected microhabitats on or near the soil surface and only emerge in response to stimulation by CO_2 (Norval *et al.*, 1987, Pegram and Banda, 1990). In nature, ticks become active in the presence of large mammals, which give out large amounts of CO_2 (Norval *et al.*, 1987), but will be attractive only if they have pheromone emitting males attached to them.

The purpose of this study was to investigate the effect of CO_2 on the efficacy of pheromone baited traps.

5.1.2 Materials and methods

A dose of 6.6mg of the AAAP, which was found to have the highest percentage of attraction of the ticks in the previous experiment was used in this study. Six microlitres of the stock solution of AAAP containing 6.6mg of the AAAP were added to 1ml of paraffin oil placed in a test tube and mixed well by shaking the tube. The mixture was then placed on whatman's filter paper attached to the flat side of a petridish using laboratory parafilm. The petridish was then placed at the centre of the

circular plot and a plastic lunch box containing 500g of dry ice with openings on the opposite sides was placed next to the AAAP (Plate 11). Marked ticks were then released from the various premarked distances in the plot from a downwind position and observed from the downwind position. Two controls were set using AAAP alone and pure paraffin oil alone in separate plots. The experiments were set in triplicates and were carried out in the morning between o6oo hrs and 1000hrs when soil tempertures were low.

Different weights (50g and 5g) CO_2 were also used and similar experiments performed alongside those with 500g CO_2 .Ticks that were attracted to the targets were collected and their numbers, colours and sexes recorded.

Plate 11. Photograph showing a tin containing dry ice placed next to the AAAP source

5.1.3 Data analysis

The data on the respponce of ticks to AAAP combined with various weights of Co_2 were recorded and entered on lotus 1-2-3 and Analysis of Variace (ANOVA) carried out using Statistical Analysis Systems (SAS, 1988). Mean separation was carried out using SNK.

5.1.4 Results

Appendix 4 shows the ANOVA results on the attraction of *A. variegatum* to AAAP combined with CO_2 in the field. The treatment (AAAP + CO_2, AAAP alone, CO_2 alone and the paraffin oil control), distance and the interaction between the treatment and the distance were all highly significant ($P<0.001$).

With the incorporation of 500g of CO_2, there was a significant increase in the number of ticks attracted from all the distances and the range of pheromone perception was extended to 5m. The number of ticks attracted from 1m (98.33%) was significantly higher than that from 2m(93.33%) and 3m(88.33%) which was in turn significantly higher than that attracted from 4 m(78.33%) which was also significantly higher than that attracted from 5 m(29.17%) at the 5% level. However, there were no significant differences at the 5% level, in the number of ticks attracted from all the distances except 5m, when AAAP alone was used (Table 5). Only 0.83% ticks were attracted from 5m and 1.67% from 3m when CO_2 alone was used. However, there were no significant differences between these two distances and the other distances from where no ticks were attracted. Paraffin oil did not attract ticks from any of the distances.

Fig. 7 shows the attraction of ticks to AAAP combined with CO_2 and AAAP

Table 5. Mean percentage number (±SE) of *A. variegatum* ticks attracted to AAAP combined with carbon dioxide from different distances in the field

	Distance(m)				
Treatment	1	2	3	4	5
AAAP+ 500gCo_2	98.33±1.05a	93.33±2.47ab	88.33±3.33ab	78.33±11.59b	29.17±13.19c
AAAP	34.17±3.00a	19.17±5.23a	18.33±5.72a	22.50±9.89a	0.83±0.83b
Co_2	0.83±0.83a	0.00±0.00a	1.67±1.67a	0.00±0.00a	0.00±0.00a
Par. oil	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a

Par. = Paraffin

Means within the same row followed by different letters are significantly different at the 5% level based on the SNK test.

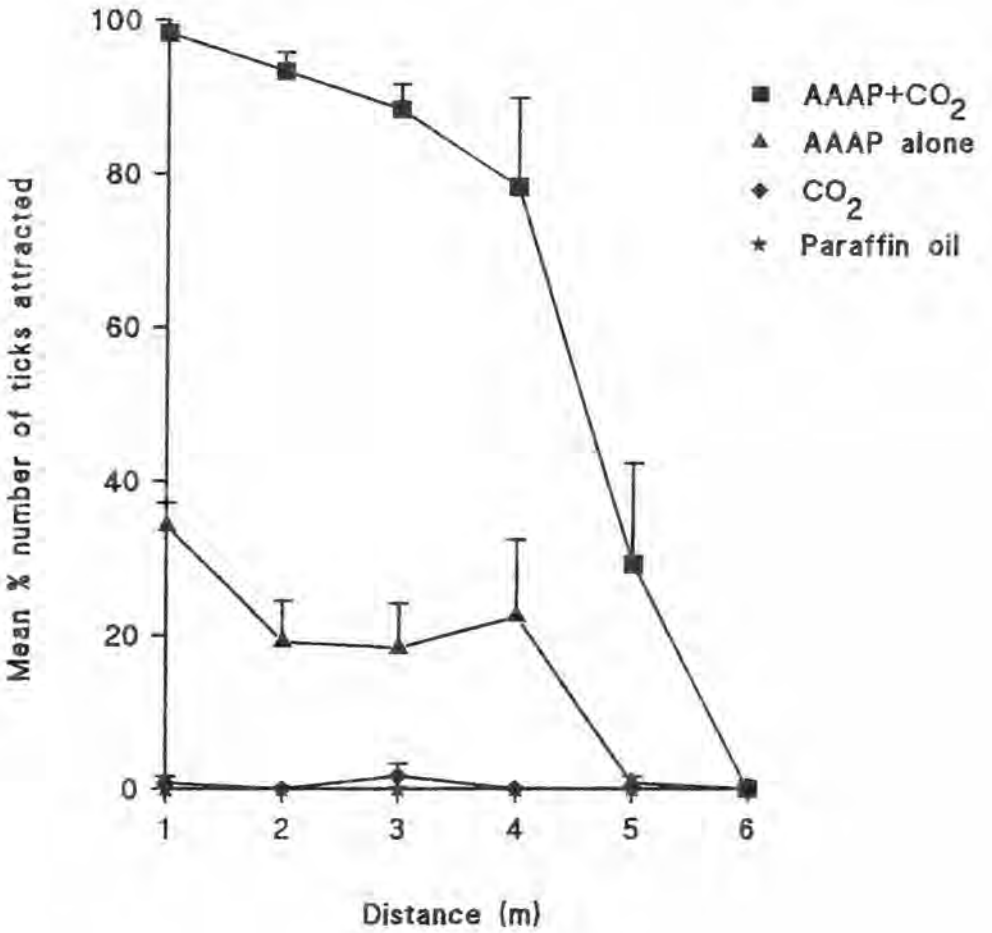

Fig. 7 Attraction of *A. variegatum* from various distances using AAAP with/without CO_2 compared to controls

alone with increasing distance from the source of the pheromone.The highest percentage number of ticks were attracted from 1m and the attraction generally decreased with increasing distance from the source of the pheromone except with the 4m distance for the AAAP alone from where more ticks were attracted copmared to the 3m distance. This could be attributed to inadequate tick stimulation in the absence of CO_2.

The ANOVA comparison results of the attraction of *A. variegatum* to AAAP over different weights of CO_2 in the field are presented on Appendix 5. The results indicate that the treatment and the distance of the pheromone source were both highly significant and so was the interaction between the treatment and the distance ($P<0.001$).

With the 500g CO_2, there were no significant differences at the 5% level, among distances 1m, 2m and 3m but the number of ticks attracted from these three distances were significantly higher from those attracted from 4m and 5m. However, when 50g of CO_2 was used, only a few ticks were attracted from 1m distance (Table 6).

Fig. 8 shows the percentage number of ticks attracted to AAAP combined with various weights of CO_2 from various distances. The mean percentage number of ticks attracted by AAAP combined with different weights of CO_2 reduced with increasing distance from the source of the pheromone. The number of ticks attracted reduced steadily with increasing distance when 500g of CO_2 was used up to the 5m distance and no ticks were attracted from 6m. A similar trend was observed when 50g of CO_2 was used. However, when 5g of CO_2 was used, a few ticks were attracted from the 1m only.

Table 6. Mean percentage number (±SE) of *A. variegatum* ticks attracted to AAAP combined with different weights of Co_2 from varying distances in the field

	Distance(m)				
Treatment	1	2	3	4	5
AAAP+ 500g Co_2	98.33±1.67a	93.33±4.41a	85.00±2.89a	60.18±8.02b	58.33±4.41b
AAAP+ 50g Co_2	91.67±4.41a	81.68±7.26ab	73.33±8.82b	21.67±7.26c	8.33±4.41d
AAAP+ 5g Co_2	23.33±7.26a	0.00±0.00b	0.00±0.00b	0.00±0.00b	0.00±0.00b
Par. oil	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a

Par=Paraffin
Mean within the same row followed by different letters are significantly different at the 5% level based on the SNK test.

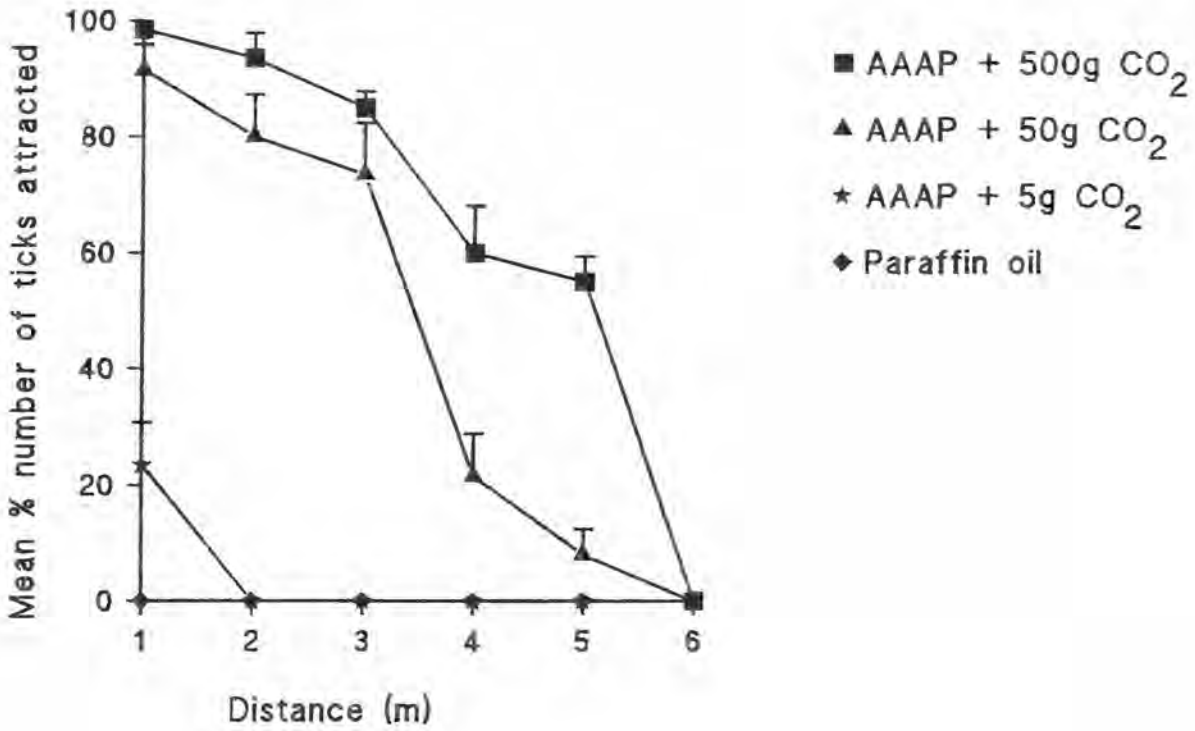

Fig. 8 Attraction of *A. variegatum* from various distances using AAAP combined with varying weights of CO_2

5.1.5 Discussion

The longest distance from which the ticks were attracted was 5 m and no ticks were significantly attracted from beyond this distance. The addition of carbon dioxide only slightly extended the range of pheromonal perception. This was probably because co stimulated ticks and which were in turn able to respond to the pheromone from a wider range.

The incorporation of 500g CO_2, an equivalent of CO_2 production emitted by one bovine of 600Kg for 8 hours (Berbigier, 1988) with the AAAP, increased the number of ticks attracted (Fig. 7). This is in agreement with the findings of Yunker *et al.* (1990) who found that 1.9% of adult *A. variegatum* released at 4m from a source of CO_2 were attracted compared to 54% when a mixture of pheromone extracts and CO_2 were used. CO_2 only stimulated the ticks as plots with CO_2 alone as a stimulus had very active ticks that moved randomly while in the controls ticks showed no activity. These findings are in line with those of Norval *et al* .(1987, 1989a), who working on *A. hebraeum*, reported that in nature, ticks were attracted by large mammals which give out large amounts of CO_2, but which become attractive only if they have pheromone emitting feeding males attached to them.

The use of decreased weights of CO_2 with AAAP resulted to significantly attracting few number of ticks. The findings of this study are in agreement with those of Barre *et al.* (1997) who reported a 66% recovery of Guadeloupe strain of *A. variegatum* adult ticks in one trial with 500g of dry ice and 43% in another with 250g. However this strain is attracted by CO_2 alone unlike the African strain which needs pheromone components to get attracted (Norval *et al.*, 1987).

CHAPTER SIX

6.0 CONTROL OF *AMBLYOMMA VARIEGATUM* USING *BEAUVERIA BASSIANA* AND *METARHIZIUM ANISOPLIAE*

6.1 The effect of different formulations and concentrations of the fungi, *Beauveria bassiana* and *Metarhizium anisopliae* on the mortality of *Amblyomma variegatum*

6.1.1 Introduction

Although the entomopathogenic fungi *B. bassiana* and *M. anisopliae* have been widely used for the biological control of agricultural pests (Ferron, 1981, Anderson *et al.*, 1988), their use in the control of livestock ticks has not been documented. However, Mwangi *et al.*(1994), reported mortalities induced by *B. bassiana* and *M. anisopliae* in all the instars of *R. appendiculatus* in the laboratory. They also observed that when adults of *R. appendiculatus* were exposed to spores of *B. bassiana* and then fed on rabbits, 74% of them failed to lay eggs, while fecundity in those few which produced eggs was decreased by 90%.

Kaaya *et al.* (1996), reported that *B. bassiana* and *M. anisopliae* induced reductions in engorgement weights, fecundity and egg hatchability in adult *A. variegatum* and caused mortalities of between 76-85% in *R. appendiculatus* and a reduction of fecundity of between 85-99% with a reduction in egg hatchability of between 94-100%. In the same study, both fungi induced mortalities of approximately 100%, 76-95% and 36-64% in larvae,nymphs and adults respectively, of *R. appendiculatus* seeded in grass in the field.

The purpose of this study was to determine a suitable formulation and concentration of the fungi, *B. bassiana* and *M. anisopliae* that can be used in the control of *A. variegatum*.

6.1.2 Materials and methods

Water and oil formulations were investigated for use in the traps. Ticks were treated with different concentrations of these two formulations.

The fungi, *M.anisopliae* and *B. bassiana* were harvested as described earlier (see section 2.3.2). The water formulations of the fungi were prepared by centrifuging the harvested spores. The supernatant was then decanted and distilled water added to the remaining spores in the centrifuge tubes. The mixture was thoroughly mixed using a vortex mixer. The mixture was then centrifuged and the supernatant decanted. This procedure of washing the spores was repeated three times to remove all the triton. The washed spores were then dispensed in distilled water and this formed the stock concentration. The stock concentration was then diluted by adding 10mls of the stock concetration to 90mls of distilled water. A drop of this dilution was placed on a Neuber chamber and covered with a coverslip. The spores were then counted and the stock concentration worked out (see section 2.3.3). Using distilled water, serial dilutions of the stock concentration were done to obtain the various water formulation concentrations that were used in the treatment of the ticks.

In preparing the oil formulation, 15% of peanut oil and 1% of emulsogen (1900T) were added to 84% distilled water solution containing the fungal stock concentration of the water formulation. This formed the stock concentration of the

oil formulation. Serial dilutions were then done using a mixture of 15% peanut oil, 1% emulsogen and 84% distilled water to obtain the different concentrations.

6.1.3. Treatments

Vials whose diameter was 3mm and with a height of 8mm, each containing 15 males and 15 females of adult *A. variegatum* ticks were prepared. Triplicates of these tubes were placed on separate petri dishes containing the stock concentration of the water formulation. The ticks were then allowed to wade for 5 seconds after which they were removed using a pair of forceps and placed back into their separate vials. This was repeated for all the concentrations of the water and oil formulations.Separate treatments were done using *M. anisopliae, B. bassiana* and a mixture of the two fungi. In preparing the stock concentration of the mixed fungi, 100mls of the stock concentration of *B. bassiana* was added to 100mls of *M. anisopliae* both containing equal spore concentrations and the mixture was thoroughly mixed using the vortex mixer. 100mls of the mixture was then removed and used as the stock concentration of the mixed fungi on which serial dilutions were carried out to obtain the various concentrations of the mixed fungi. The controls of distilled water, triton in distilled water, peanut oil in distilled water and plain ticks were similarly set. The tubes were then labelled and incubated at 28°C and 75% relative humidity for three weeks. Similar groups of ticks were placed in nylon mesh tetrapaks which were sealed using a hot soldering iron and were placed in a nylon net whose opening was then closed by tying. The bags were then taken to the field and placed in the grass randomly. On the second and third weeks, the

number of dead ticks in each treatment was recorded. The experiment was carried out during both the dry and wet seasons.

6.1.4 Data analysis

Data on tick mortality were recorded, entered on lotus 1-2-3 and analysed using Analysis of variance (ANOVA) procedure on the Statistical Analysis Systems (SAS, 1988). Mean separation was done using the SNK.

6.1.5 Results

ANOVA results of the laboratory experiments on the effect of water and oil formulations of different concentrations of fungi on the mortality of *A. variegatum* are shown on Appendix 6. The effect of formulation, concentration of fungi,type of fungi and the interaction between formulation,fungi and concentration were all significant ($P<0.001$). However, the interactions between: formulations and concentrations, fungi and concentration were not significant ($P>0.05$).

Table 7 shows the mean percentage mortality of *A . variegatum* due to various concentrations of the water and oil formulations of *B. bassiana, M. anisopliae* and a mixture of *B. bassiana* and *M. anisopliae* in the laboratory. There were significant differencces in tick mortality between lower fungal concentrations ($1x10^5$,$1x10^6$, $1x10^7$ spores/ml) and higher concenttrations ($1x10^8$, $1x10^9$, $1x10^{10}$ and $1x10^{11}$ spores/ml) for both *B. bassiana* and *M. anisopliae* at the 5% level. However, there were no significant differences in tick mortality among the fungal concentrations for the oil

Table 7. Mean percentage number (±SE) of *A. variegatum* killed due to infections caused by varying concentrations of the oil and water formulations of *B. bassiana, M. anisopliae* and the mixture of the two fungi in the laboratory

	B. bassiana		*M.anisopliae*		*B.bassiana+ M.anisopliae*			
	Formulation							
Concentration (spores/ml)	oil	water	oil	water	oil	water	Distilled water	Peanut oil
0							0.33±0.00b	2.22±1.11b
$1x10^5$	80.00±1.92b	3.33±1.92b	82.22±8.68bc	1.11±1.11b	95.56±1.11a	2.22±1.11b		
$1x10^6$	84.44+2.22b	6.67±1.92b	88.88±5.88ab	1.11±1.11b	96.67±1.92a	4.44±1.11b		
$1x10^7$	83.33±0.00b	5.56±1.11b	75.56±5.88c	14.11±1.11ab	100.00±0.00a	4.44±2.94b		
$1x10^8$	94.44±1.11a	7.78±1.11b	90.00±5.09ab	7.76±1.11ab	100.00±0.00a	7.78±2.94b		
$1x10^9$	100.00±0.00a	15.56±4.84ab	100.00±0.00a	3.33±0.00b	100.00±0.00a	24.44±10.85a		
$1x10^{10}$	100.00±0.00a	10.00±6.94b	100.00±0.00a	11.11±4.45ab	100.00±0.00a	22.22±1.11a		
$1x10^{11}$	98.89±1.11a	21.11±7.29a	100.00±0.00a	18.89±8.02a	100.00±0.00a	25.56±2.94a		

Means within the same column followed by the different letters are significantly different at the 5% level based on the SNK test.

formulation of the mixture of the two fungi at the 5% level. The water formulation had significant differences among the fungal concentrations in all the treatments while the water controls were significantly different from all the treatments.

There was a general trend of increased tick mortality with increase in fungal concentrations for both the oil and water formulations (Fig. 9). The mixed fungi showed higher tick mortality than the separate fungi when the fungal concentrations were increased in the case of the water formulation with highest tick mortality being attained at $1x10^{11}$ spores/ml. The oil formulation showed higher tick mortalities for the mixed fungi compared to separate fungi with concentrations below $1x10^{8}$ spores/ml but there were no differences in mortality with concentrations above this.

The ANOVA comparisons of formulations and concentrations of fungi and mortality of *A. variegatum* during the dry season are shown on Appendix 7. The formulation and concentration were highly significant ($P<0.001$) while fungi, the interaction between formulation and concentration, fungi and concentration as well as formulation, fungi and concentration were not significant ($P<0.05$). Table 8 shows the mean percentage mortality of *A. variegatum* due to various concentrations of water and oil formulations of *B. bassiana*, *M. anisopliae* and a mixture of *B. bassiana* and *M. anisopliae* during the dry season. There were no significant differences in tick mortalities due to various fungal concentrations in all the treatments except for *M. anisopliae* at a concentration of $1x10^{9}$ spores/ml and the mixed fungi which showed significant differences in tick mortality with the oil formulations at the 5% level.

The ANOVA comparisons for the effect of formulation and concentration of fungi on *A. variegatum* during the wet season are shown on Appendix 8. The formulation, concentration and fungi were all significant ($P<0.001$) and so were the interactions between formulation and concentration, fungi and concentration as well as formulation, fungi and concentration.

Table 9 shows the mean percentage mortality of *A. variegatum* due to various concentrations of water and oil formulation of *B. bassiana, M. anisopliae,* and the mixture of *B. bassiana* and *M. anisopliae* during the wet season. There were significant differences in tick mortality among the various concentrations of all the treatments at the 5% level. For both oil and water formulations of the separate and mixed fungi, tick mortality generally increased with increasing spore concentration. However, mortality was much higher with the oil formulation compared to water formulation.

Figure 10 shows tick mortality due to differnt formulations and concentrations of fungi, during the dry season (October-November, 1996) and the wet season (December, 1996-January 1997).

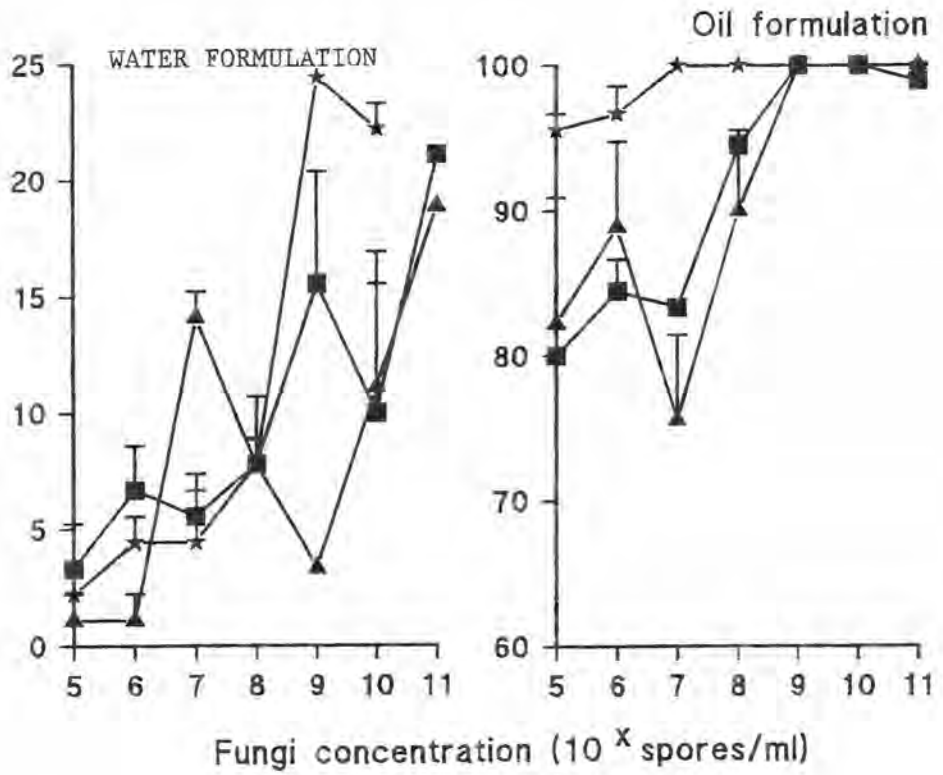

Fig. 9 Effect of different formulations and concentrations of *B. bassiana* (■), *M. anisopliae* (▲) and the mixture of *B. bassiana* and *M. anisopliae* (★) on the mortality of *A. variegatum* in the laboratory

Table 8. Mean percentage numbers (±SE) of *A. variegatum* killed due to infections caused by varying concentrations of the oil and water formulations of *B bassiana, M. anisopliae* and the mixture of the two fungi during the dry season

	B. bassiana		*M. anisopliae*		*B. bassiana* + *M. anisopliae*			
Concentration (spores/ml)	Formulation oil	water	oil	water	oil	water	Distilled water	Peanut oil
0							0.00±0.00a	0.33±1.92b
$1x10^5$	7.78±1.11a	3.33±1.92a	10.00±3.33b	1.11±1.11a	3.33±1.92b	3.33±0.00a		
$1x10^6$	6.67±3.85a	5.56±1.11a	8.89±5.55b	2.22±2.22a	10.00±5.77ab	3.33±0.00a		
$1x10^7$	6.67±0.00a	2.22±1.11a	10.00±5.09b	0.00±0.00a	11.11±1.11ab	1.11±1.11a		
$1x10^8$	5.56±2.22a	3.33±1.92a	7.78±2.94b	6.67±3.33a	15.56±1.11ab	5.56±1.11a		
$1x10^9$	11.11±1.11a	8.89±2.94a	30.00±21.69a	3.33±1.92a	24.44±1.11a	3.33±1.92a		
$1x10^{10}$	10.00±0.00a	5.56±4.00a	17.78±4.00b	7.76±2.22a	20.00±0.00ab	13.33±3.85a		
$1x10^{11}$	15.56±1.11a	11.11±2.94a	15.56±2.22b	10.00±0.00a	24.44±1.11a	16.67±.5.09a		

Means within the same column followed by different letters are significantly different at the 5% level based on the SNK test.

Table 9. Mean percentage numbers (±SE) of *A. variegatum* killed due to infections caused by varying concentrations of the oil and water formulations of *B. bassiana*, *M. anisopliae* and the mixture of the two fungi during the wet season

	B.bassiana		*M. anisopliae*		*B. bassiana*+*M. anisopliae*			
	Formulation							
Concentration (spore/ml)	oil	water	oil	water	oil	water	Distilled water	Peanut oil
0							1.11±1.11f	3.33±3.33f
$1x10^5$	22.22±1.11e	10.00±0.00c	18.89±1.11e	11.11±1.11c	31.11±1.11d	11.11±2.94d		
$1x10^6$	23.33±1.92e	21.11±2.94b	26.67±1.92d	13.33±0.00c	26.67±1.92d	12.22±1.11d		
$1x10^7$	25.56±2.22e	11.11±1.11c	31.11±1.11d	11.11±1.11c	54.44±1.11c	21.11±2.94c		
$1x10^8$	35.56±4.00d	10.00±0.00c	43.33±5.09c	12.22±2.22c	60.00±33.85c	38.89±2.22b		
$1x10^9$	45.56±4.00c	28.89±2.22b	68.89±4.44a	26.67±1.92b	81.11±2.22b	35.56±1.11b		
$1x10^{10}$	60.00±0.00b	25.56±2.22b	68.89±4.00a	26.67±1.92b	81.11±2.22b	35.56±1.11b		
$1x10^{11}$	66.67±1.92a	42.22±4.00a	73.33±1.92a	38.89±1.11a	92.22±1.11a	48.89±1.11a		

Means within the same column followed by different letters are significantly different at the 5% level based on the SNK test.

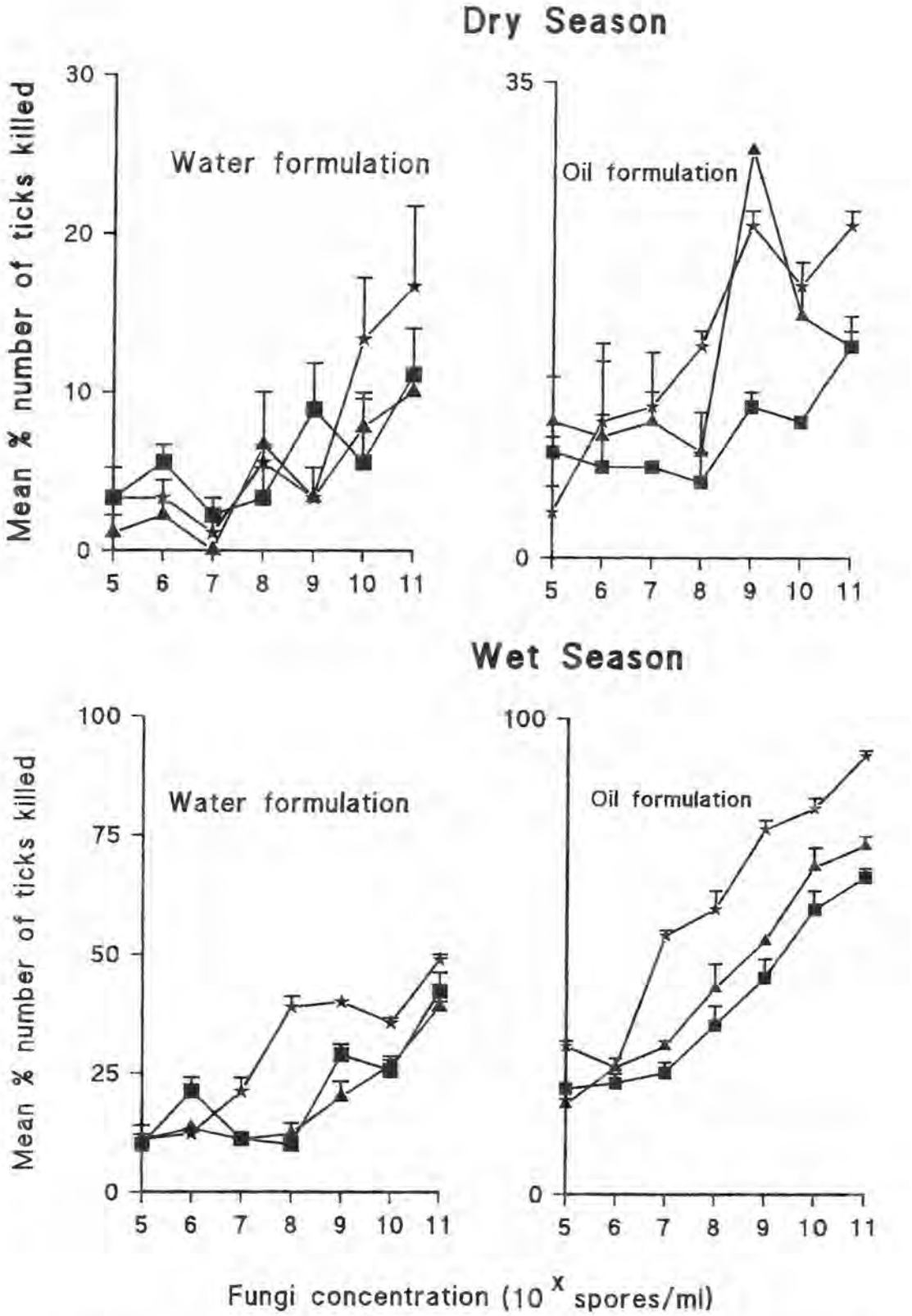

Fig.10 Effect of different formulations and concentrations of *B. bassiana* (■), *M. anisopliae* (▲) and the mixture of *B. bassiana* and *M. anisopliae* (★) on the mortality of *A. variegatum* during the dry and wet seasons

During the dry season, the water formulation had very low tick mortality reaching a maximum of only 16.7% with a concentration of $1x10^{11}$ spores/ml using *B. bassiana* combined with *M. anisopliae*, while the highest tick mortality due to *B.* bassiana and *M. anisopliae* was only 11% and 10% respectively with the same concentration. The oil formulation had a trend similar to the one of the water formulation except that the highest tick mortality was attained by *M. anisopliae* (30%), at a concentration of $1x10^{9}$ spores/ml followed by *B. bassiana* combined with *M. anisopliae* (24%) at the concentrations of $1x10^{9}$ and $1x10^{11}$ spores/ml while *B. bassiana* had its highest tick mortality of 15.5% at a spore concentration of $1x10^{11}$ spores/ml.

During the wet season, the water formulation had its highest tick mortality of 49% at a concentration of $1x10^{11}$ spores/ml using *B. bassiana* combined with *M. anisopliae* while *B. bassiana* and *M. anisopliae* had their highest tick mortalities of 42% and 39% respectively at a similar concentration. The oil formulation had its highest tick mortality of 92% with the mixed fungi while those of the separate fungi were 66.6% and 73% for *B. bassiana* and *M. anisopliae* respectively.

6.1. 6 Discussion

The use of fungi for the control of livestock ticks is still at its early stages but its use in the control of agricultural and forest pests has been well documented (Ferron, 1981; Anderson *et al.*, 1988; Maniania, 1993). The mode of entry of entomopathogenic fungi gives them an advantage over the other pathogens because fungi directly penetrate the cuticle unlike other pathogens which require ingestion to cause disease in the arthropod.

In these studies, mortality of ticks due to different formulations and concentrations of *B. bassiana* and *M. anisopliae* was investigated in the laboratory and in the field. In the laboratory it was observed that oil formulation of the fungi gave upto 100% mortality in *A. variegatum* adults at a concentration of $1x10^9$ spores/ml. Mwangi *et al* . (1994) reported 73% and 35% mortality by *B. bassiana* and *M. anisopliae*, respectively, in unfed adults of *Rhipicephalus appendiculatus*. In the same study, the mortality in engorged *R. appendiculatus* was 74% for *B. bassiana* and 81% for *M. anisopliae*.

Kaaya et al.,(1996) reported that the water formulation of *B. bassiana* and *M. anisopliae* induced mortality of 37% at a concentration of $1x10^8$ spores/ml while *B. bassiana* induced no mortality in the adults of *A. variegatum* when sprayed on ticks engorging on rabbits. In the current study, a mortality of 21% and 18% for *B. bassiana* and *M. anisopliae* respectively was induced using a concentration of $1x10^{11}$ spores/ml with water formulation while with the combination of the two fungi at a similar concentration, a mortality of 25% of unfed adult ticks was induced in the laboratory. The results of this study differ from those of Kaaya *et a.l.*(1996), in that a lower mortality was obtained even with a higher spore concentration probably because unfed ticks were used while Kaaya *et al* . (1996) used engorging ticks.

In the field experiments which were carried out during the dry and wet seasons, *A. variegatum* mortality induced by fungi of the oil formulation was higher compared to the water formulation in both seasons. The combined fungi formulation induced higher mortality in both seasons compared to the separate fungi.There were significant differences between the seasons (i.e laboratory, wet and dry season) as well as the

formulation and the concentrations. There was also a significant difference between the combined fungi and the separate fungi formulations, but these showed no significant differences between themselves. In both the dry and wet seasons, the highest tick mortality was induced by the combined fungi with oil formulation . During the dry season, the highest tick mortalities recorded were 16% and 24% for water and oil formulations respectively, both by combined *B.bassiana* and *M. anisopliae* with a concentration of $1x10^{11}$ spores/ml. During the wet season, the combined fungi gave the highest tick mortality of 92% and 48% for oil and water formulations respectively. Samsinakova *et al.* (1974) observed that the infection rates of *Ixodes ricinus*, *Dermacentor marginales* and *D. reticulatus* in the field by various pathogens including *B. bassiana* was only 6% in winter while it was between 45-57% in engorged *Ixodes ricinus* females during summer when temperatures were high.

In the laboratory, it was observed that there was 100% mortality of ticks due to infection by the combined fungi with the $1x10^{11}$ spores/ml concentration the second week following exposure of ticks to the fungi while it took three weeks to obtain the highest mortalities for both *B. bassiana* and *M. anisopliae* when treated separately at that concentration. These observations could be attributed to the competition between the fungal species which could have led to a faster growth rate. This was evidenced by more sporulation of the fungi in the mixed infections than in the separate infections even when the same concentration of fungi was used (Plates 12,13,and14). Combination of fungi was found to be more effective in killing the ticks compared to individual treatments.

In the laboratory, oil formulation of the concentrations between $1x10^{9}$

spores/ml and $1x10^{11}$ spores/ml gave upto 100% mortality for both *M.anisopliae* and *B.bassiana*. Lower concentrations gave mortalities between 75% and 94% and the mortality increased with increasing concentration. The fungal mixture showed 100% mortality at a much lower concentration of $1x10^{7}$ spores/ml. Water formulation had a highest mortality of 21% and this was at a concentration of $1x10^{11}$ spores/ml for *B.bassiana* and 19% for *M.anisopliae* while that of the mixed fungi was 25% at the same concentration.

From the results obtained in this study, it was therefore concluded that in the laboratory a concentration of $1x10^{9}$ spores/ml was good enough for the control of *A. variegatum* by both *M. anisopliae* and *B. bassiana* with the oil formulation while a higher concentration of $1x10^{11}$ spores/ml was fairly good if a water formulation was to be used.

Field experiments carried out during the dry period showed little mortality. Water formulation gave a mortality of 10% at a concentration of $1x10^{11}$ spores/ml for both *M.anisopliae* and *B. bassiana* while that of the mixed fungi at the same concentration was 17%. Oil formulation also showed the highest mortality of 17% at a concentration of $1x10^{11}$ spores/ml for both *M.anisopliae* and *B.bassiana*. For the mixed formualtion during the wet period, the highest mortality was 23% at a concentration of $1x10^{11}$ spores/ml . The water formulation gave the highest tick mortality of 40% for *M.anisopliae* and 43% for *B.bassiana* at a concentration of $1x10^{11}$ spores/ml while the mixture showed the highest mortality of 50% at the same concentration. For the oil formulation, the highest mortality of 73% and 67% were obtained for *M.anisopliae* and *B.bassiana* respectively at a concentration of $1x10^{11}$

spores/ml.

There were greater mortality rates in mixed infections as compared to separate infections of the fungi. Microscopic observations revealed that, in mixed infections *M.anisopliae* outgrew *B.bassiana* and that sporulation was more intense and occurred faster in mixed infections than in separate infections of the fungi (Plates 12, 13 and 14) which may be attributed to competition between the two species of fungi.

Plate 12. Tick infected by *M. anisopliae*

Plate 13. Tick infected by *B. bassiana*

Plate 14. Tick showing mixed infections of

M. anisopliae and *B. bassiana*

6.2 Compatibility of *Beauveria bassiana* and *Metarhizium anisopliae* with AAAP and lateral transfer of fungal infection in the mortality of *Amblyomma variegatum*

6.2.1 Compatibility of *Beauveria bassiana* and *Metarhizium anisopliae* with AAAP

6.2.1.1 Introduction

Prior to this study, no work has been reported on pheromone/fungi mixtures and their compatibility. However, Norval *et al* (1996) reported excellent efficacy of acaricide/AAAP mixtures on tick mortality on animals. Gladney *et al* (1974a) also demonstrated that females of *Amblyomma maculatum* which had been placed on cattle were killed when they were lured to a spot on an animal with a pheromone/acaricide mixture.

In this study, *B. bassiana*, *M. anisopliae* and the mixture of the two were each combined with various concentrations of the AAAP to study the effect of the latter on their infection efficiency on *A. variegatum*.

6.2.1.2 Materials and methods

The oil formulation of both fungi *B. bassiana* and *M. anisopliae* were prepared as in section 4.1.2 and the synthetic AAAP as in section 2.4. Adult 15 male and 15 female ticks were counted and placed in a tube and 160 such tubes prepared. Fungi concentrations of $1x10^9$ spores/ml were prepared for both *B. bassiana* and *M. anisopliae* (see section 6.1.2) and the mixture of the fungi prepared by taking equal volumes of both fungi and thoroughly mixing them using a vortex mixer. Ten millilitres of *B. bassiana, M. anisopliae* and the mixture of the two fungi were each placed on separate petri dishes . Some petri dishes consisting of distilled water and the peanut oil with emulsogen and distilled water alone were prepared to be used as controls. Eighty such tubes were prepared and then labelled.

One microlitre of AAAP containing 0.2mg ortho-nitrophenol, 0.1mg methyl salicylate and 0.8mg nonanoic acid were added to the 10mls of the oil formulation of *M. anisopliae* in a petri dish and mixed well using a stirer. Ticks in one of the tubes were released on to the petri dish and allowed to wade for 5 seconds after which they were removed using a pair of forceps and placed back into the tube and labelled.Triplicates of this preparation were made and the procedure repeated for *B. bassiana* and the mixture of *B. bassiana* and *M. anisopliae.*

This procedure was repeated with increasing concentrations of the AAAP by 1.1mg each time upto 11mg of the AAAP. The ticks were then incubated in the laboratory at 28°C and 75% relative humidity for two weeks. The control experiments were similarly set using distilled water and peanut oil.

6.2.1.3 Data analysis

The data on the effect of various conncenttratiions of AAAP on the mortality of *A. variegatum* due to *B. bassiana, M. anisopliae* and their mixture were recorded, entered on lotus 1-2-3 and Analysis of Variance carried out on the Statistical Analysis Systems (SAS, 1988). Mean separation was done using the SNK.

6.2.1.4 Results

The ANOVA comparisons on the fungi and the concentration of AAAP are shown on Appendix 9. The results indicate that the effect of the concentration of AAAP on the tick mortality was significant ($P< 0.001$) while the interaction between the fungi and the concentration was not significant ($P<0.05$). The mean percentage mortalities of *A. variegatum* due to *B. bassiana, M. anisopliae* and the mixture of *B bassiana* and *M. anisopliae* combined with various concentrations of AAAP are shown on Table 10. These results indicate that AAAP had no significant effect on tick mortality due to fungi with all the concentrations that were experimented upon and even with the control, at the 5% level, except for *M. anisopliae* which showed significant difference at an AAAP dose of 11mg.

Table 10. Mean percentage number (±SE) of ticks killed due to infections caused by *B. bassiana*, *M. anisopliae* and the mixture of the two fungi treated with varying concentrations of AAAP.

AAAP dose(mg)	*B. bassiana*	*M. anisopliae*	*B.bassiana*+ *M.anisopliae*	Distilled water	Peanut oil
0	98.89±1.11a	100.00±0.00a	100.00±0.00a	0.33±0.33b	0.00±0.00b
1.1	100.00±0.00a	96.67±1.92a	100.00±0.00a	0.00±0.00b	3.33±3.33b
2.2	98.89±1.11a	97.78±1.11a	100.00±0.00a	0.00±0.00b	3.33±3.33b
3.3	96.67±0.00a	94.44±2.22a	97.78±1.11a	0.00±0.00b	3.33±3.33b
4.4	97.78±1.11a	95.56±2.22a	97.78±1.11a	3.33±0.00b	4.44±2.94b
5.5	95.56±1.11a	92.22±1.11a	94.44±1.11a	2.22±1.11b	2.22±1.11b
6.6	90.00±0.00a	93.33±1.92a	96.67±0.00a	0.33±0.33b	0.00±0.00b
7.7	85.56±4.00a	93.33±0.00a	94.44±1.11a	0.33±0.00b	3.33±0.00b
8.8	91.11±1.11a	90.00±3.33a	93.33±0.00a	1.11±1.11b	0.33±0.33b
9.9	87.78±2.94a	87.78±1.92a	91.11±1.11a	0.33±0.33b	3.33±3.33b
11	85.89±4.00a	65.56±26.13b	91.11±2.94a	3.33±3.33b	3.33±3.33b

Means within the same column followed by the same leter are not significantly different at the 5% level based on the SNK test.

Fig. 11 shows a general trend of decreasing tick mortality with increasing concentration of AAAP. Lowest level of tick mortality was observed with *B. bassiana* followed by *M. anisopliae* while the mixed fungi showed higher tick mortality rates with all concentrations of AAAP copmpared to the separate fungi. The regression equations for the tick mortality due to fungi are: *B. bassiana*, $Y = -1.434x + 101.222$, $r = 0.911$, $r^2 = 0.83$; *M. anisopliae* ,$Y = -1.895x + 101.768$, $r = 0.717$, $r^2 = 0.514$; and the mixed fungi, $Y = -0.891 + 100.961$, $r = 0.96$, $r^2 = 0.922$. The untreated fungi had 99%, 100% and 100% for, *B. bassiana*, *M. anisopliae* and the combined fungi respectively.

6.2.1.5 Discussion

The infection efficiency of *B. bassiana*, *M. anisopliae* and their mixture decreased with increasing concentration of AAAP. The mixed fungi showed slightly higher tick mortality rates compared to that of the separate fungi with almost all the AAAP concentrations that were experimented on. However, *B. bassiana*, *M. anisopliae* and their mixture with varying AAAP concentrations had significant differences with the controls. This means that it is appropriate to use *B. bassiana*, *M. anisopliae* and their mixture with varying AAAP concentrations of as much as 11mg to attract and kill the ticks. The results of this study have also shown that the infection efficiency of *B. bassiana*, *M. anisopliae* and their mixture continue to reduce gradually with increasing concentrations of AAAP, with the mixed fungi causing slightly higher mortalities than the separate fungi (Fig,11). However, in the case of AAAP /acaricide mixtures, AAAP has not been reported to have any effect on the efficacy of the acaricide. This is probably because fungi are living micro-organisms and the AAAP

components may be interfering with their metabolic reactions and as a result there is decreased infection efficiency. When using *B. bassiana*, *M. anisopliae* and their mixture and AAAP for the control of *A. variegatum*, they could be used separately for maximum benefit.

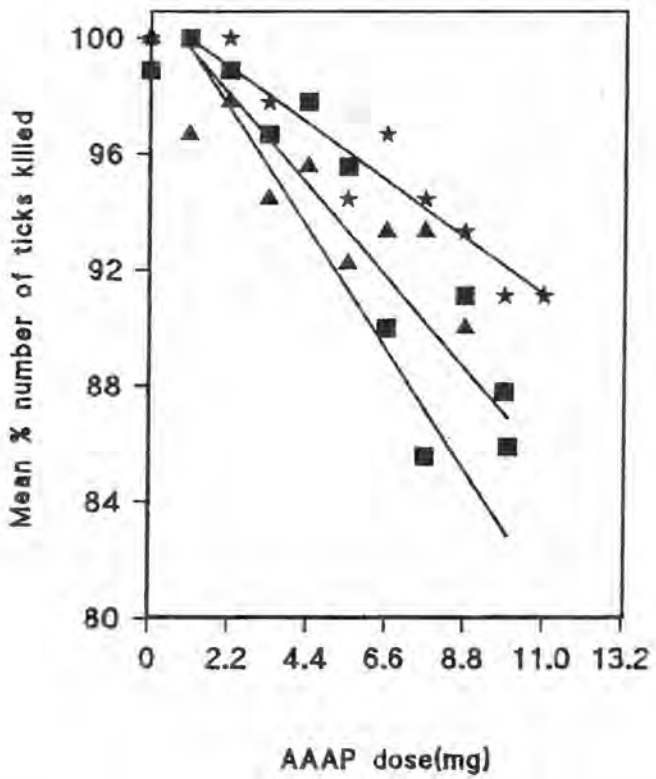

Fig. 11 Mortality of *B. bassiana* (■) *M. anisopliae* (▲) and the mixture of *B.bassiana* and *M.anisopliae* (★) with AAAP

6.2.2 Lateral transfer of fungal infection and mortality of *A. variegatum*

6.2.2.1 Introduction

The possibility of horizontal transfer of the pathogenic fungi among ticks could enhance the efficacy of using entomopathogenic fungi in the control of *A. variegatum*.

Kaaya and Okech (1990), demonstrated that adult tsetse when exposed to conidia of *B. bassiana* and *M. anisopliae* acquired fungal infection and were capable of transmitting the infections to the non-infected tsetse, irrespective of their sex combination in the laboratory in small cages. This suggested that transmission took place not only during the process of mating, but probably at any time when there was physical contact between the infected and non-infected tsetse. Brobyn and Wilding (1983), successfully infected houseflies with *Entomophthora muscae* by releasing healthy flies into cages containing flies that had died of *E. muscae* infection and then removed the dead flies 16 hrs later.

6.2.2.2 Materials and Methods

Adult *A. variegatum* ticks were sorted out according to their sexes in groups of ten. Oil formulations of *M. anisopliae* and *B. bassiana* of ($1x10^9$ spores/ml) were prepared as described earlier (see section 6.1.2). In the first group, ten males were exposed to *B. bassiana* by allowing them to wade on a petri dish containing the fungus for five seconds. The exposed ticks were then placed in a petri dish containing ten female ticks which were unexposed to the fungus(ratio of exposed:non-exposed = 1:1). The petri dish was then covered with cotton mesh which was firmly held to the petri dish by an elastic band. In the second group, females were exposed instead of males and treated as above. In the third group, ten marked exposed males were added to ten

unexposed males and in the fourth group ten marked exposed females were added to ten unexposed females.In the fifth group, ten adult males and ten adult females were exposed to fungi as explained above and then placed in separate petri dishes each containing ten nymphs of *A. variegatum*. Group six was treated with distilled water while group seven was untreated. Six replicates of each treatment were prepared. This procedure was repeated for *M. anisopliae* as well as their mixture.

The ticks were left in their petri dishes for 24hrs and on the following day, in three replicates, of each of the groups, the treated ticks were separated from the untreated ticks while in the other three replicates the treated ticks were not separated from the untreated ticks. The various groups of ticks were then tranferred into separate tubes which were then covered with cotton wool and incubated at 28° C and 75% relative humidity for two weeks. This procedure was repeated with a duration of 30minutes and a ratio of 1:3 for exposed: nonexposed.

6.2.2.3 Data analysis

Tick mortality data in relation to ratio, duration, fungi and types (males, females and nymphs) were recorded, entered on lotu 1-2-3 and analysed using the General Linear Models (GLM) procedure on the Statistical Analysis Systems (SAS, 1988). Mean separation was done using the Student-Newman-Keuls (SNK) test. All the values were subjected to square root transformation ($\sqrt{\log_{10}(x+0.5)+1}$) prior to analysis.

6.2.2.4 Results

The results of the GLM comparisons on the lateral transfer of *B. bassiana*, *M. anisopliae* and their mixture by *A. variegatum* are presented on Appendix 10. The

effects of ratio, duration, fungi, and type were found to be significant ($P< 0.001$) and the interactions between duration and fungi, ratio and type, were also significant ($P< 0.05$) and so was the interaction between ratio and duratiion ($P<0.001$).The ratio of 1:1 of the exposed ticks to the non-exposed ticks resulted in a higher tick mortality compared to a ratio of 1:3 of the exposed ticks to the non-exposed ticks and these differences were significant at the 5% level (Tables11a and 11b). Mortalities due to the mixed fungi were significantly higher than those due to separate fungi which in turn caused higher mortalities than the ticks treated with distilled water whose difference was also significant at the 5% level. Long term(24 hrs) exposure of ticks to fungi resulted in higher lateral transfer of ticks than the short term exposure (30 minutes) and the differences were significant at the 5% level (Tables 11a, 11b, 11c and 11d). The mortality of nymphs resulting from infection due to lateral transfer of fungi by adult ticks was the highest compared to the other types of transfer and nymphal mortality was highest when fungal transfer was by the females. Mortality due to infection as a result of lateral transfer between males and females and vise versa was not significantly different at the 5% level. Mortality of ticks in a situation where exposed ticks were incubated together with non-exposed ticks was not significantly different from that of the ticks which were incubated separately at the 5% level. There was a significant difference in tick mortality due to mixed infection and separate infections but there was no significant difference between the tick mortality due to *B. bassiana* and *M .anisopliae* but both of them were significantly different from that of the distilled water (control) at the 5% level. Mortality of ticks in a situation where exposed ticks were incubated together with non-exposed ticks was not significantly different from that of the ticks which were incubated separately at the 5% level. There was a significant difference in tick mortality

due to mixed infection and separate infections but there was no significant difference between the tick mortality due to *B. bassiana* and *M .anisopliae* but both of them were significantly different from that of the distilled water (control) at the 5% level

Table 11a. Mean percentage number (±SE) of *A. variegatum* killed due to infections caused by the lateral transfer of *B. bassiana, M. anisopliae* and the mixture of the two fungi.

Duration: 24 hours Ratio: 1:1

Type of transfer	*B.bassiana*	*M.anisopliae*	*M.anisopliae*+ *B.bassiana*	Distilled water
male to fem	33.33±16.67mnop	26.67±3.33opqr	43.33±3.33ijklm	6.67±0.67uvw
male to male	36.67±12.02lmno	36.67±8.82lmno	60.00±5.77defg	0.00±0.00w
Fem to male	20.00±10.00qrst	30.00±10.00nopq	26.67±6.67opqr	0.00±0.00w
Fem to fem	30.00±0.00nopq	36.67±8.82lmno	36.67±14.53lmno	0.00±0.00w
Fem to nymph	56.67±3.33efgh	73.33±8.82bc	80.00±0.00ab	3.33+3.33vw
male to nymph	56.67±8.82efgh	66.67±6.67cd	86.68±3.33a	10.00±5.77tuvw
Fem to male(tog)	46.67±3.33hijk	20.00±10.00qrst	56.67±8.82efgh	3.33±3.33vw
Fem to fem(tog)	36.67±12.02lmno	33.33±3.33mnop	56.67±6.67efgh	3.33±3.33vw
male to male(tog)	43.33±3.33ijklm	36.667±3.33lmno	46.667±12.02hijkl	3.33±3.33vw
male to fem(tog)	30.00±5.77nopq	40.00±11.55klmn	46.67±6.67hijkl	0.00±0.00w

Fem= Female Tog= Together

Means followed by different letters within the same row of each set of type of transfer are significantly different at the 5% level based on the SNK test.

Table 11b. Mean percentage number (±SE) of *A. variegatum* killed due to infections caused by the lateral transfer of *B. bassiana*, *M. anisopliae* and the mixture of the two fungi.

Duration: 24 hours Ratio: 1:3

Type of transfer	*B.bassiana*	*M.anisopliae*	*M.anisopliae*+ *B.bassiana*	Distilled water
male to fem	11.11±6.41stuvw	22.22±6.41pqrs	29.63±9.80nopq	0.00±0.00w
male to male	22.22±11.11pqrs	11.11±11.11stuvw	22.22±6.41pqrs	0.00±0.00w
Fem to male	18.52±13.35qrstu	25.93±13.35opqr	37.04±19.60lmno	0.00±0.00w
Fem to fem	37.04±9.80lmno	22.22±6.41pqrs	48.15±3.71hijkl	0.00±0.00w
Fem to nymph	33.33±0.00mnop	40.74±3.71jklmn	62.96±9.80def	3.33±3.33vw
male to nymph	44.44±0.00ijklm	40.74±9.80klmn	51.85±3.71ghij	3.33±3.33vw
Fem to male(tog)	14.81±7.41rstuv	29.63±3.71nopq	29.63±9.80nopq	0.00±0.00w
Fem to fem(tog)	25.63±9.80opqr	33.33±6.41mnop	29.63±3.71nopq	3.33±3.33vw
male to male(tog)	25.93±7.41opqr	22.22±0.00pqrs	40.74±3.71klmn	0.00±0.00w
male to fem(tog)	29.63±3.71nopq	44.44±0.00ijklm	48.15±3.71hijkl	3.33±3.33vw

Fem= Female Tog= Together

Means followed by different letters within the same row of each set of type of transfer are significantly different at the 5% based on the SNK test

Table 11c. Mean percentage number (±SE) of *A. variegatum* killed due to infections caused by the lateral transfer of *B. bassiana, M. anisopliae* and the mixture of the two fungi.

Duration: 30 minutes Ratio: 1:1

Type of transfer	*B.bassiana*	*M.anisopliae*	*M.anisopliae*+ *B.bassiana*	Distilled water
male to fem	50.00±5.77ghijk	40.00±0.00klmn	60.00±5.77defg	3.33±3.33vw
male to male	36.67±3.33lmno	36.67±8.82hijkl	46.67±3.33lmno	3.33±3.33vw
Fem to male	33.33±8.82mnop	30.00±0.00nopq	50.00±5.77hijk	3.33±3.33vw
Fem to fem	43.33±8.82ijklm	53.33±3.33fghi	53.33±12.02ghi	0.00±0.00w
Fem to nymph	76.67±3.33b	63.33±6.67de	80.00±5.77b	3.33±3.33vw
male to nymph	66.67±8.82cd	73.33±3.33bc	76.67±8.82b	3.33±3.33vw
Fem to male(tog)	20.00±5.77qrst	36.67±12.02lmno	33.33±5.77mnop	0.00±0.00w
Fem to fem(tog)	36.67±3.33lmno	26.67±12.02opqr	30.00±5.77nopq	0.00±0.00w
male to male(tog)	26.67±6.67opqr	23.33±8.82pqr	26.67±8.82opqr	0.00±0.00w
male to fem(tog)	16.67±3.33rstu	40.00±11.55klmn	43.33±6.67ijklm	3.33±3.33vw

Fem= Female Tog= Together

Means followed by different letters within the same row of each set of type of transfer are significantly different at the 5% level based on the SNK test.

Table 11d. Mean percentage number (±SE) of *A. variegatum* killed due to infections caused by the lateral transfer of *B. bassiana, M. anisopliae* and the mixture of the two fungi.

Duration: 30 minutes Ratio: 1:3

Type of transfer	*B.bassiana*	*M.anisopliae*	*M.anisopliae*+ *B.bassiana*	Distilled water
male to fem	3.70±3.71vw	11.11±6.41stuvw	0.00±0.00w	0.00±0.00w
male to male	7.41±3.71uvw	0.00±0.00w	11.11±0.00stuvw	0.00±0.00w
Fem to male	7.41±3.71uvw	7.41±3.71uvw	7.41±7.41uvw	0.00±0.00w
Fem to fem	40.74±40.74klmn	11.11±6.41stuvw	7.41±3.71uvw	0.00±0.00w
Fem to nymph	22.22±0.00pqrs	18.52±9.80qrstu	25.93±0.00opqr	0.00±0.00w
male to nymph	3.70±3.71vw	11.11±6.41stuvw	7.41±3.71uvw	0.00±0.00w
Fem to male(tog)	14.81±3.71rstuv	14.81±9.8rstuv	18.52±3.71qrstu	3.33±3.33vw
Fem to fem(tog)	7.41±3.71uvw	0.00±0.00w	3.70±3.71vw	0.00±0.00w
male to male(tog)	18.52±7.41qrstu	18.52±13.35qrstu	14.81±3.71rstuv	3.33±3.33vw
male to fem(tog)	14.81±3.71rstuv	3.70±3.71stuvw	11.11±6.41vw	0.00±0.00w

Fem =Female Tog =Together

Means followed by different letters within the same row of each set of type of transfer are significantly different at the 5% level based on the SNK test.

6.2.2.5 Discussion

The results of this study have demonstrated that when *A. variegatum* ticks were exposed to the spores of *B. bassiana, M. anisopliae* and the mixture of *B. bassiana* and *M. anisopliae* they were able to pick up the spores and then transmited them to previously non-exposed ticks and eventually caused infection and death in those ticks. The transfer from one sex to another were equally efficient but low while that from adults to nymphs was significantly high (Table 11a, 11b, 11c and 11d). This shows that transmission does not only occur during mating but any time the exposed ticks are in contact with the non- infected ticks. The transfer of fungal infection from infected ticks to non-infected ticks could enhance the efficiency of the use of fungi for the control of ticks. Fungal infections have been shown to be transmitted from infected to non-infected arthropods through physical contact (Brobyn and Wilding, 1983). Kaaya and Okech, 1990, have demonstrated that adult tsetse when exposed to conidia of *B.bassiana* and *M. anisopliae* acquired fungal infection and were capable of transmitting the infections to non infected healthy tsetse in small cages in the laboratory. The findings of this study are in line with those of Kaaya and Okech (1990) for tsetse. The transmission between ticks which had a longer term exposure (24hrs), was significantly higher than that in ticks which were exposed for 30 minutes. This was probably because the ticks had enough time for the fungi to establish on the cuticle.

The ratios of the contaminated ticks to non-infected ticks were found to influence the level of transmission. A ratio of 1:1 of the contaminated ticks to non-exposed ticks caused significantly higher tick mortality than that of a ratio of 1:3 (Table 11a, 11b, 11c, and 11d). Like in the previous experiments, mixed fungi caused significantly higher tick mortalities than separate infections. It was concluded that mixed

fungi with a 24hr exposure and a ratio of 1:1 was prefered in the lateral transfer of fungi in *A. variegatum*. Although mixed fungal infection was more effective, the overall efficiency of lateral transfer was relatively low even in the confined places. In animals, transmission may be better because of clustering as no aggregation pheromone was used in this experiment. It is possible that the pheromone may improve transmission since it causes aggregation in *A. variegatum* and hence enhace the spread of the disease. However, this requires further studies.

CHAPTER SEVEN

7.1 MORTALITY OF TICKS EXPOSED TO *M. ANISOPLIAE* AND *B. BASSIANA* IN TRAPS BAITED WITH AAAP COMBINED WITH CARBON DIOXIDE IN THE FIELD.

7.1.1 Introduction

The use of pheromone traps for the control of ticks has not been well exploited. So far pheromone traps have been used in the sampling of *A. variegatum* in the vegetation (Barre *et al.* 1997). The use of pheromone/acaricide mixtures in the control of *Amblyomma hebraeum* has been extensively investigated (Rechav and Whitehead, 1978, Sonenshine *et al.*, 1979 and Norval *et al.*, 1991) while the use of pheromone/acaricide impregnated decoys has also been documented. Norval *et al.* (1994a), have described a decoy impregnated with AAAP and used it to attract unfed males, females and nymphs of *A. hebraeum* to a location where an acaricide could kill them. Pheromone/acaricide impregnated plastic tail-tag decoys have demonstrated excellent efficacy of the bont tick, *A. hebraeum*, on cattle in Zimbabwe (Norval *et al.* 1996).

Most of this work has been carried out on *A. hebraeum* but not on *A. variegatum* and no work has been done using pheromone/fungi mixtures. The aim of this experiment was therefore, to investigate the efficacy of fungi traps baited with the AAAP for the control of *A. variegatum* in vegetation.

7.1.2 Materials and methods

Fifteen plots were prepared as in section 4.1.2.2 except that in this case the grass was not cut short but instead it was left to grow naturally. A total of 400 ticks were released from four directions (North, South, East and West) with 100 ticks being released from each direction and twenty ticks were released from each of the five distances (1m,2m,3m,4m and 5m) in each of the fifteen plots. The ticks were then left in the vegetation for five days to stabilise. On the sixth day, traps containing the oil formulation of a mixture of *B. bassiana* and *M. anisopliae* of a concentration of $1x10^{11}$ spores/ml and baited with 6.6mg of AAAP and 500g of CO_2 were set at the centre of each of the three experimental plots (Plate 15). Control plots were similarly set with three of the plots containing the oil formulation without fungi, the other three had distilled water instead of the fungi while three plots had empty traps and the remaining three were only seeded with ticks. The experiment was divided into two phases whereby in the first phase the ticks that were attracted to the traps and were exposed to the fungi as well as the controls were collected and they were incubated in the laboratory for three weeks at 28°C and 75% relative humidity and their mortality assessed. In the second phase, the plots were cleared of the old ticks using AAAP and CO_2 traps before releasing a new set of ticks and then setting the traps as above. The traps were left for three weeks after which sampling of ticks was done using AAAP combined with 500g of CO_2 in each of the plots.

Plate 15. Photograph showing the tick trap set in the field

7.1.3 Data analysis

Data on ticks attracted to the traps and that of their mortality were recorded, entered on lotus 1-2-3 and Analysis of Variance(ANOVA) carried out on the Statistical Analysis Systems (SAS, 1988). Mean separatiion was done using SNK. Data on ticks recaptured was similarly handled.

7.1.4 Results

The ANOVA results on the efficiency of the pheromone trap in the field are shown on Appendix 11. The results indicate that the effect of the treatment was highly significant ($P<0.001$). The mean percentage number of ticks exposed to the fungi in the field and killed by the fungi are presented on Table 12. The highest number of ticks killed were from the group where the fungi were present and these results were significantly different at the 5% level. There was minimal mortality in ticks which were not exposed to the fungi which clearly shows that AAAP, CO_2, peanut oil and distilled water did not kill the ticks but fungi killed them.

Appendix 12 is the ANOVA table on the efficiency of the pheromone trap and the results indicate that the effect of treatment was highly significant ($P<0.001$). The mean percentage numbers of ticks recovered after treatments are presented on Table 13. There was a significant difference at the 5% level, between the number of ticks recovered from the paddocks which had the fungi trap baited with the AAAP pheromone and CO_2 and all the other treatments including the untreated controls, whereby the paddocks with the fungi trap had the lowest number of ticks (33.77%) and this was less than half the number of ticks recovered in the other paddocks. However, there were no significant differences between all the other treatments. Fig. 12 shows the

percentage number of ticks exposed to the fungi and those which were killed by the fungi. There were no significant differences in the number of ticks exposed to all the treatments but the number of ticks killed by the fungi was much higher compared to the other treatments and it was almost as high as the number of ticks exposed to the fungi which means that oil, distilled water and traps did not kill the ticks. The percentage number of ticks (33.77%) recovered from the paddocks containing the fungi were significantly different at the 5% level from the control paddocks which ranged between 76% and 84% (Fig.13).

Table 12. Mean percentage numbers (±SE) of *A. variegatum* killed, due to infections caused by the mixture of *B. bassiana* and *M. anisopliae* 3 weeks after exposing them to the fungi using the traps.

Treatment	Mean % number of ticks	
	Exposed	Killed
AAAP+CO_2+fungi	79.00±1.39a	77.83±1.26a
AAAP+ CO_2+oil formulation	85.00±3.72a	1.00±0.29b
AAAP+ CO_2+distilled water	83.00±2.24a	0.67±0.22b
AAAP+ CO_2+ Traps	86.00±3.28a	0.08±0.08b
None	77.00±3.33a	0.00±0.00b

Means within the same column followed by the same letter are not significantly different at the 5% based on the SNK test.

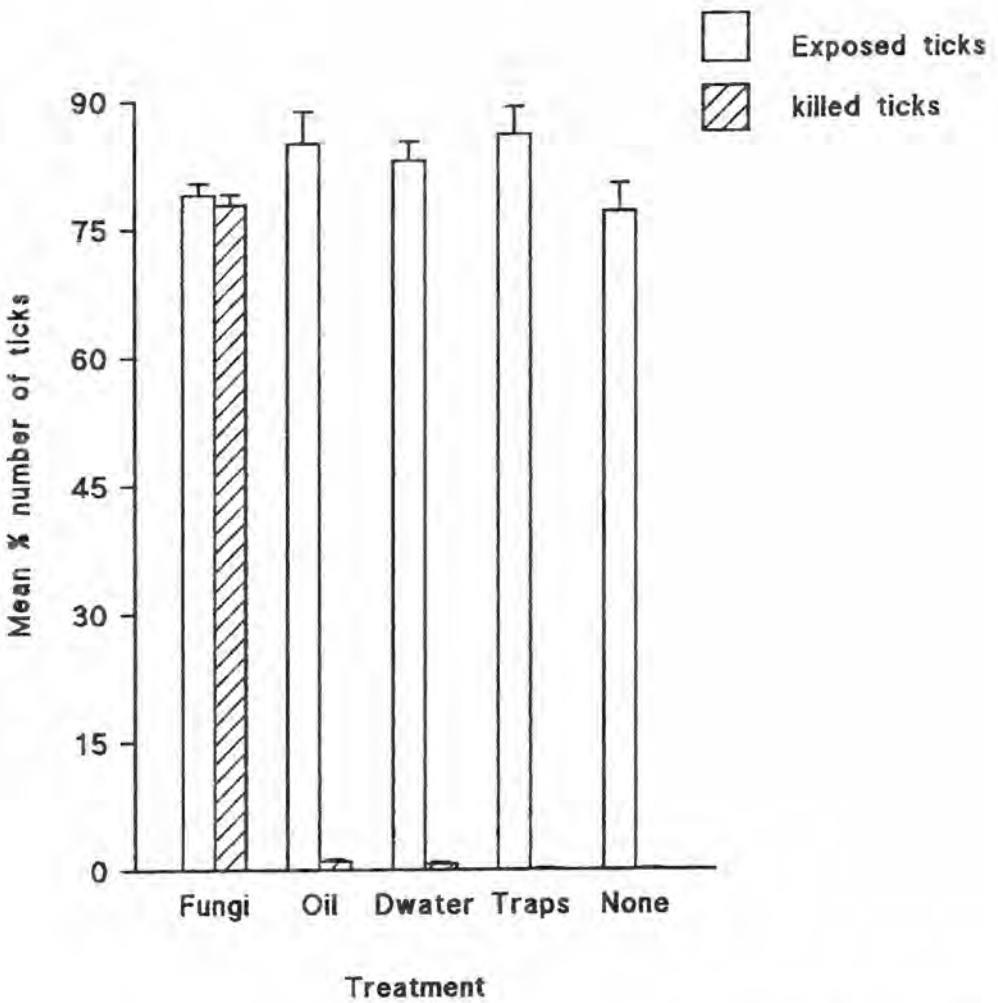

Fig. 12 Mean number of ticks exposed to fungi and controls in the field and those killed due to fungal infection in the laboratory

Table 13. Mean percentage numbers (±SE) of *A. variegatum* recovered, 3 weeks after setting the traps in the paddocks.

Treatment	Mean % number of ticks recovered
AAAP+ CO_2+fungi	33.77±9.96b
AAAP+ CO_2+oil formulation	76.33±2.04a
AAAP+CO_2+distilled water	80.42±4.07a
AAAP+CO_2+Traps	84.08±2.56a
None	83.83±2.62a

Means within the same column followed by the same letter are not significantly different at the 5% level based on the SNK test.

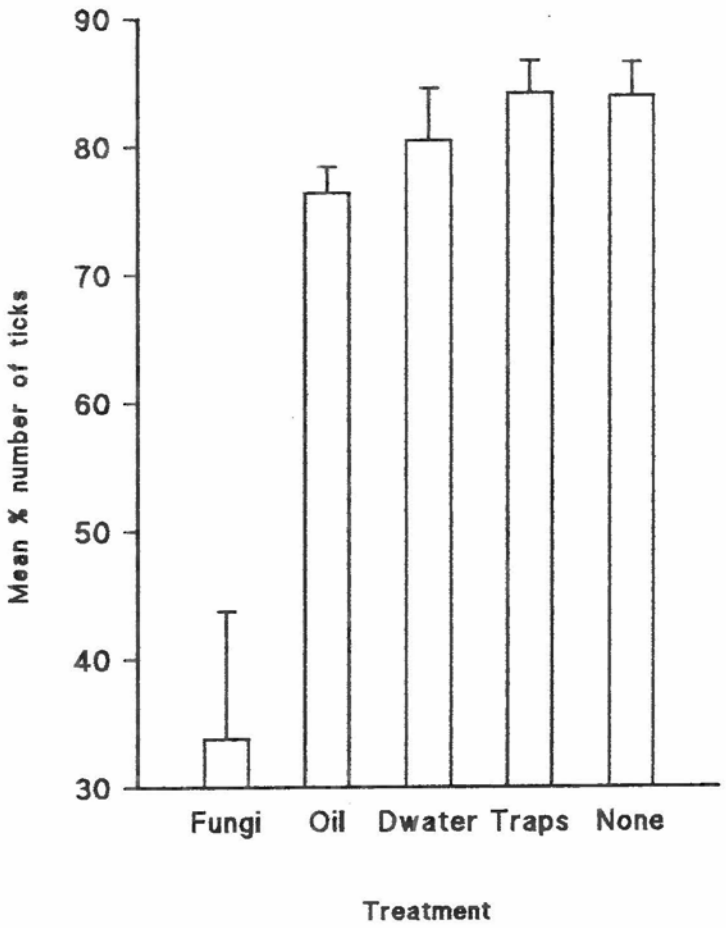

Fig. 13 Mean number of ticks recovered, 3 weeks after setting the traps in the field

7.1.5 Discussion

In this study, 33.8% of the ticks that were initially seeded in the vegetation in the paddocks containing AAAP, oil formulated fungi was recovered while between 76% and 84 % were recovered from the control paddocks. This clearly shows that the fungi influenced tick mortality resulting in the lower recovery rate of ticks in the test paddocks. However some of the ticks may have been preyed upon while others may have died as a result of desiccation or may not have responded to the pheromone during sampling. In this study, 66.2% tick mortality less those which may have died of other causes was achieved in a period of three weeks when ticks were exposed to the fungi using the device and left in the vegetation while ticks that were exposed to fungi in the field using the device and then incubated in the laboratory had 78% mortality. The high mortality recorded in the laboratory may have been due to the ideal conditions for fungi growth in the laboratory unlike in the field situation where the prevailing weather conditions could vary from day to day thus affecting the growth of fungi. The current study has shown that pheromone/fungi mixtures could be used to reduce *A. variegatum* populations in the vegetation.

The use of fungi for the control of ticks has an advantage over the acaricides because, fungi are cheap to obtain, environmentally friendly with no major side effects on other organisms, they have the possibility of being transferred from infected to non-infected ticks which may eventually raise the number of deaths over a period of time and in a way they are self propagating since once they have been introduced into the environment and there may be no need of further applications. The method used in this study is far much safer than most conventional methods because it does not involve spraying the animals and therefore contamination of consumable animal products is not

possible.

The method can be especcially useful in small scale farms where rotational grazing is practised. The traps can be set after the animals have been moved to other paddocks for a period of time and this can clear the paddock off ticks before the animals return. Reduced tick populations in the vegetation will mean that there will be less numbers of ticks on the animals which will boost milk production as result of reduced animal stress caused by large numbers of ticks on the animals, increased meat production, and reduced anaemic animals as the *Amblyomma* species are known to draw large amounts of blood from the animals (Norval, 1990) and also there will be reduced incidences of heartwater disease.

Among all the current tick control methods, none of them is completely efficient on its own. This method could therefore be incooperated in ITM programmes for better tick management. The method could also be suitable for use in game parks and game reserves to control *Amblyomma* ticks in these areas where the conventional methods are not quite applicable.

CHAPTER EIGHT

General Discussion and Conclusions

This study has shown that using an AAAP dose of 6.6mg, it is possible to attract up to 79% of released *A. variegatum* from a radius of 5m to a point where they can be exposed to fungal infections. Lesser or higher doses of AAAP did not attract many ticks probably because it is the species's way of avoiding unsuitable hosts (feeding and breeding sites on the host) in one situation and over-infested hosts in the other situation both of which are detrimental to its survival.

Since the results of this study revealed that extreme soil temperatures reduced the response of *A. variegatum* to the pheromone, it was found necessary to set the traps when the soil temperatures were moderate between (22-35)°C for maximum benefit. Norval *et al.* (1989a) working with *A. hebraeum* reported that no ticks were attracted when soil temperatures were above 35°C and similar observations were made by Barre *et al.* (1997), in the Guadeloupe. Norval *et al.* (1989a) reported lowest recoveries in cool (20-22)°C weather with *A. hebraeum* but no such low temperatures were experienced in this study.

The number of ticks recaptured in the absence of CO_2 reached a maximum of 53% in three days but when 500g of dry ice was incorporated, 79% of the released ticks were recovered within a period of three hours only. It, therefore, means that in order to maximise exposure of ticks to the fungi in the traps, the use of CO_2 is necessary. When the amount of CO_2 was varied by reducing the weight of dry ice used, it was observed that the number of ticks attracted also decreased (Fig. h). In this study, it was therefore found appropriate to use 500g of dry ice as a source of CO_2 in the traps because it

attracted a reasonable number (79%) of ticks and more so because this is the equivalent of CO_2 emitted by one bovine of 600Kg for 8hours (Berbigier, 1988).In nature, ticks become active in the presence of large mammals, which give out large amounts of CO_2 (Norval *et al.*,1987),but which will be attractive only if they have pheromone-emitting fed males attached to them. As pointed out earlier, this is an adaptation to prevent loss of adults through attachment to unsuitable hosts.It will also mean that the ticks will be less likely to attach to cattle that are regularly treated with acaricides and hence male-free, than untreated infested cattle.The presence of pheromone also brings the sex together and ensures that they attach on parts of the host that are suitable for feeding and are protected from grooming.

If the use of fungi is to be effective, a suitable formulation is required and the fungi application must be done at the right time.Unlike most insects, ticks have a tough cuticle and it is therefore necessary that the formulation used should enable the fungal spores to remain moist and attached to the cuticle for a longer period of time to germinate compared to insects with a softer cuticle. This is even more necessary when dealing with unfed ticks whose non-extended cuticle is tougher than that of the fed ticks. In this study, it was shown that an oil formulation was an effective medium for delivering *B. bassiana* and *M. anisopliae* fungi to the unfed adults of *A. variegatum*. Mortalities of up to 92% were achieved using the oil formulation and it was therefore preferred for use in the traps.

The results of this study also have shown that during the dry season when it was sunny and windy with less moisture in the air, tick mortality was very low. This may have been due to poor spore germination as a result of less moisture in the environment which then resulted in a low rate of infection by the fungi while application during

favourable weather conditions (the wet period) led to higher tick mortalities. It was therefore concluded that the pheromone/fungi traps should be used during the wet season in order to optimize their use. In areas that are generally hot, it may be advisable to set traps very early in the morning by 0600hrs or very late in the evening after 1700hrs, when the soil temperatures are low. Alternatively, traps could be set under a shade to keep them cool so that the ticks are not repelled by the hot traps and also because this could reduce evaporation of the fungi formulation. This also reduces the rate of evaporation of the fungal formulation and the rate of AAAP emission thus prolonging their activity. In addition, a fungus formulation that conserves moisture might be helpful.

Prior to this study, no work had been done to compare tick or insect mortalities due to separate and mixtures of fungi. Although the use of the separate fungi was shown to be effective in this study, it was shown that higher tick mortalities can be achieved when using the mixture of the two fungi (Fig. j) with the oil formulation during the wet period. This could probably be attributed to faster growth rate of the fungi forming more myceliae (Plate 14) which in turn produced more toxins thus killing the ticks faster and in larger numbers compared to infections due to separate fungi (Plates 12 and 13). The underlying mechanisms which are responsible for the faster growth of myceliae in mixed infections are not clear and warrant futher investgations. In order to maximise the benefit of the trap, it was found better to use the mixed fungi in the field experiments.

The results of this study have demonstrated that the fungi and AAAP are compatible. These results have shown that at low concentrations, AAAP has no significant effect on the mortality of *A. variegatum* due to fungi. However, there was a

general trend of decreased tick mortality when the AAAP concentration was increased (Fig. 11). It is therefore advisable not to mix the AAAP and the fungi in the traps in the field since long term contact would reduce the virulence of the fungi.

The studies of Kaaya and Okech (1990) demonstrated that adult tsetse when exposed to conodia of *B. bassiana* and *M. anisopliae* acquired fungal infection and were capable of transmitting the infections to the non-infected tsetse irrespective of their sex .This is in agreement with the findings of this study because lateral transfer of fungal infection took place among ticks irrespective of their sex. The same workers also reported that 30 minute exposure was sufficient to transmit infection from the infected tsetse to the healthy ones. The findings of this study differ from those of Kaaya and Okech (1990) in that, although there was transmission of infection from exposed/infected ticks to non-exposed/uninfected ticks, mortality was lower with a 30 minute exposure compared to a 24hr exposure. It was therefore found necessary to have a 24hr exposure for better fungal transmission to occur. This is probably because unfed ticks have a tough cuticle compared to tsetse and because it requires a longer period of exposure to transfer enough spores as the mobility of *A. variegatum* is relatively limited.

The results of this study also have shown that at least a ratio of 1:1 for the exposed ticks to non-exposed ticks is necessary for reasonable transfer of infection to take place and that mixed infection with mixed fungi caused higher mortality than separate infections. However, mortality achieved due to the lateral transfer of fungi was low but significant (Tables 11a, 11b, 11c and 11d).

The device that was used as a trap for luring and infecting adult *A. variegatum* ticks with fungi achieved 79% tick mortality for ticks exposed to fungi in the field using

the device and then incubated in the laboratory and 66.2% for ticks which were exposed to the fungi in the field and then left in the vegetation for three weeks. The device has a high degree of efficiency and it may be suitable for use to reduce *A. variegatum* populations in the field on small scale farms. A farmer may only require about three traps which can be moved from one place to another to infect *A. variegatum* on a 2 acre plot within one week. The trap should be used alongside other ITM packages especially in rotational grazing where the field is not under use can be cleared of ticks before letting back the animals.Since the device is cheap, it may be suitable for the resource poor farmers with small farms as is the case in many African countries. The device can also be used in any type of climate where fungi can thrive. In places where the fungi cannot grow, it can be replaced by other suitable pathogens or even acaricide. The use of acaricide in the device may not be very harmful because it will be localised in the traps which will minimise environmental pollution and will not be harmful to non-target animals including livestock since the devices can be removed immediately after use. Since there is also no direct contact between the acaricide and livestock animals, this eliminates the possibility of consumable products from the animals being contaminated.

However, the device as it is now, needs a number of modifications in order to achieve more efficiency.

1. The device should be made of either plastic or wooden material to avoid heating up during the hot weather which would otherwise prevent ticks from entering the trap.
2. The slow release method for the AAAP could be modified in the form of solid balls made of suitable materials for easy use, packaging and marketing.
3. Similarly, the fungal oil formulation may be packaged in a more commercially acceptable manner for easy use.

4. The device should be placed under a natural shade or should be fitted with a shade so as to reduce direct sunlight and rain.

The use of pheromone/fungi for tick control could be more advantageous over most conventional methods of tick control for a numbeer of reasons. Firstly, fungi directly penetrate the cuticle of the arthropod unlike most other pathogens which require ingestion for them to cause diseases in the arthropod. This means that it is easy to apply the fungi and chances of them infecting the tick are much higher compared to the other pathogens.The method is cost effective because the fungi is cheap and easy to obtain and also because only little amounts of fungi are required. The pheromone used is also not expensive and only little amount of it is used and the device can be locally made by the farmer. Since the pheromone used attracts only *A. variegatum* ticks, the method is target specific unlike the conventional methods which are indescriminate. In addition, this method is environmentally safe causing no environmental pollution as is the case with the toxic chemical acaricides. The method uses very little amount of water, for the preparation of the fungi formulations and it is therefore very convinient for use in drought-prone areas as it is the case in most African countries and does not require specific training for its application. This technique can maintain a situation whereby, *A. variegatum* is reduced in numbers to a level below the threshold for economic damage, yet maintaining sufficient tick numbers on the animals for maintainance of endemic stability of heartwater. It is therefore, a technique that is quite acceptable for the resource poor farmer and can save large sums of money in foreign currency used by governments to purchase the much harmful chemical acaricides.

With the current emphasis on rural electrification in many African countries, dry ice is expected to be readily available to most small scale rural farmers for use in this

device. It is hoped that this technology can be transferred to small hold farms where it can be incorporated with other ITM packages to help reduce *A. variegatum* populations in the field. This will in turn reduce heartwater incidences and the resulting economic losses due to less milk production associated with stressed and anaemic animals. It will also reduce the effects of damaged udders, skins and hides brought about by massive feeding of the tick.

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APPENDICES

Appendix 1. ANOVA table on the attraction of *A. variegatum* to decreasing doses of AAAP in a choice olfactometer

Source	DF	TYPE III SS	MSS	F	Pr > F	Sig
DOSE	9	2.242	0.249	4.56	0.0001	***
TYPE	2	3.063	1.532	28.04	0.0001	***
TREAT	1	0.006	0.006	0.11	0.7440	NS
TYPE*TREAT	2	0.295	0.148	2.70	0.0710	NS
DOSE*TYPE	18	1.515	0.084	1.54	0.0880	NS
DOSE*TREAT	9	0.458	0.051	0.93	0.5000	NS
DOSE*TYPE*TREAT	18	1.151	0.064	1.17	0.2970	NS

DF = Degrees of freedom
SS = Sum of squares
MSS = Mean sum of squares
Sig = Significance
*** = Highly significant (P< 0.001)
NS = Not significant
F = Variance ratio
TREAT = Treatment
TYPE = Males, Females and Nymphs

GLM comparisons based on square root ($\sqrt{(\log_{10}(x+0.5)+1)}$ transformed values

Appendix 2 ANOVA table on the attraction of *A. variegatum* to increasing doses of AAAP in a choice olfactometer

Source	DF	TYPE III SS	MSS	F	Pr > F	Sig
DOSE	9	1.113	0.124	1.95	0.0510	*
TYPE	2	0.862	0.431	6.79	0.0020	**
TREAT	1	4.630	4.630	72.89	0.0001	***
TYPE*TREAT	2	1.140	0.570	8.97	0.0002	***
DOSE*TYPE	18	0.940	0.052	0.82	0.6712	NS
DOSE*TREAT	9	0.794	0.881	1.39	0.2023	NS
DOSE*TYPE*TREAT	45	2.579	0.072	0.90	0.6465	NS

DF	= Degrees of freedom
SS	= Sum of squares
MSS	= Mean sum of squares
Sig	= Significance
*	= Significant (P<0.05)
**	= Significant (P<0.01)
***	= Highly significant (P< 0.001)
NS	= Not significant
F	= Variance ratio
TREAT	= Treatment
TYPE	= Males, Females and Nymphs

GLM comparisons based on square root ($\sqrt{(\log_{10}(x+0.5)+1)}$ transformed values

Appendix 3. ANOVA table on the attraction of *A. variegatum* to AAAP from varying distances

Source	DF	SS	MSS	F	Pr > F	Sig
TEMP	2	1.022	0.511	16.95	0.0001	***
DISTANCE	5	4.353	0.871	28.89	0.0001	***
DOSE	6	1.445	0.241	7.99	0.0001	***
TEMP*DOSE	12	0.762	0.063	2.11	0.0273	*
DISTANCE*DOSE	30	1.988	0.066	2.20	0.0036	**

DF = Degrees of freedom
SS = Sum of squares
MSS = Mean sum of squares
Sig = Significance
* = Significant (P<0.05)
** = Significant (P<0.01)
*** = Highly significant (P< 0.001)
TEMP = Temperature
F = Variance ratio

Appendix 4. ANOVA table on the attraction of *A. variegatum* to AAAP combined with CO_2 in the field

Source	DF	SS	MSS	F	Pr > F	Sig
TREAT	3	100381.250	33460.417	268.73	0.0001	***
DISTANCE	5	21050.000	4210.000	33.81	0.0001	***
TREAT*DISTANCE	15	33070.833	2204.722	17.71	0.0001	***

DF = Degrees of freedom
SS = Sum of squares
MSS = Mean sum of squares
Sig = Significance
*** = Highly significant (P< 0.001)
TREAT = Treatment
F = Variance ratio

Appendix 5. ANOVA table on the attraction of *A. variegatum* to AAAP over different weights of CO_2 in the field

Source	DF	SS	MSS	F	Pr > F	Sig
TREAT	5	76304.861	15260.972	281.74	0.0001	***
DISTANCE	5	15907.639	3181.528	58.74	0.0001	***
TREAT*DISTANCE	25	29960.417	1198.417	22.12	0.0001	***

DF = Degrees of freedom
SS = Sum of squares
MSS = Mean sum of squares
Sig = Significance
*** = Highly significant (P< 0.001)
TREAT = Treatment
F = Variance ratio

Appendix 6. ANOVA table on the effect of different formulations and concentration of fungi on *A. variegatum* in the laboratory

Source	DF	SS	MS	F	Pr > F	Sig
FORM	1	219027.866	219027.866	6899.38	0.0001	***
FUNGI	2	956.790	478.395	15.07	0.0001	***
CONC	6	4349.206	724.868	22.83	0.0001	***
FORM*CONC	6	396.825	66.138	2.08	0.0637	NS
FUNGI*CONC	12	177.778	14.818	0.47	0.9287	NS
FORM*FUNGI*CONC	14	1860.494	132.892	4.19	0.0001	***

DF = Degrees of freedom
SS = Sum of squares
MSS = Mean sum of squares
Sig = Significance
NS = Not significant(P>0.05)
*** = Highly significant (P< 0.001)
FORM = Formulation
CONC = Concentration

Appendix 7. ANOVA on the effect of different formulations and concentration offungi on *A.variegatum* during the dry season

Source	DF	SS	MSS	F	Pr >	Sig
FORM	1	1703.792	1703.792	31.83	0.0001	***
FUNGI	2	292.593	146.296	2.73	0.0708	NS
CONC	6	2112.875	352.146	6.58	0.0001	***
FORM*CONC	6	507.937	84.656	1.58	0.1626	NS
FUNGI*CONC	12	450.617	37.551	0.70	0.7457	NS
FORM*FUNGI*CONC	14	716.049	51.146	0.96	0.5049	NS

DF = Degrees of freedom
SS = Sum of squares
MSS = Mean sum of squares
Sig = Significance
NS = Not significant
*** = Highly significant (P< 0.001)
FORM = Formulation
CONC = Concentration
F = Variance ratio

Table 8. ANOVA on the effect of different formulations and concentration of fungi on *A. variegatum* during the wet season

Source	DF	SS	MSS	F	Pr > F	Sig.
FORM	1	19812.698	19812.698	1214.46	0.0001	***
FUNGI	2	5295.767	2647.884	162.31	0.0001	***
CONC	6	27744.098	4624.015	283.44	0.0001	***
FORM*CONC	6	3208.289	534.715	32.78	0.0001	***
FUNGI*CONC	12	2125.225	177.102	10.86	0.0001	***
FORM*FUNGI*CONC	14	1319.753	94.268	5.78	0.0001	***

DF = Degrees of freedom
SS = Sum of squares
MSS = Mean sum of squares
Sig = Significance
*** = Highly significant (P< 0.001)
FORM = Formulation
CONC = Concentration
F = Variance ratio

Appendix 9. ANOVA table for the compatibility of fungi and the AAP of *A. variegatum*

Source of variation	DF	SS	MSS	F	Pr > F	sig
FUNGI	2	343.659	171.829	2.43	0.0955	ns
CONC	10	2788.777	278.878	3.95	0.0003	***
FUNGI*CONC	20	1063.749	53.187	0.75	0.7563	ns

DF = Degrees of freedom
SS = Sum of squares
MSS = Mean sum of squares
Sig = Significance
NS = Not significant
*** = Highly significant (P< 0.001)
F =Variance ratio
Conc = Concentration

Appendix 10. GLM analysis table on the lateral transfer of *B. bassiana*, *M. anisopliae* and a mixture of *B. bassiana* and *M. anisopliae* by *A. variegatum*

Sources of variation	DF	Type III SS	F	Pr>F	Sig
RATIO	1	4.624	114.26	0.0001	***
DURATION	1	1.575	38.91	0.0001	***
TYPE	9	2.158	0.24	0.0001	***
TYPE*FUNGI	27	1.876	1.72	0.0162	*
FUNGI	3	7.540	62.11	0.0001	***
RATIO*DURATION	1	1.877	46.39	0.0001	***
RATIO*TYPE	9	0.836	2.30	0.0164	*

DF = Degrees of freedom
SS = Sum of squares
MSS = Mean sum of squares
Sig = Significance
* = Significant (P<0.05)
*** = Highly significant (P< 0.001)
F = Variance ratio
TYPE = Males, Females and Nymphs

General Linear Models (GLM) comparisons based on square root ($\sqrt{(\log_{10}(x+0.5)+1)}$

Appendix 11. ANOVA table on the mortality of *A. variegatum* exposed to the fungi using traps baited with the AAAP andd CO_2 in the field and incubated in the laboratory.

Source	DF	SS	MSS	F	Pr>F	Sig.
Treatment	4	14378.3333	3594.5833	3464.66	0.0001	***

DF = Degrees of freedom
SS = Sum of squares
MSS = Mean sum of squares
F = Variance ratio
*** = Highly significant (P<0.001)
Sig. = Significance

Appendix 12. ANOVA table on the efficiency of the fungi trap baited with the AAAP and CO_2 in the field.

Source	DF	SS	MSS	F Value	Pr>F	Sig.
Treatment	4	55110.818	1377.704	17.21	0.0002	***

DF = Degrees of freedom
SS = Sum of squares
MSS = Mean sum of squares
F = Variance ratio
*** = Highly significant (P<0.001)
Sig. = Significance