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EVALUATION OF THE INTERACTION BETWEEN *BEAUVERIA BASSIANA* AND AN APHICIDE FOR THE MANAGEMENT OF THE TOBACCO APHID *MYZUS PERSICAE NICOTIANAE*

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ABSTRACT

Aphids (*Myzus persicae* nicotianae) cause damage by sucking sap from the plants leading to distortion and wilting of the foliage, and eventually reducing economic yields. In order to minimise losses to viral diseases, it is important to use insecticides in association with fungi to control insect pests in an environmental and ecological friendly way without contaminating the environment. A study was carried out from October, 2013 at Kutsaga Research Station in the laboratory to evaluate the efficacy of *Beauveria bassiana* (contact insecticide)and imidacloprid (systemic insecticide) 200 SL for the control of tobacco aphid, *Myzus persicae nicotianae.* Three doses of 200 SL Imidacloprid (Imida) namely: Imida low rate 0.55 ml per 500ml water (LR); Imida medium rate 1.1 ml per 500ml water (MR) and Imida high rate (2.2 ml per 500ml water (HR) were mixed with Broadband® *Beauveria bassiana* (B. Bass) at three different rates namely: B. bass 4 x 10^3 (LR); B. bass.4 x 10^4 (MR) or B. bass 4 x 10^5 (HR) to evaluate the most effective application combination rate of *Beauveria bassiana* and imidacloprid for the control of the tobacco aphid. Treatments were: water (control); Imida MR; B. bass MR; B. bass LR + Imida LR; B. bass LR + Imida MR; B. bass LR + Imida HR; B. bass MR + Imida LR; B. Bass MR + Imida MR; B. Bass MR + Imida HR; B. Bass HR + Imida LR; B. Bass HR + Imida MR and B. Bass HR + Imida HR. Inoculation of aphids was done and data collected from day 1 to day 7 after inoculation. Mortality of aphids was recorded at 8 o'clock in the morning cumulatively as a percentage of the initial number infested (10 per petri dish). Results showed that there were significantly high $p<0.001$ cumulative mortality from B. Bass HR + Imida HR and B. Bass HR + Imida MR in comparison with the control and other treatments. The number of deaths on day one indicated clearly that the level of mortality was dose-dependent and the presence of the contact insecticide *(Beauveria bassiana)* compared with systemic insecticide (imidacloprid) which took three days to show mortality. In Imida MR death rates started at day 3 and accumulated up to 98% at day 7 whilst B. Bass started at day 1. However, death in day 1 increased as the dosage of B. Bass increased. It was also evident that as the rates of B. Bass and Imida increased the cumulative mortality also increased significantly ($p<0.001$). For controlling aphids B. bass HR + Imida MR was the best because its efficacy was the same as B. bass HR + Imida HR showing the same mortality in day one and by day five, both mortality had reached 100%.

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DEDICATION

To my wonderful parents Miriam and Godwin Mtetwa, and my siblings Answer, Evidence and Beloved.

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1.0 INTRODUCTION

1.1 Background

Tobacco originated as a natural hybrid in South and Central America and has been under cultivation for many centuries (Breen, 1985). Tobacco is a plant within the genus *Nicotianae* of the *Solanaceae* family. While there are more than 70 species of tobacco, the chief commercial crop is *Nicotiana tabacum*. The more potent species *Nicotiana rustica* is also widely used around the world. There are over 50 species of the botanical genus *Nicotianae*, with all being derived from one of the two species *N. tabacum* and *N. rustica* (Breen, 1985). By far, the most important is *N. tabacum* which is an allotetraploid that resulted from natural hybridization of the two wild species *Nicotiana sylvestris* and *Nicotiana tementosiformis* (Shew, 1991).

Tobacco is grown for commercial purposes in at least 97 countries around the world. It is a cash crop and is grown in warm climates. Tobacco contains the alkaloid nicotine which is used as a stimulant. The leaf turns from green to lemon gold at harvest when nicotine, its major product is extracted (Tucker, 1982). Major tobacco producing countries include China, Brazil, United States of America (U.S.A) and Zimbabwe (Takada, 1981). Zimbabwe produces some of the finest flavoured tobacco in the world owing to the climate and physical conditions which favour farming of the crop in the country (The Herald, 2005). It is grown as an annual from unusually small seeds. It has very large leaves which are pointed at the tip. There are many variations of tobacco under cultivation but this classification is mainly based on the curing method used (e.g. fire, air, sun and flue curing) (Breen, 1985).

Three types of tobacco have been traditionally grown in Zimbabwe: flue-cured barley and oriental tobacco (www.fao.org). Flue-cured tobacco constitutes over 95% of Zimbabwe's tobacco yield while barley constitutes about 4%. Tobacco rose to become a major export in Zimbabwe in the 1990's. According to Masuka, Cole and Mguni (1998), Zimbabwean tobacco is grown as a summer crop and it requires a 7-9 month growing season in order to produce a full crop. Income from tobacco in 2013, accounted for at least 10.7% of the Zimbabwe's gross domestic product and 21.8% of all exports, compared to 9.2% for other agricultural commodities.

The tobacco market worldwide is under threat from pests like various Lepidoptera such as cutworms (*Agrotis* species) and budworm (*Heliothis* species) while aphids (*Myzus* species) Black and White flies (*Bemisia tabaci*) suck nutrients and transmit viral diseases to the tobacco plant hence causing yield loses (Blair, 1990). *Myzus persicae* is a major vector in the transmission of Tobacco Bushy-top virus (TBTV) and potato virus Y (PVY) (DiFonzo, Ragsdale, Radcliffe, Gudmestad and Secor, 1997). Because of rapid acquisition and transmission times for these non-persistent viruses, a few aphids, through probing, can transmit these viruses to many plants in a short time, and greatly reduce the effectiveness of chemical control in limiting the spread of viruses (Chamberlin, 1958).

Currently, management of these cosmopolitan pests is achieved by a combination of chemical, biological and cultural methods (Blair, 1990). And of these, chemical control has been the most frequently used. However, this intensive spraying of chemical pesticides has left a lot of negative impacts on the environment and human health (Tamaki and Weeks, 1968). Most importantly, aphids also develop general resistance to almost all classes of chemical insecticides (Ye, Dun and Feng, 2005). Consequently, there is increased interest in using alternative and integrated approaches to control aphids and therefore, biological controls have been initiated (Blair, 1990).

The incorporation of fungal biological control agents into management systems has been considered as a feasible alternative to frequent chemical use (Feng and Johnson, 1990). The most practical integrated approach is incorporation of fungal and chemical agents into combined formulations with enhanced efficacy on the control of target pests (Ye *et al*., 2005). Imidacloprid, a versatile neonicotinoid insecticide, has been widely used to combat the sucking insect pests such as aphids over the past decade (Weichel and Nauen, 2004) and is one of few insecticides recommended for insect control on residue sensitive crops. Previous studies have shown that imidacloprid is highly compatible with *Beauveria bassiana* and may enhance fungal action in laboratory bioassays or field trials (Ye *et al*., 2005).

Several entomopathogenic fungi including *B. bassiana (Balsamo) Vuellemi* and *Metarhizium anisopliae* (Metschnikoff) Sorokin,*Paecilomyces fumosoroseus* (Wize) Brown & Smith and *Verticillium lecani* (Zimmermann) are potential biocontrol agents of various aphids (Feng and Johnson, 1990; Feng, Johnson and Kish, 1990a; Dorschner, Feng and Baird, 1991;Stary, 1988; Chandler, 1997).Both *B. bassiana* and *P. fumosoroseus* have established certain aphid control in the field (Ye *et al*., 2005, Vandenberg, Sandvol, Jaronski, Jackson, Souza and Halbert, 2001;

Hatting, Wraight and Miller, 2004). As true pathogens, the fungal biological agents usually have a latent period of several days to kill aphid hosts after infection (Milner, 1997). Due to this inherent feature, fungal formulations lack knockdown action, kill target pests slower than chemical insecticides, and often discourage commercial efforts (Ye *et al*., 2005, Feng, Poprawski and Khachatourians, 1994; Wraight, Jackson and de Kock, 2001). For successful control, there is a strong need to evaluate interaction between *B. bassiana* and an applicable commercially available pesticide such as imidacloprid for the improved management of the tobacco aphid *Myzus persicae nicotianae.*

1.2 Justification

Zimbabwe is the largest producer of tobacco leaf in Africa and the world's fourth producer (www.fao.org) after China, Brazil and U.S.A. This is because the country does not have a large tobacco manufacturing industry and so it exports 98% of the tobacco it produces (Banga, 2012). Agriculture emerged as one of the nation's leading economic performers in 2011 with tobacco being the highest contributor (England and Hawkins, 2011). Tobacco is the crop of economic importance in Zimbabwe since tobacco production makes an important contribution to gross domestic product and to export revenue, and plays a major role in the national economy (www.fao.org). The tobacco aphid *Myzus persicae nicotianae* is an economically important pest of tobacco in Zimbabwe and is the most damaging pest (Chamberlin, 1958). Thus, it is in every grower's interest to minimise yield losses due to the attack by aphids so as to maintain high yields.

Aphids cause damage by sucking sap from the plants leading to distortion and wilting of the foliage, and reduction in crop yields. Aphids excrete large amounts of honey-dew on which a sooty mould develops. In addition to interfering with photosynthesis, the sooty mould reduces the quality of the cured leaf. Winged aphids act as vectors of viral diseases. In Zimbabwe, viruses transmitted by aphids include TBTV, PVY and Tobacco Mosaic Virus (TMV). If plants are infected at an early stage of growth, 100% yield losses can occur. In order to minimise losses to viral diseases the use of chemical insecticides in association with fungi to control insect pests can be regarded as a solution to environmental and ecological threats posed by using chemical insecticides only.

The use of chemical pesticides without considering the complexities of the soil environment has been a major case of disruption. This is because the target pest species in the process becomes tolerant to the pesticide leading to pesticide resistance and can no longer be controlled economically with chemicals (Dixon, 1998). Aphids develop general resistance to all classes of chemical insecticides (Ye *et al*., 2005). This has led to the population of the target species quickly recovering from the pesticide action and for a variety of reasons, rising to new resurgence.

The continuous cultivation of tobacco in Zimbabwe has resulted in chemical control methods being heavily relied on for the control and prevention of aphid transmitted bushy top virus disease (FAO, 2010). Evidence suggests that the aphid in Zimbabwe is already developing some complex resistance due to the over-use of chemicals (FAO, 2010) and there is great need to evaluate other formulations to ensure successful control of the aphid. The new methods need to be effective, less expensive and environmentally friendly. There is therefore need to evaluate the most effective application rates to effectively control aphids without adversely affecting the overall quality and yield of the tobacco crop.

1.3 Objectives

1.3.1 Main objective:

- to evaluate the efficacy of the combination of*B. bassiana* and imidacloprid for the control of the tobacco aphid.

1.3.2 Specific objective:

- to evaluate the most effective application combination rate of *B. bassiana* and imidacloprid for the control of the tobacco aphid.

2.0 LITERATURE REVIEW

2.1 The tobacco aphid (*Myzus persicae nicotianae*)

2.1.1 Taxonomic classification

Myzus persicae nicotianae (Blackman) is classified as follows:

Kingdom: Animalia

Phylum: Arthropoda

Class: Insecta

Order: Homoptera

Family: Aphididea

Genus: *Myzus*

Species: *Myzus persicae*

Subspecies: *Myzus persicae nicotianae*

2.1.2 Description

Aphids are small (1-10 mm), soft-bodied plant-sucking insects and they have an intricate life cycle. It is believed that all generations of aphids comprise parthenogenetic females which reproduce without egg fertilization and are viviparous (i.e. produce live young) (Dixon, 1998). Embryos developing in parthenogenetic females also have embryos developing within them. This parthenogenesis and telescoping of generations enables aphids to achieve very high rates of increase which increases the complexity of controlling the pest (Dixon, 1998).

Aphids have a proboscis through which they suck plant juices from the phloem. The skin of the aphid is more or less provided with glands that secrete either waxy or mealy matter. The form is generally oval or globose, sometimes flattened below, the head is more or less distinct in the wingless females than winged females (Theobald, 1926). Three forms of females occur in the

majority of aphids, an alate viviparous female, an apterous viviparous female and an apterous oviparous female. *M. persicae* and its tobacco adapted subspecies *M. persicae nicotianae* are pests of economic importance in Zimbabwe and they are practically indistinguishable from each other morphologically (Clements, Sorenson, Wiegmana, Neese and Roe, 2000). Based on the distinctive morphometric and anholocyclic development, Blackman (1987) first identified the tobacco feeding form of *M. persicae* as *M. nicotianae*.

However, the general consensus among aphidologists now is to treat this as a subspecies of *M. persicae* hence the name in common usage *M. persicae nicotianae* (Capinera, 2005). The body structure of aphids is simplified to perform only the function of feeding and reproduction, while retaining the ability to walk is aided by their gressorial legs (Blackman, 1987). Aphids exist in two body forms, the wingless (apteral) and the winged (alate). The tobacco aphid is found in two distinct colour forms, green and red. Colour variation between aphids is common, and it appears to be interclonal and sympatric in occurrence (Takada, 1981; Miyazaki, 1987).

The success of *M. persicae* in colonizing different host plants has been related to the presence of aphid enzymatic mechanisms of detoxification, which are responsible for the metabolism of host plant allelochemicals (Francis, Gerkens, Harmel, Mazzucchelli, DePauw and Haubruge, 2006). The performance of *M. persicae nicotianae* on tobacco should therefore have a genetic/ biochemical base, which could be related to the ability of the subspecies to colonize a well-defended host plant (i.e. tobacco with glandular trichomes and cuticular sucrose esters) (Cabrera-Brandt, Fuentes-Contreras and Figueroa, 2010).

2.1.3 Life cycle of aphids

Aphids are often parthenogenetic (asexual) for part or all of their life and both viviparous and oviparous at different times of the year (www.insect-world.com). Parthenogenetic reproduction generally results in vivipority (live birth) (www.insect-world.com). In temperate countries, the typical life cycle of an aphid species is an alternation between a primary host, usually woody, on which the over wintering eggs are laid on an herbaceous secondary host (Dixon, 1977). Sexual reproduction is induced by low temperature and shortening day length in many aphids(Tamaki and Weeks, 1968). This is not known to occur among tobacco aphids in Zimbabwe. All aphids in Zimbabwe are females and their potential for rapid expansion is unique among insects (Tamaki

and Weeks, 1968). The life cycle of aphids in Zimbabwe consists essentially of the infestation of a host plant by winged migrants which give birth to living young. Seven to ten days after its birth, the nymph becomes a mature female. A mature female can live for up to 30days during which time she can produce from 30 to 80 nymphs. These nymphs develop to wingless adults by a series of four moults, and they in turn give birth to a number of nymphs (Moran, 1990). The embryonic development of the young begins before the mothers birth, in the body of the grandmother, so that the food intake of one individual sustains three generations of about 6 000 individuals (Blair, 1990).

It is this telescoping of generations combined with a rapid life cycle of 7 to 14 days that results in the extremely high reproductive potential of aphids (Blair, 1990). By this life cycle, colonies quickly develop but eventually a physiological change in the host plant, caused by the feeding aphids themselves or the natural senescence of the plant tissues, a less degenerate, darker winged form of the insect is produced. When mature these animals fly from the host plant and start fresh infestations on other suitable plants (Moran, 1990).

2.2 Feeding ecology and damage

Aphids feed from the phloem of plants which they tap into with the stylets of their proboscis (www.insect-world.com). They gain access to the phloem vessels from three main parts of the plants: stems, leaves and roots (www.insect-world.com). Their stylets which are contained within the proboscis when the aphid is not feeding, are very thin and could suffer damage while being pushed into the plant or bent in an unwanted direction (www.insect-world.com). When the stylets reach a phloem tube the aphid injects saliva into the plant cell to avoid the plant cell sealing the puncture (www.insect-world.com). Sucking the sap from plants leads to distortion and wilting of the foliage, which leads to reduction in crop yields. Aphids excrete large amounts of honeydew because of the amount of sap they need to suck up in order to get enough nitrogen in the diet which means they have far more sugar and liquid than they need (www.insect-world.com). This honeydew does not only clog the pores of the leaves interfering with its assimilation, but also encourages the growth of black, sooty mould, which can prevent light from reaching the photosynthetic tissues of the plant (Tamaki and Weeks, 1968).

2.3 Aphid-Transmitted Diseases

Potato virus Y belongs to the *Potyvirus* genus. The genus is currently known to be the largest of all the plant virus genera and is thought to constitute the most destructive families of plant viruses affecting potato crops (England and Hawkins, 2011) and many other economically important plant species. These plants include tobacco, tomato and pepper (Miyazaki, 1987). The level of damage to a crop is determined by the strain of PVY infecting the plants, the viral load, the time at which infection occurs, as well as the tolerance the host possesses toward the virus (Warren, Kruger and Schoeman, 2005). Resistance to PVY infection by hosts is low in many cases. Infection of a potato field with PVY may ultimately result in 10-100% loss in yield (Warren *et al*., 2005).

2.3.1 Tobacco Bushy-top disease (TBTV)

Tobacco Bushy-top virus was first reported in Zimbabwe in 1958 (Gates, 1962). The virus usually occurs as a complex with other viruses, for example PVY. The virus is readily transmitted by mechanical inoculation from plants that are infected with another virus (e.g. Tobacco Vein Distorting Virus), but not from plants infected with it alone (Gates, 1962). Tobacco Bushy-top disease is caused by a complex of the Tobacco Bushy-top virus, a member of the genus *Umbravirus* and Tobacco Vein Distorting Virus, a member of the genus *Poleovirus*, which acts as a vector encapsidating the Tobacco Bushy-top genomic RNA (Mo,Qin, Wu, Yang, Wu, Duan, Li and Chen, 2011). Tobacco Bushy-top virus causes stunted growth in plants, and leaves show symptoms of vein distortion, vein clearing and mottling, and rounding (Mo, Qin, Tan, Li, Wu and Chen, 2002). It also stimulates the sprouting of axillary shoots from the main stem (Gates, 1962). These early sprouts form lateral shoots on which other shoots are produced, resulting in a ‘bushy’ appearance (Mo *et al*., 2002).

2.3.2 Sooty mould and secondary infections

The occurrence of sooty mouldon tobacco has always been linked to the presence of parasitic insects on plants (Gates, 1962). Among them, aphids and whiteflies are most commonly associated with black mould on the lamina. This is related to the feeding behaviour of the insects. The aphids must collect large amounts of sap for the protein they need. This makes them reject the excess sugar in the form of honeydew (Gates, 1962). It can be found in large amounts in

places where aphid colonies grow and taint the surface of the invaded lamina. The honeydew is an ideal substrate for several phylloplane fungi that use it for food and thus, progressively, the dew is covered by black mould(*Alternaria* spp. *Cladosporium* spp or *Capnodium* spp).

2.3.3 Tobacco Mosaic Virus (TMV)

TMV was the first virus to be discovered(nist.rcsb.org). Late in the 19th century, researchers found that a tiny infectious agent, too small to be a bacterium, was the cause of a disease of tobacco plants(nist.rcsb.org). TMV is very stable, so stable that it can survive for years in cigars and cigarettes made from infected leaves (nist.rcsb.org). The viral RNA is infectious by itself, but the addition of a protein coat protects the RNA from enzymes that would destroy it. TMV uses two tricks to release its RNA. As with many viruses, TMV has a chemical switch that causes the proteins to change when the environment changes. The capsid protein has several clusters of acidic amino acids that are stable outside of cells, where calcium levels are high, but repel one another in the low-calcium conditions inside cells. This is enough to loosen the first few capsid proteins, releasing the end of the RNA. TMV then uses ribosomes as the engines to finish the job. As the ribosomes move down the strand, creating the first set of virus proteins, they displace the remaining capsid proteins (nist.rcsb.org). This disease is also transmitted by aphids (Mo *et al*., 2011).

2.3.4 Potato Virus Y (PVY)

PVY mostly infects plants in the family Solanaceae (potatovirus.com)**.** The Solanaceous plants include economically important ones like potato (*Solanum tuberosum*) and tobacco(potatovirus.com).Aphids can carry viral particles and spread them between plants. PVY can be spread by many species of aphids(potatovirus.com). Within minutes of starting to feed on a PVY-infected plant, the PVY particles get stuck in the aphid's stylet (potatovirus.com). If the aphid then moves to a healthy plant and soon starts to feed, the virus particles are transmitted to the healthy plant. This process is termed "non-persistent" transmission(potatovirus.com). When PVY infects the tobacco plant, it replicates by assuming control of some of the plant's proteins and enzymes to make more PVY viruses. This disrupts the normal functioning of the plant. Tobacco cultivars vary in how susceptible they are to the effects of PVY

infection(potatovirus.com). If a plant cannot become infected at all, we call this immunity(potatovirus.com).

2.4 Migration

Most of the time aphids do not have wings, and in general they move very little, and life consists of feeding and giving birth (www.insect-world.com). The number of winged aphids in a given population is influenced by: death of the host plants which stimulates aphids to migrate in search of the new host, environmental changes and over population also stimulate migration to either primary or secondary host (www.insect-world.com). Aphids are weak fliers, and in still air they move about 1.6 to 3.2 km per hour (www.insect-world.com).

Their migration can be quiet extensive and they often take advantage of favourable wind to enhance their flight efficiency (www.insect-world.com). Aphids land on leaves, because they are attracted to the yellowish light emitted from young actively growing crops or older senescing ones. This is because the flow of nutrients to and from these leaves is greater (www.insect-world.com).

2.5 Tobacco Aphid Management in Zimbabwe

The control of aphid pests still involves large amounts of pesticides, but other ecologically friendly methods have been in use in other places for some time (www.insect-world.com). Biological and cultural control tools are being extensively researched. Also most aphid populations are controlled or moderated by natural controls that include even stress and natural enemies like lady beetles and other wide variety of Hymenopteran parasitoids (Stary, 1988). When conditions are favourable, aphids' populations grow very rapidly resulting in problems if not controlled. Tobacco varieties resistant to the aphid and which also produce acceptable leaf quality are not yet available in Zimbabwe despite numerous breeding efforts (Cottrell, 1994).

Characteristically, aphid populations normally drop significantly immediately following topping (the removal of the apical region of the plant before flowering structures are developed). Topping increases leaf thickness and the concentration of alkaloids which cause heavy aphid mortality. Consequently, no aphid management is cost-beneficial to a farmer after this

operation. Control strategies can be in the form of cultural, biological or chemical techniques (Cottrell, 1994).

2.5.1 Cultural control

In Zimbabwe, cultural control is regarded as the most feasible control strategy for the aphid (McDonald *et al*., 2003). Tobacco aphids primarily come into the crop in late November up till late January which corresponds to the wettest periods of the country (Gates, 1962). Consequently, early-planted tobacco suffers less aphid damage but any tobacco grown after the onset of the rainy season is susceptible to aphid damage. As a result, mandatory cultural stalk destruction dates have been enforced in Zimbabwe to ensure that specific host free periods are created to reduce the populations of the aphid significantly from year to year (Reynolds and Volk, 2007). The effectiveness of this control strategy has been limited by the failure of small scale farmers to adhere to these stalk destruction dates, thereby creating a continuous host for the parasite and enabling it to establish sufficient population numbers to spread bushy top every season (Tamaki and Weeks, 1968).

Cultural control measures are important in producing strong disease free seedlings in tobacco farming. Good sanitation such as elimination of weeds which act as reservoir hosts to aphids is also important (Cottrell, 1994). Large aphid populations are most likely to build up on irrigated crops particularly when temperature rises rapidly at the beginning of the rains (September/ October). Crops must be scouted once a week and if found infested must be treated immediately (Cottrell, 1994).

Legislation are regarded as components of cultural methods and it is important to adhere to legislated plant destruction and sowing dates in order to control transmission of diseases. In Zimbabwe, legislation states that (Tamaki and Weeks, 1968):

- ✓ No sowing of seedbed should be done before June 1 each year,
- ✓ no planting before September 1 and after December 31,
- ✓ destroy seedbeds as soon as possible but before January 1 and
- ✓ destroy stalk/ regrowth in the field by May 15 each year.

2.5.2 Chemical control

Aphids may be controlled by a number of insecticides which are classified as contact or systemic aphicides. Stomach poisons which are applied to the surface of the plant are ineffective because the aphids pierce the plant tissue with their specially adapted mouthparts and suck out non-toxic sap (Villacarios, 1987). Among the organophosphates, Parathion and Malathion are contact insecticides which are widely used for aphid control. Demeton-s-methyl, dimethoate and phosdrin are systematic organophosphates which are effective against most aphid species. Work done over the years at Kutsaga to establish a suitable method of applying aphicides for the control of tobacco aphids in the field, shows that aphicides placed in the planting hole, slit-in-ridge and slit adjacent ridge controlled aphids effectively (TRB annual report, 2000). Some of the most widely used aphicides in Zimbabwe are shown in the table below:

Table 1: Aphicides used in Zimbabwe

Chemical	Active ingredient	Route of entry	Target site of action
Acephate 75 SP	Triazamate	contact	Acetylcholine esterase inhibitors
Dimethoate 40% EC	Triazamate	contact	Acetylcholine esterase inhibitors
Monocrotophos 40% EC	Triazamate	contact	Acetylcholine esterase inhibitors
Methamidophos 60% SL	organophosphate	contact	Acetylcholine esterase inhibitors
Imidacloprid 200 SL	neonicotinoid	systematic	Nicotine acetylcholine antagonists
Thiamethoxam 25% WG	neonicotinoid	systematic	Nicotine acetylcholine antagonists
Aldicarb 15% granules	carbamate	systematic	Acetylcholine esterase inhibitors

Because of the ease with which aphids develop resistance, rotation of aphicides is imperative to prevent resistance by the tobacco aphid. Consequently growers must take responsibilities to use available products carefully and judiciously (Tamaki and Weeks, 1986).

Chemical control remains the effective means of controlling the aphid pest in Zimbabwe. Several chemicals have been tested and proven efficacious to the control of the pest in both early planted and late planted tobacco. However, continued use of chemicals has led to the resistant strains of the aphid, a case that scholars (McDonald *et al*., 2003)alike in Zimbabwe agree to be directly linked to improper application rates coupled with failure to adhere to the cultural stalk destruction dates. The issue of chemical residues is also negatively associated with the chemical control strategy. Tobacco companies are increasingly sensitive concerning insecticide residues. Specifically, they do not want Thiodan residues in the tobacco. Some companies spot-test for Thiodan residues on the market floors and, if certain markets are exhibiting Thiodan residues, they will shift their buying to other markets. Available alternatives to thiodan based chemicals include Acephate (Orthene 75S), endosulfan (Phaser 3EC, Thiodan 3EC), Methomyl (Lannate 90 SP, 2.4 LV) and Imidacloprid (Pravado 1.6F).

2.5.3 Biological control

Biological control involves the use of predators and parasitoids which feed on aphids. There are many predatory insects which prey or parasitize aphids (www.insect-world.com). These include the bush crickets (Orthoptera), wasps (Vespidae) and spiders (Aranae). Predators of aphids are also sold commercially in the U.S.A and these include ladybird beetles (e.g. *Coccinella septempunctata*) and laceworms (*Chrysoperla cornea*) (Stacy, 2003). *Aphidus* species are important natural enemies of aphids. The adult female lays its eggs in aphids and the larvae develop within the aphid eventually killing it (Miyazaki, 1987).

2.6 *Beauveria bassiana* for insect management

B. bassiana is a fungus that controls insect pests by causing a disease known as the white muscadine disease in the insects (www.hort.uconn.edu). When the fungal spores of *B. bassiana* come in contact with the cuticle of specific insects that are susceptible to it, they germinate and

grow directly through the cuticle to the inner body of the host insect. The fungus then proliferates throughout the insect's body, producing harmful toxins and depriving the insect of nutrients until eventually the insect dies (www.simplynetworking.es).

Unlike bacterial and viral pathogens of insects, *B. bassiana* and other fungal pathogens infect the insect with contact and do not need to be consumed by their host to cause infection (Das, 2013). Once the fungus has killed its host, it grows back out through the softer portions of the cuticle, covering the insect with a layer of white mould (hence the name white muscadine disease). This downy mould produces millions of new infective spores that are released to the environment(www.simplynetworking.es).

2.6.1 Efficacy of *B. bassiana* for insect pest control

B. bassiana can be used as a biological insecticide to control a number of pests such as termites, whiteflies, thrips, aphids and a wide range of beetles (danpritchard.com). *B. bassiana* parasitizes a very wide range of arthropod hosts. However, different strains vary in their host ranges, some having rather narrow ranges (danpritchard.com), it is important to identify the correct strain for a targeted species of pest for efficient control (Xu, Ying and Feng, 2002). Some strains do have a wide host range and should therefore be considered nonselective biological insecticides (danpritchard.com).

2.6.2 Recent work on *B. bassiana* for insect control

B. bassiana was evaluated in combination with insecticides in Maryland USA and a notable reduction in two spotted spider mites, *Tetranychus urticae* and green peach aphids *Myzus persicae by* an average of 83% compared with other organic insecticides was observed (www.angr.umd.edu). It was noted in the same trial that certain strains of *B. bassiana* were more efficaciousthan others and this was more pronounced under rising temperatures. The authors argued that using Azadirachtin, a chemical pesticide, alone or in combination with *B. bassiana* did not increase the control of aphids or mites as compared to using *B. bassiana* alone (www.angr.umd.edu). Furthermore, because of high costs associated with Azadirachtin, it did

not appear to be a cost-effective method of controlling aphids and mites at that time (www.angr.umd.edu).

3.0 MATERIALS AND METHODS

3.1 Study location

The study was conducted at Tobacco Research Board, Kutsaga Research Station in Harare, Zimbabwe. Tobacco Research Board (Kutsaga Research Station) is located 15 km East of Harare at latitude $17^0$55′S and longitude $31^0$08′E with an altitude of 1479 m above sea level. The average temperatures were 32ºC and 18ºC in summer and winter respectively, with annual rainfall between 800-1000mm. The soils at Kutsaga Research Station are on average light textured sandy loam.

3.2.1 *Beauveria bassiana* and aphid cultures used in the experiment

An emulsifiable spore concentration of *Beauveria bassiana* 4 x 10^9strains PPRI 5339, a fungal contact insecticide from Broadband® a company in South Africa was used in this study (Fig 1).It is highly compatible with imidacloprid (Ye *et* al., 2005; Xu*et al.*, 2002). Serial dilutions were done to reduce the concentrations to low, middle and high (Fig 2). For high, 0.05ml of 4 x 10^9 was mixed in 500ml of water to obtain 4 x 10^5. For middle, 0.05ml of 4 x 10^9 was mixed in 5 L of water to obtain 4 x 10^4. For low, 50ml was drawn from 4 x 10^4 solutions and was added to 450ml of water obtaining 4 x 10^3.

Tobacco aphids used in this study were maintained in screen cages on Chinese cabbage (*Brassica pekinensis*) (Fig 1). To prepare age uniformity of aphids, five days before the experiment, six wingless adults were taken from the colony and transferred to six clean potted Chinese cabbage plants. The adults were allowed to freely produce nymphs for 120 hours. Six-hundred, five-day old aphids were taken for the experiment. Ten, five-day old aphids per petri dish (12cm diameter) were used. New nymphs with age variation of ≤ 2days received the treatments in table 2.

3.2.2 Tobacco leaves used in the experiment

Fifteen upper tender leaves, from healthy tobacco plants (variety KRK 26) were taken from one of Kutsaga fields and were carefully observed to ensure the leaves were clean (have no aphids) (Figure 3) to produce 60 leaf discs of 5cm diameter. A pill box of 5cm diameter was used to cut

the leaves into leaf discs, four discs per leaf. The leaf discs were placed in petri dishes, one per petri dish. Twelve treatments were applied to the leaf discs and five blocks were conducted.

Figure 1: *B. bassiana* used in the experiment and Chinese cabbage used to rear aphids

3.2.3 Imidacloprid used in the experiment

The aphicide used was imidacloprid 200 SL. The insecticide came with the instruction of mixing 220 ml of imidacloprid per 100 litres (L) of water**.** Imidacloprid had three levels in this experiment low, middle and high. Scaling down was done since the experiment was done at a smaller scale in the laboratory (Fig 4).

For low, 110ml imidacloprid was applied to 100 L of water, by scaling down; 0.55ml imidacloprid per 500ml of water was used. For middle, instead of 220 ml of imidacloprid per 100 L of water, 1.1 ml of imidacloprid per 500ml of water was used. For high, instead of 440ml of imidacloprid per 100 L of water, 2.2ml of imidacloprid per 500ml of water was used.

Table 2: Treatments used in the experiment

Treatment	concentration	treatment	concentration	Coded treatment
1. Control	water	**-**	-	**Control**
2. Imidacloprid 200 SL	Medium rate (1.1 ml per 500ml water)	**-**	-	**Imida MR**
3. *B. bassiana*	Medium rate (4 x 10^4)	**-**	-	**B. bass MR**
4.*B. Bassiana*	low rate (4 x 10^3)	**+Imidacloprid**	low rate (0.55 ml per 500ml water)	**B. bass LR + Imida LR**
5. *B. bassiana*	low rate (4 x 10^3)	**+ Imidacloprid**	medium rate (1.1 ml per 500ml water)	**B. bass LR + Imida MR**
6. *B. bassiana*	low rate (4 x 10^3)	**+ Imidacloprid**	high rate (2.2 ml per 500ml water)	**B. bass LR + Imida HR**
7. *B. bassiana*	medium rate (4 x 10^4)	**+Imidacloprid**	low rate (0.55 ml per 500ml water)	**B. bass MR + Imida LR**
8. *B. bassiana*	medium rate (4 x 10^4)	**+ Imidacloprid**	medium rate (1.1 ml per 500ml water)	**B. Bass MR + Imida MR**
9. *B. bassiana*	medium rate (4 x 10^4)	**+ Imidacloprid**	high rate (2.2 ml per 500ml water)	**B. Bass MR + Imida HR**
10. *B. bassiana*	high rate (4 x 10^5)	**+Imidacloprid**	low rate (0.55 ml per 500ml water)	**B. Bass HR + Imida LR**
11. *B. bassiana*	high rate (4 x 10^5)	**+ Imidacloprid**	medium rate (1.1 ml per 500ml water)	**B. Bass HR + Imida MR**
12. *B. bassiana*	high rate (4 x 10^5)	**+ Imidacloprid**	high rate (2.2 ml per 500ml water)	**B. Bass HR + Imida HR**

Figure 2: Serial dilutions for *Beauveria bassiana*

Figure 3: Tobacco variety (KRK 26) from land 10 used to make leaf discs

Figure 4: Dilution of imidacloprid

3.2.4 Treatments and inoculation on tobacco disc

For each block, (block 1-5) fungal sprays consisting three concentration treatments, low, medium and high incorporated with one of the three concentration treatments of imidacloprid, low, medium and high were applied to tobacco leaf discs. The three imidacloprid and *B. bassiana* treatment concentrations were, 0.55, 1.1 and 2.2 ml per 500ml of water and 4×10^3, 4×10^4 and 4×10^5, respectively. Water was sprayed in control petri dishes. Treatments with medium rates of imidacloprid only and *B. bassiana* only were also considered (Table 2). Treatments were collected in 1 L beakers. Leaf disks were dipped in respective beakers with treatments using metal forceps.

Leaf discs were dipped for five seconds to allow the leaf discs to be soaked fully. After five seconds, leaf discs were pulled out of the beaker using metal forceps and allowed to dry on filter papers at room temperature to prevent aphids from drowning. After drying the leaf discs were transferred to the petri dishes with a damp filter paper to keep the leaf disc moist. Five day old aphids were then infested using a paint brush. Petri dishes were then covered with their lid to prevent aphids from moving out of petri dishes and were maintained in the lab at room

temperature at the same regime for seven days. Ten aphids were placed on each leaf disc with a paint brush and mortality recorded daily for seven days.

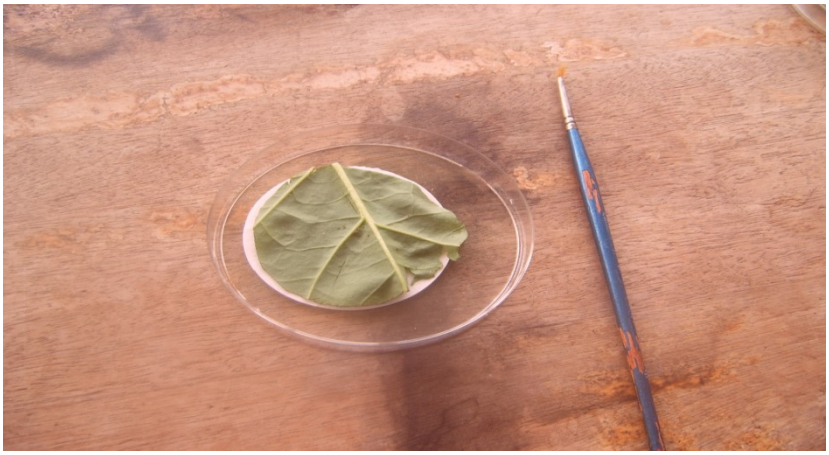


Figure 5: Leaf discs used and inoculation of aphids using a paint brush

3.2.5 Analysis of aphid mortality

Mortality of aphids was recorded starting from 24 hours after inoculation up to 168 hours after inoculation (7 days). Recordings were done at 8 o'clock in the morning for uniformity. Data sheets indicating the cumulative mortality rates were prepared and recorded. The mortality of the aphids was recorded as a percentage of the initial number infested (10 per petri dish).

3.2.6 Data analysis

ANOVA was used to compare the mortality rates between treatments and blocks and was calculated using Genstat Release 17th edition. For control correction, Abbott's formula was used to calculate corrected efficacy % in pesticide trials.

Abbott's formula

$$\text{Corrected \%} = \left(1 - \frac{\text{n in T after treatment}}{\text{n in Co after treatment}}\right) * 100$$

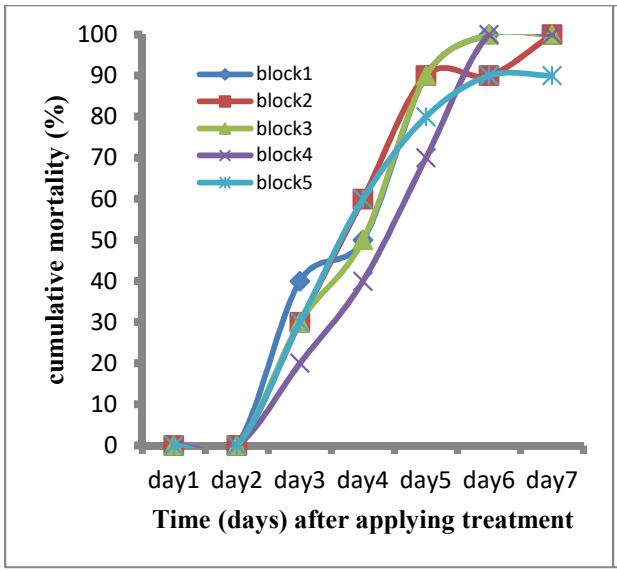
Where : n = Insect population , T = treated , Co = control

4.0 RESULTS

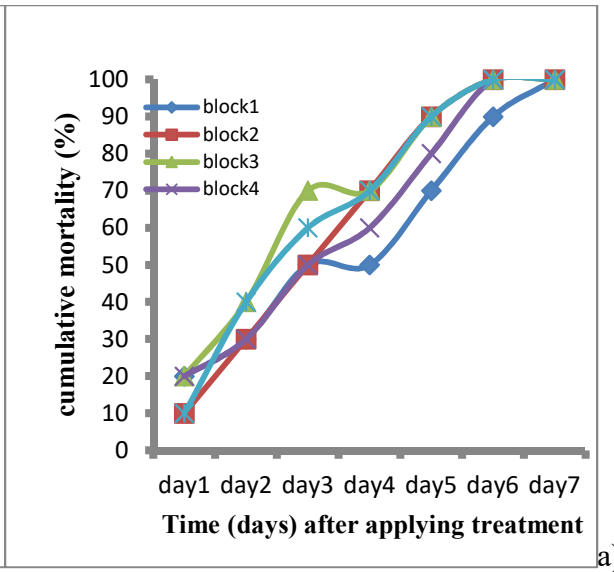
4.1. Trends in aphid mortality

In the control there were deaths observed (Table 4), but these did not show any significant difference and they were due to natural variance ($p>0.05$ appendix 2). There were no deaths observed with Imida MR during the first two days (Fig. 6a). Thereafter deaths started to occur in day 3. B. bass HR + Imida HR showed significantly high rates ($p<0.001$ appendix 3) of mortality in day1 compared to all other treatments except B. bass HR + Imida MR (Table 3). Deaths in B. bass MR treatment were observed from day one. A white mould was also seen wrapping the aphid body. In treatments B. bass LR+ Imida LR; B. bass LR + Imida MR and B. bass LR + Imida HR mortality was observed from day one.

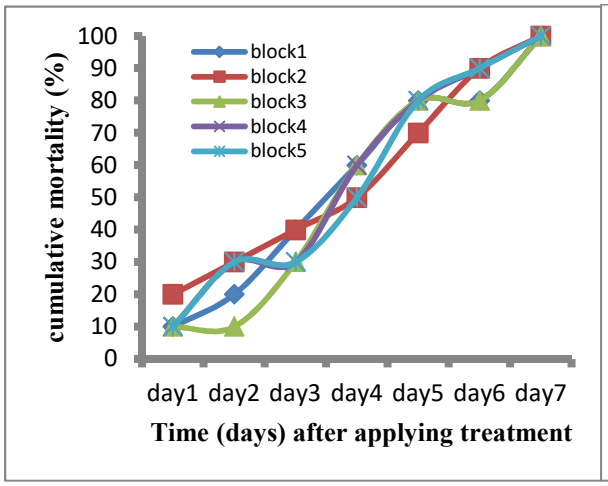
From day three the mortality rate accelerated in B. bass LR + Imida MR and B. bass LR + Imida HR. A white mould was also seen wrapping the aphid body. The mortality rate was average compared to B. bass LR+ Imida LR; B. bass LR + Imida MR and B. bass LR + Imida HR. Mortality rate also began to accelerate after 72 hours. A white mould was also observed wrapping the aphid body. In B. bass HR + Imida LR; B. bass HR + Imida MR and B. bass HR + Imida HR chemical control, mortality was observed from day one. The mortality rate started to accelerate after twenty- four hours. The mortality rate was high ($p<0.001$ appendix 6) in treatmentsB. bass HR + Imida MR and B. bass HR + Imida HR. In B. bass HR + Imida HR chemical control (Fig 6k) by day five almost all aphids were dead.



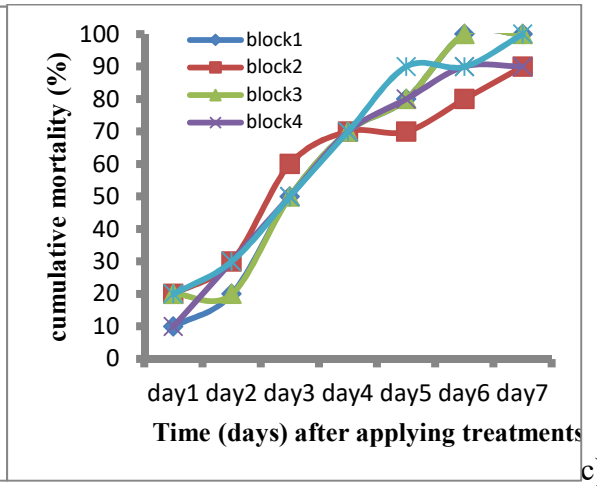
a) Imida MR



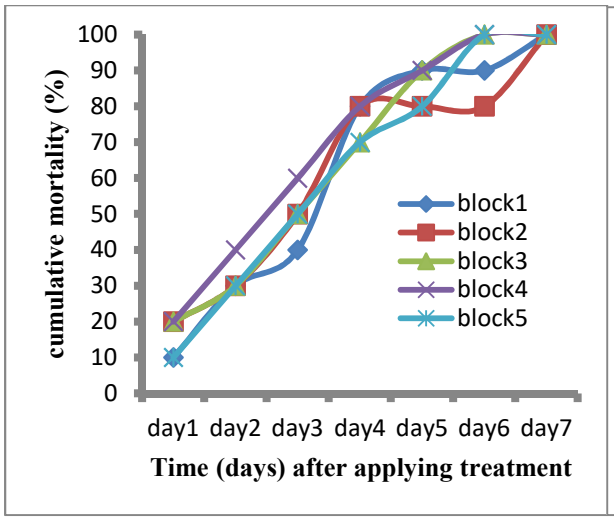
b) B. bass MR

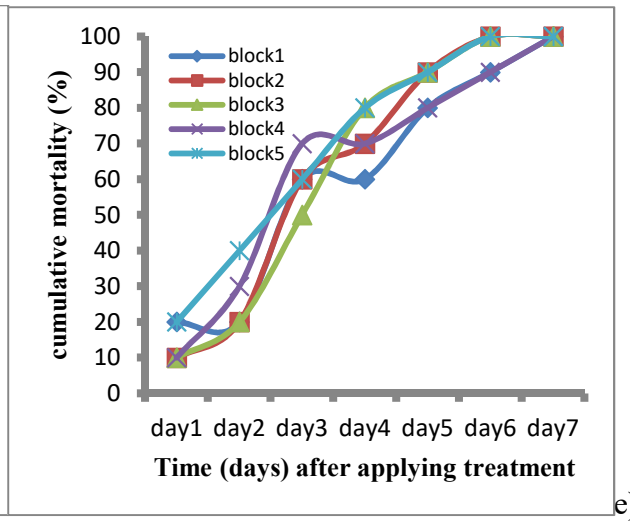


c) B. bass LR+ Imida LR



d) B. bass LR + Imida MR

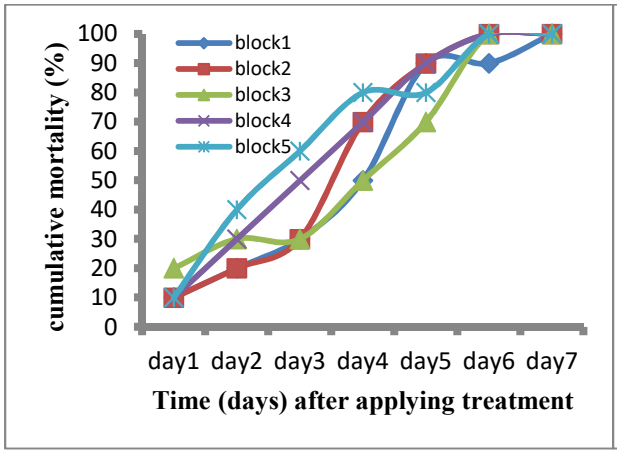


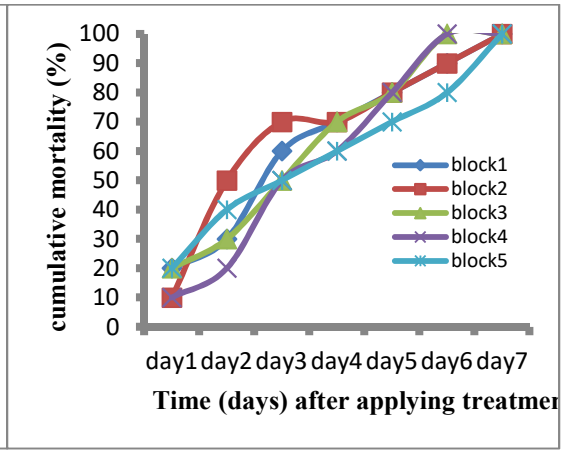


e)

B. bass LR + Imida HR

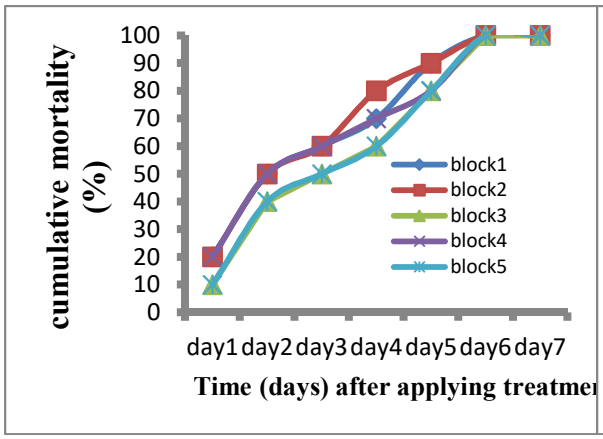
f) B. bass MR + Imida LR

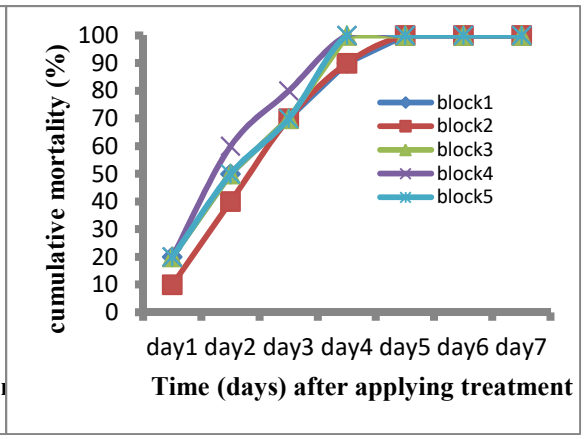




g) B. bass MR + Imida MR

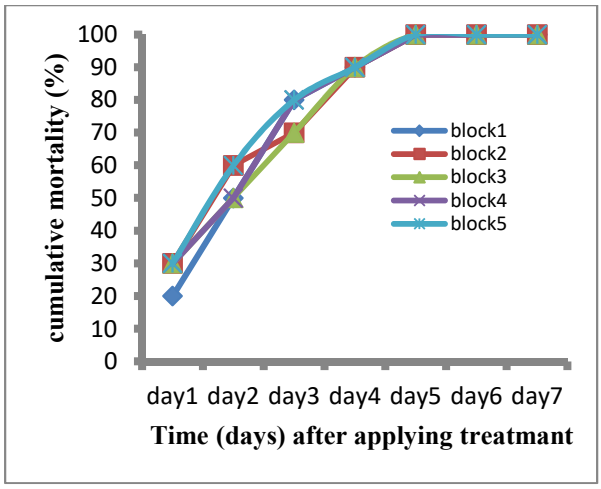
h) B. bass MR + Imida HR





i) B. bass HR + Imida LR

j) B. bass HR + Imida MR



k) B. bass HR + Imida HR

Figure 6: Trends in percent mortalities of *M. persicae nicotianae* days after exposure to different treatments.

4.1.1 Mortality rates

After calculating corrected efficacy percentage in pesticide trials using Abbott's formula, there were significant differences shown among treatments ($p<0.001$ appendix 3-8). On day 1 B. bass HR + Imida HR showed significantly high death rates to all other treatments except B. bass HR + Imida MR (Table 3). The control and Imida MR showed significantly lower rates compared to the all other treatments. Day 2, control and Imida MR continued to show significantly lower rates to all other treatments ($p<0.001$ appendix 3). B. bass HR + Imida HR showed high death rates to all other treatments except B. bass HR + Imida LR and B. bass HR+ Imida MR. Day 3, Imida MR started to show death rates but in control plates death rates were still not observed. B. bass HR +Imida HR continued to show high death rates than all other treatments, but death rates were not significantly different with those of B. bass HR + Imida LR and B. bass HR + Imida MR (table 3). Day 4, B. bass HR + Imida MR showed high death rates though the death rates were not significantly different to B. bass HR + Imida LR and B. bass HR + Imida HR (table 3). Imida MR still showed lower death rates to all other treatments and in control plates no deaths were recorded.

Day 5, in control plates no death had been recorded, Imida MR showed death rates which had no significant differences to all other treatments except B. bass HR + Imida MR and B. bass HR +

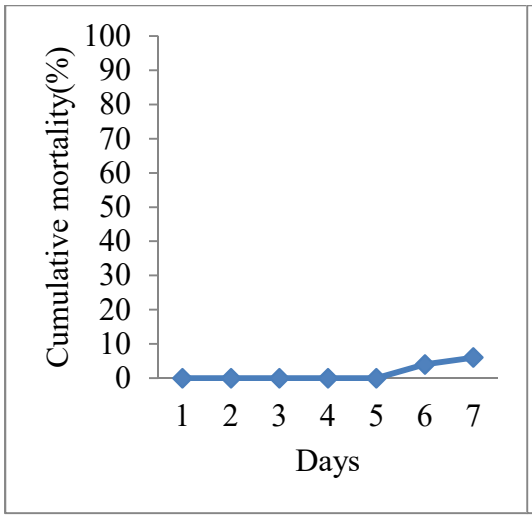
Imida HR (table 3). By day 5, B. bass HR + Imida MR and B. bass HR + Imida HR had reached 100% mortality rate. Day 6, deaths started to occur in control plates and all other treatments showed high significant death rates with B. bass HR + Imida LR, B. bass HR + Imida MR and B. bass HR + Imida HR showing 100% mortality rates ($p<0.001$ appendix 7). Day 7, an increase in mortality rate in control plates was observed; all other treatments reached 100% mortality rates except in the control, Imida MR and B. bass LR + Imida MR.

Table 3: Percent mortality after using Abbott's formula to correct for mortality in the control plates

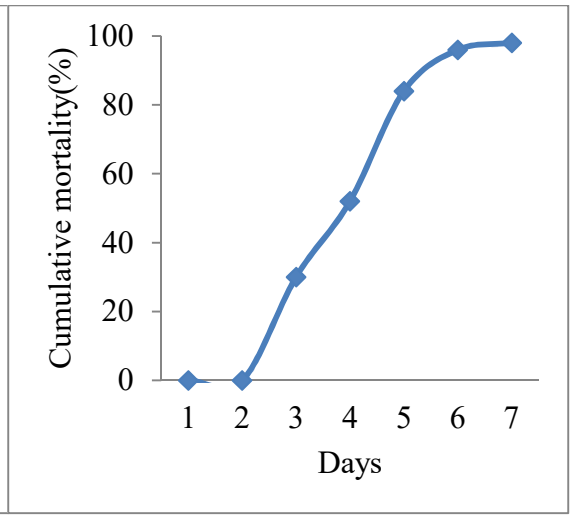
TREATMENT	**DAY**						
	1	2	3	4	5	6	7
Control	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	4^{a}	6^{a}
Imida MR	0^{a}	0^{a}	30^{b}	52^{b}	84^{b}	96^{bc}	98^{b}
B. bass MR	16^{b}	34^{bc}	56^{def}	64^{bcd}	84^{b}	97.8^{bc}	100^{b}
B. bass LR+ Imida LR	12^{b}	24^{b}	34^{bc}	56^{bc}	78^{b}	85.6^{b}	100^{b}
B. bass LR + Imida MR	16^{b}	26^{b}	52^{de}	70^{cd}	80^{b}	92^{bc}	96^{b}
B. bass LR + Imida HR	16^{b}	32^{b}	50^{cde}	76^{de}	86^{b}	93.8^{bc}	100^{b}
B. bass MR + Imida LR	14^{b}	26^{b}	60^{efg}	72^{de}	86^{b}	95.8^{bc}	100^{b}
B. bass MR + Imida MR	12^{b}	28^{b}	40^{bcd}	64^{bcd}	84^{b}	97.8^{bc}	100^{b}
B. bass MR + Imida HR	16^{b}	34^{bc}	56^{def}	66^{bcd}	79^{b}	91.8^{bc}	100^{b}
B. bass HR + Imida LR	16^{b}	46^{cd}	56^{def}	68^{cd}	84^{b}	100^{c}	100^{b}
B. bass HR + Imida MR	18^{bc}	50^{d}	72^{fg}	96^{f}	100^{c}	100^{c}	100^{b}
B. bass HR + Imida HR	28^{c}	54^{d}	76^{g}	90^{ef}	100^{c}	100^{c}	100^{b}
MEAN	13.7	29.5	48.5	64.5	78.7	87.9	91.7
F- PROB	<.001	<.001	<.001	<.001	<.001	<.001	<.001
S.E.D.	3.04	4.05	4.71	4.455	3.88	3.63	1.61
L.S.D.	6.35	8.17	9.49	8.98	7.81	7.32	3.25
CV (%)	35.1	21.7	15.3	10.9	7.8	6.5	2.8

4.1.2 Effect of different chemical treatments on cumulative mortality rates of *M. persicae nicotianae*

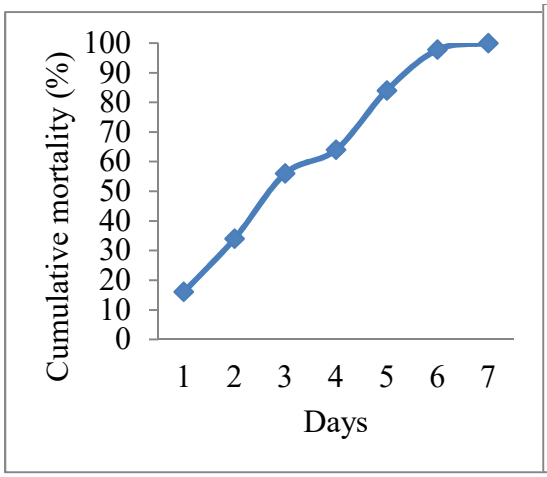
Control treatment showed zero cumulative rates up to day 5 (Fig. 7a). However, the death rates started to accumulate on day 6. In Imida MR death rates started to accumulate at day 3 and reached 98% at day 7 (Fig 7b). B. bass MR (Fig 7c) and all other treatments (Fig 7d- 7i) death rates started to accumulate from day 1 and reached 100% by day 7 except B. bass LR + Imida MR (Fig 7f) which reached 96% at day 7, B. bass HR + Imida LR (Fig 7j) which reached 100% mortality rate at day 6 and B. bass HR + Imida MR (Fig 7k) and B. bass HR + Imida HR (Fig 7l) which reached 100% mortality rate by day 5.



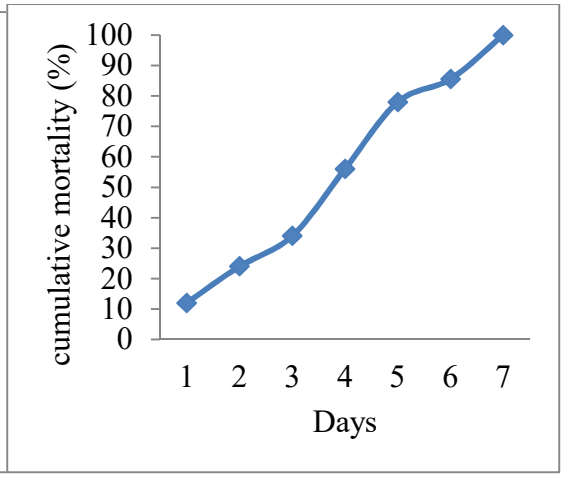
a) Control



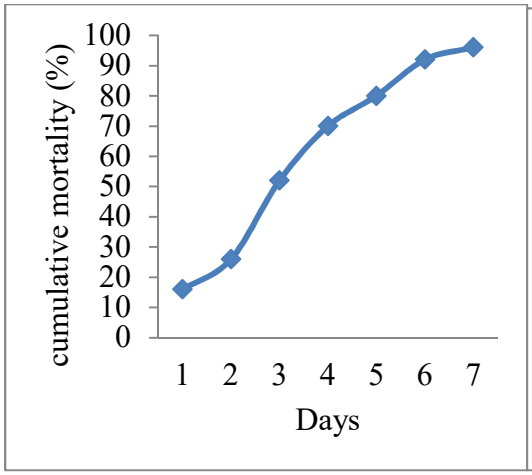
b) Imida MR



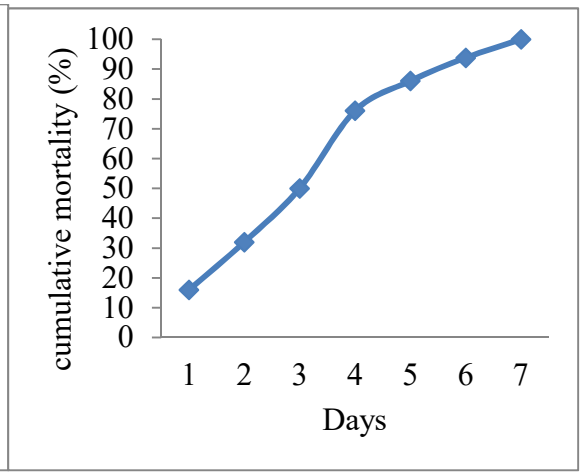
c: B. bass MR



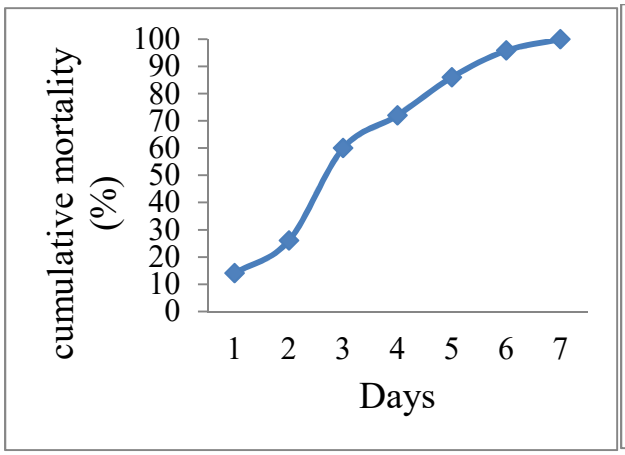
d) B. bass LR+ Imida LR



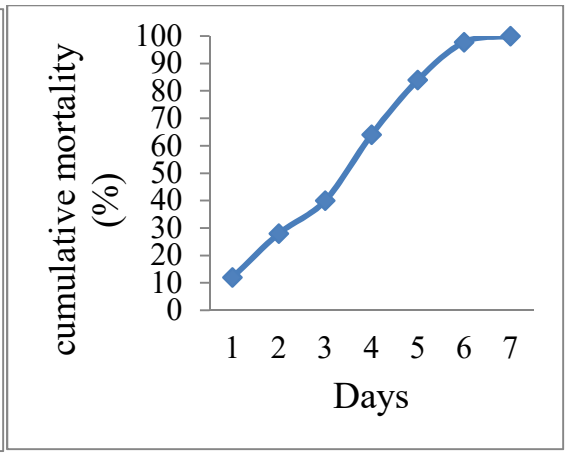
e) B. bass LR + Imida MR



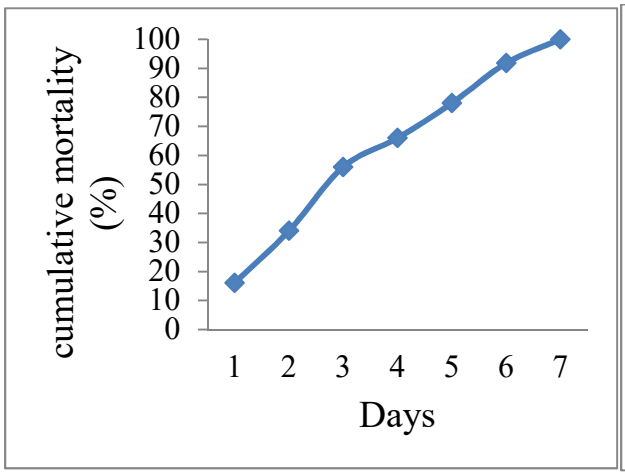
f) B. bass LR + Imida HR



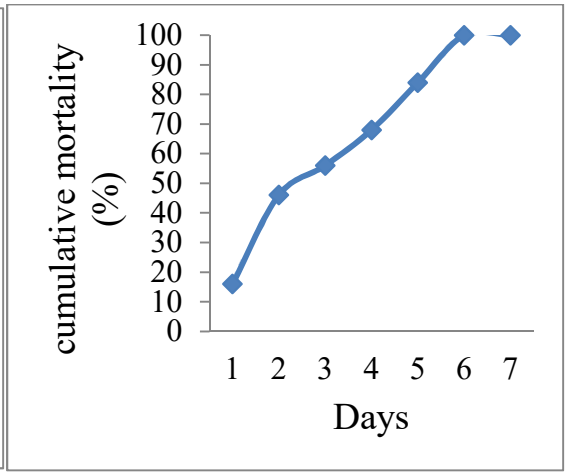
g) B. bass MR + Imida LR



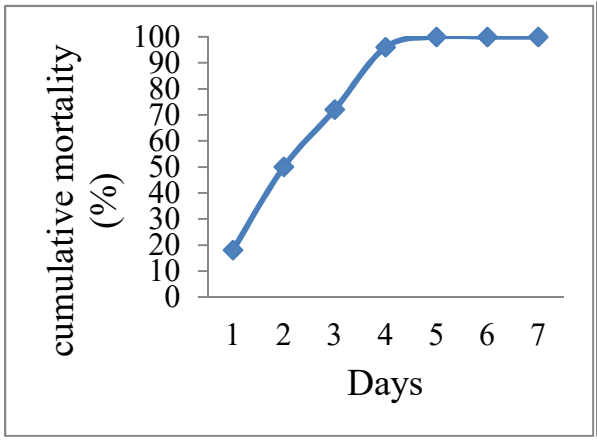
h) B. bass MR + Imida MR



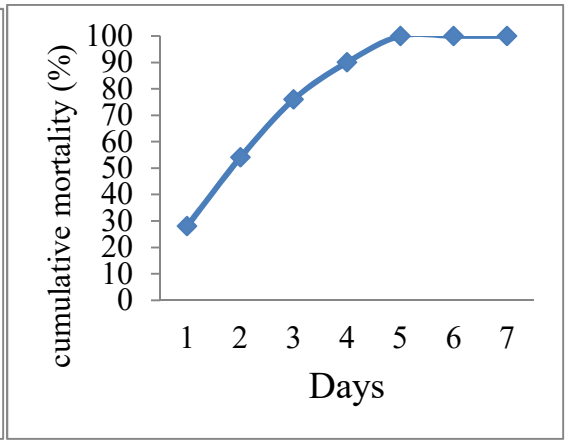
i) B. bass MR + Imida HR



j) B. bass HR + Imida LR



k) B. bass HR + Imida MR



l) B. bass HR + Imida HR

Figure 7: Effect of different chemical treatments on cumulative mortality rates of *M. persicae nicotianae*

5.0DISCUSSION

The control treatment showed deaths, which were due to natural occurrence because there were no significant differences from day one up to day seven. There were no deaths observed with Imida MR during the first two days. This was because imidacloprid is a systematic insecticide, it should be absorbed first by the plant and this takes about 48 hours. Once the chemical is assimilated, aphids when feeding ingest the chemical and die. This finding clearly suggested that the neonicotinoid insecticides like imidacloprid take slightly longer time for their maximum effect against sap-sucking insect like aphids and could persist up to 10 days (Das, 2013).

Once applied, the insecticide enters into the plant system by translaminar action, after which it enters into the insect body by feeding and finally killing the aphid by binding it with nicotinic acetylcholine receptor(Das, 2013).B. bass HR + Imida HR showed significantly higher rates of mortality on day one compared to all other treatments except B. bass HR + Imida MR. Deaths in B. bass MR treatment were observed from day one, this was because *B. bassiana* is a contact insecticide and it kills aphids on contact. This chemical does not need to be consumed by aphids to cause effect. A white mould was also seen wrapping the aphid body. *B. bassiana*is a fungus that kills aphids by causing a disease known as white muscadine disease. When the spores of *B. bassiana* come in contact with the cuticle of specific insects that are susceptible to it, they germinate and grow directly through the cuticle to the inner body of the host (www.hort.uconn.edu).

The fungus then proliferates through the insect's body, producing harmful toxins and depriving the insect of nutrients until the insect dies (www.hort.uconn.edu). In treatments B. bass LR+ Imida LR; B. bass LR + Imida MR and B. bass LR + Imida HR mortality was observed from day one because there was a contact insecticide in the formulation, but the mortality rate was slow because the contact insecticide rate used was low. From day three the mortality rate accelerated in B. bass LR + Imida MR and B. bass LR + Imida HR treatments, this was due to low rates of the contact insecticide and medium to high rates of the systematic insecticide which were to be assimilated by the plant first then fed by the aphid eventually killing the aphid by binding it with nicotinic acetylcholine receptor.

This number of deaths on day one indicated clearly that the level of mortality was dose-dependent and the presence of the contact insecticide (Ye*et al*., 2005). The higher rates of .B. bass and Imida MR and HR indicated that by four to five days cumulative mortality had reached 100% unlike higher rate of B. bass with lower rate of imidacloprid which took 6 days to reach 100%. A white mould was also seen wrapping the aphid body in this treatment. The mortality rate was moderate compared to B. bass LR + Imida LR; B. bass LR + Imida MR and B. bass LR + Imida HR. Mortality rate also began to accelerate after 72 hours because the systematic insecticide had been absorbed into the plant system and also absorbed by the system of the pest after feeding.

In B. bass HR + Imida LR; B. bass HR + Imida MR and B. bass HR + Imida HR chemical control, mortality was observed from day one because the contact insecticide concentration was high. The mortality rate was high in treatments B. bass HR + Imida MR and B. bass HR + Imida HR this was because the systematic insecticide rate used was medium to high. In B. bass HR + Imida HR chemical control (Fig 7l) by day five cumulative mortality had reached 100%.

6.0 CONCLUSIONS AND RECOMMENDATION

6.1 Conclusion

Formulations of fungal and chemical agents are sustainable options for *Myzus persicae nicotianae* control. Adopting combined formulations of fungal and chemical agents can effectively control *Myzus persicae nicotianae* and sustain pesticide usage at lower levels. B. bass HR + Imida MR had significantly high rates of mortality in day one and by day five, mortality had reached 100%. However, there would be no need to use the higher rates if the same results could be attained without using higher rates of Imidacloprid. Uses of this formulation will benefit agrochemical users economically, while maintaining a friendly environment and reducing exposure when handling the pesticides.

6.2 Recommendation

I strongly recommend the use of B. bass HR + Imida MR because this treatment had significantly high rates of mortality in day one and by day five, mortality had reached 100% which was the same as B. bass HR + Imida HR. Such a combination undoubtedly increase practicability of fungal formulation and may help to manage aphid resistance to chemical insecticides. It is also economical viable to use this formulation compared to B. bass HR + Imida HR because efficacy was the same. Experiments should be conducted further with the aim of developing our own strain of *B. bassiana* suitable for tobacco aphid control. *B. bassiana* is environmental friendly and it has worked well on controlling aphids, experiments on the control of other pests using *B. bassiana* should be conducted since its wide application as a biological pesticide could be taken up after exploring its toxicity and field trials.

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APPENDICES

APPENDIX 1: cumulative mortality raw data

Table 4: Cumulative mortality trends of aphids in all treatments and blocks before using Abbott's formula

		day1	day2	day3	day4	day5	day6	day7
control	block1	0	0	0	0	0	1	1
	block2	0	0	0	0	0	0	0
	block3	0	0	0	0	0	0	1
	block4	0	0	0	0	0	0	0
	block5	0	0	0	0	0	1	1
Imida MR	block1	0	0	4	5	9	10	10
	block2	0	0	3	6	9	9	10
	block3	0	0	3	5	9	10	10
	block4	0	0	2	4	7	10	10
	block5	0	0	3	6	8	9	9
B. bass MR	block1	2	3	5	5	7	9	10
	block2	1	3	5	7	9	10	10
	block3	2	4	7	7	9	10	10
	block4	2	3	5	6	8	10	10
	block5	1	4	6	7	9	10	10
B. bass LR + imida LR	block1	1	2	4	6	8	8	10
	block2	2	3	4	5	7	9	10
	block3	1	1	3	6	8	8	10
	block4	1	3	3	6	8	9	10
	block5	1	3	3	5	8	9	10
B. bass LR + imida MR	block1	1	2	5	7	8	10	10
	block2	2	3	6	7	7	8	9
	block3	2	2	5	7	8	10	10
	block4	1	3	5	7	8	9	9
	block5	2	3	5	7	9	9	10
B. bass LR + imida HR	block1	1	3	4	8	9	9	10
	block2	2	3	5	8	8	8	10
	block3	2	3	5	7	9	10	10

	block4	2	4	6	8	9	10	10
	block5	1	3	5	7	8	10	10
B. bass MR + imida LR	block1	2	2	6	6	8	9	10
	block2	1	2	6	7	9	10	10
	block3	1	2	5	8	9	10	10
	block4	1	3	7	7	8	9	10
	block5	2	4	6	8	9	10	10
B. bass MR + imida MR	block1	1	2	3	5	9	9	10
	block2	1	2	3	7	9	10	10
	block3	2	3	3	5	7	10	10
	block4	1	3	5	7	9	10	10
	block5	1	4	6	8	8	10	10
B. bass MR + imida HR	block1	2	3	6	7	8	9	10
	block2	1	5	7	7	8	9	10
	block3	2	3	5	7	8	10	10
	block4	1	2	5	6	8	10	10
	block5	2	4	5	6	7	8	10
B. bass HR + imida LR	block1	2	5	6	7	9	10	10
	block2	2	5	6	8	9	10	10
	block3	1	4	5	6	8	10	10
	block4	2	5	6	7	8	10	10
	block5	1	4	5	6	8	10	10
B. bass HR + imida MR	block1	2	5	7	9	10	10	10
	block2	1	4	7	9	10	10	10
	block3	2	5	7	10	10	10	10
	block4	2	6	8	10	10	10	10
	block5	2	5	7	10	10	10	10
B. bass HR+ imida HR	block1	2	5	8	9	10	10	10
	block2	3	6	7	9	10	10	10
	block3	3	5	7	9	10	10	10
	block4	3	5	8	9	10	10	10
	block5	3	6	8	9	10	10	10

APPENDIX 2: ANOVA mortality for day 1

Variate: Percent mortality at 24hours

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	4	8875.0	2218.8	4.34	
block.*Units* stratum					
treatment	7	8337.5	1191.1	2.33	0.053
Residual	28	14325.0	511.6		
Total	39	31537.5			

APPENDIX 3: ANOVA mortality for day 2

Variate: Percent mortality at 48 hours

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	4	535.00	133.75	2.20	
block.*Units* stratum					
treatment	7	5057.50	722.50	11.87	<.001
Residual	28	1705.00	60.89		
Total	39	7297.50			

APPENDIX 4: ANOVA mortality for day 3

Variate: Percent mortality at 72 hours

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	4	231.283	15.419	2.78	
block.*Units* stratum					
treatment	7	1411.350	1411.350	5.57	<.001
Residual	28	230.050	20.914		
Total	39	1872.683			

APPENDIX 5: ANOVA mortality for day 4

Variate: Percent mortality at 96 hours

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	4	315.017	21.001	4.23	
block.*Units* stratum					
treatment	7	2496.150	2496.150	8.9	<.001
Residual	28	313.250	28.477		
Total	39	3124.417			

APPENDIX 6: ANOVA mortality for day 5

Variate: Percent mortality at 120 hours

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	4	366.400	24.427	6.50	
block.*Units* stratum					
treatment	7	3713.067	3713.067	1.7	<.001
Residual	28	366.133	33.285		
Total	39	4445.6			

APPENDIX 7: ANOVA mortality for day 6

Variate: Percent mortality at 144hours

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	4	395.833	84.342	8.43	
block.*Units* stratum					
treatment	7	4646.400	4646.400	11.45	<.001
Residual	28	394.400	35.855		
Total	39	5436.633			

APPENDIX 8: ANOVA mortality for day 7

Variate: Percent mortality at 168 hours

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	4	401.467	26.764	4.10	
block.*Units* stratum					
treatment	7	5041.667	5041.667	12.79	<.001
Residual	28	4001.133	36.467		
Total	39	9444.267			