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Synergy In Biorational Insecticides Applied To Collard Greens , Brassica Oleracea , Infested With Diamondback Moths, Plutella Xylostella (Lepidopedra : Yponomeutidae)

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Dedication

I dedicate this work to God and my wife, Jessica Lynn Farmer Flanery.

Biographical Sketch

Matthew Conklin Flanery was born on December 8, 1979, in Danbury, North Carolina. He received a Bachelor of Science degree in Chemical Engineering from North Carolina State University in 2003. He is a candidate for a Masters in Science in Plant, Soil and Environmental Science.

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Abstract

The pest status of the diamondback moth, *Plutella xylostella*, has risen as it has become resistant to most insecticides used for its control. Insecticide mixtures could be exploited to slow down resistance development in the diamondback moth. We evaluated various mixtures comprising: Agroneem Plus®, spinosad, thiamethoxam and jalapeño pepper extract using laboratory bioassays with a view to obtaining a combination that could be adopted by small growers. These mixtures were applied to collard greens using the leaf dip method and fed to second to fourth instar larvae in bioassays. The mixtures were evaluated for their effect on larval fitness and leaf damage. Our results indicate a possible interaction between the methanolic extract of jalapeño pepper and spinosad. Agroneem Plus® and thiamethoxam also exhibited interactions. Spinosad at the recommended rate killed 100% of the exposed larvae. These mixtures are to be further evaluated to determine their ability to delay resistance in diamondback moth populations and eventually test them under field conditions.

CHAPTER 1

Introduction

1.1 The Diamondback Moth and Host Plant, Crucifers

The diamondback moth (DBM), *Plutella xylostella* (Lepidoptera: Yponomeutidae) is one of the major insect pests of crucifers (Talekar, 1992). It has the ability to rapidly become resistant to the different insecticide groups that are used for its control (Wright, 2004). However, mixtures of insecticides with proven synergistic interaction will present a diversity of toxic molecules. That diversity could be used to slow down the onset or progress of resistance development (Wirth et al., 2004). Consumers have an extremely low tolerance for damage from diamondback moths. In fact, only trace amounts of insect damage or frass is accepted in the final product (Morisak et al., 1984). Crucifers are popular vegetables grown in North Carolina. They include cabbages, broccoli, cauliflowers, radishes, kohlrabi, kale and collard greens. Collard greens, *Brassica oleracea* L (Acephala Group), play a key role in traditional southern US cuisine (Gardner et al., 2010).

1.2 Biorational Insecticides

Biorational insecticides include insecticides that act against target pest insects while causing relatively/no adverse effects on non-target organisms in the pest's environment. These insecticides include horticultural oils, insecticidal soaps and botanical and microbial insecticides. As biorational insecticides do not usually have adverse effects on natural enemies, they can be used in conjunction with biological control (Schuster & Stansly, 2005). Thus, effective biorational insecticides are key aids to farmers practicing bio-intensive integrated pest management (BI-IPM). Biorational insecticides, being low risk, are also used to keep fruits and vegetables free of harmful insecticide residues, safeguard the health of agricultural workers and reduce the environmental impact of synthetic compounds.

1.3 Interaction

Interactions between insecticides can be synergistic, additive or antagonistic. Synergy has been described as an interaction between two or more chemicals that elicits a response which is greater than the sum of the individual effects (Working Group on Synergy in Complex Mixtures, 1986). In pest management this means a synergistic mixture of insecticides has greater efficacy than its individual insecticides. Additive responses are effects of insecticide mixtures that are equivalent to the insecticides being used separately. On the other hand, antagonistic responses mean the efficacy of the insecticide mixture is worse than its individual parts. Thus, antagonism between insecticides may exacerbate resistance development (Ahmad, 2004). Biorationals have been used in isolation with some success except when resistance has evolved as with spinosad in Hawaii and also in other places with the *Bacillus thuringiensis* (Bt) strains (Shelton et al., 1993; Tabashnik et al., 1990). Hence, there is a need to investigate the effect on resistance when biorationals are combined (Wirth et al., 2004).

1.4 Hypothesis

Therefore, an initial investigation into the interactions between some biorational insecticides was performed. We evaluated various mixtures comprising: Agroneem Plus®, spinosad, thiamethoxam and jalapeño pepper extract. We used laboratory bioassays with a view to finding a combination that could be adopted by small growers. The overall goal of the research was to find a mixture of biorational insecticides that can be used in subsequent field experiments aimed at reducing resistance development in diamondback moths. The specific objectives were:

1. Determine the efficacy of three commercial insecticides and a crude methanolic extract of jalapeño pepper (*Capsicum annuum)* against diamondback moth on collard greens (Morris Heading variety).

The expected results at this step were to find the efficacy of application rates above and below the recommended rate.

2. Establish the efficacy of selected mixtures of biorational insecticides against DBM larval feeding using collard greens as the crop model in laboratory bioassays.

The goal was to find at least one mixture of biorational insecticides that demonstrated a distinctly higher efficacy than any of the insecticides used individually.

3. Determine ovicidal activity and oviposition deterrence of the most effective mixture (from Objective 2 above).

The expectation was that the selected mixture from the second objective would have greater ovicidal activity and oviposition deterrence than the individual components.

CHAPTER 2

Literature Review

2.1 Chemical Interactions in Pest Control

Chemical interactions that occur in pest control may be synergistic, additive or antagonistic. Synergy may be described as an interaction between two or more chemicals that elicits a response greater than the sum of the individual effects (Working Group on Synergy in Complex Mixtures, 1986). Meanwhile, additive responses occur when two or more chemicals mixed together have an effect equal to the sum of the individual effects. On the other hand, antagonism is an interaction between two or more chemicals that elicits a response less than the sum of the individual effects. Synergistic, additive and antagonistic interactions have been found between deltamethrin and organophosphate insecticides when applied to cotton bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae), infestations (Ahmad, 2004).

Proof of synergistic effects on humans due to chemicals used in pest management has already been found. For example, researchers at Duke University have reported that the response resulting from exposure to both DEET® and permethrin is synergistic. DEET® by itself reduced sensorimotor performance and decreased the permeability of the blood-brain barrier. Meanwhile, permethrin by itself showed no effect. Together, DEET® and permethrin had amplified effects on sensorimotor performance and the permeability of the blood-brain barrier. Additionally, these chemicals together caused amplified urinary excretions of 6B-hydroxycortisol, an indicator of chemical poisoning, and release of brain mitochondrial cytochrome c, an indicator of brain cell death (Abou-Donia et al., 2001; Abu-Qare et a., 2001; Abdel-Rahman et al., 2001).

Synergy has also been found between *Bacillus sphaericus* and *Bacillus thuringiensis* subsp. *israelensis* (Bti). *B. sphaericus* is used to control mosquito larvae particularly in areas with polluted waters. However, it has a tendency to select for resistance along with having a narrow host range. When mixed with Bti, these two bacterial strains can affect a much broader host range. In addition, the efficacy can be improved and resistance development may be reduced, while still remaining effective in polluted waters and maintaining a long residual activity (Wirth et al., 2004).

2.2 Biorational Insecticides

A relatively new term, biorational insecticides, delineate a group of insecticides that are effective against target pest insects but cause negligible harm to non-target organisms when used properly. The active ingredients or formulations effectively control pests and are derived from biological or natural origins. The term has been applied to only natural products by some scientists. Other scientists, however, apply the term much more broadly to any insecticides that are relatively innocuous to beneficial organisms (Stansly et al., 1996). By definition, biorational insecticides are well suited to be used in conjunction with biological control. Biorational insecticides include oils, soaps and botanicals among others (Schuster & Stansly, 2005).

Spinosad (Monterey Garden Insect Spray, Lawn and Garden Products, Inc., Fresno, CA) is effective against a plethora of foliar-feeding insect pests. Spinosad is made from an aerobic fermentation process involving spinosyns A and D, the two most active naturally occurring metabolites produced by the actinomycete, *Saccharopolyspora spinosa* Mertz & Yao. Structurally, these A and D compounds are macrolides with a unique tetracyclic ring. This actinomycete was first isolated from soil samples collected from the Caribbean (Sparks et al., 1998). Spinosad is a neurotoxin that utilizes a unique mode of action. It targets both the GABA receptors and the nicotinic acetylcholine receptor (Salgado, 1997). Primarily it is a stomach poison, but it also has minor contact activity. Effects begin with cessation of feeding, then paralysis and finally death. Due to very little toxicity to mammals and birds and moderate toxicity to fish (Bret et al., 1997), spinosad is classified as a reduced risk material both environmentally and toxicologically by the United States Environmental Protection Agency (Saunders & Bret, 1997). Spinosad has been proven to be effective against thrips, flies, leafminers and moth larvae. It is especially useful against diamondback moths and other caterpillars. Spinosad is used in many IPM programs due to its low activity against beneficial insects (Liu et al., 2006).

Agroneem Plus® is a multi-component (including a synergist) biorational insecticide. The Organic Materials Review Institute (OMRI) has approved it, and it is sold commercially. The formulators believe that the inherent synergy among the multiple components in neem is more effective than the single most abundant component, azadirachtin. In addition, several proprietary synergists have been added to the formula (Anonymous, 2002). Agroneem Plus® is manufactured by Agrologistic Systems, Inc., Diamond Bar, CA, USA. It has demonstrated anti-feedant, repellant, and growth-regulating characteristics. It is a broad spectrum insecticide that affects insects at different growth stages, and yet considered to be nontoxic to humans and beneficial organisms (Anonymous, 2002). However, as with other neem-based insecticides, Agroneem Plus® may be toxic to aquatic invertebrates (Goktepe & Plhak, 2004).

Since ancient times plant parts have been used to protect against insects (Karunamoorthi et al., 2009). Hot pepper and its corresponding extracts are an example that has been verified scientifically to protect plants against many different insect pests. For example, capsaicin from hot peppers has been shown to hinder the growth of spiny bullworm larvae, *Earias insulana* (Weissenberg et al., 1986) and oleoresin, a chemical compound from *capsicum*, repels cotton pests (Mayeux, 1996). Chili pepper powder has demonstrated oviposition deterrence to the onion fly, *Delia antiqua* (Cowles et al., 1989). Chili pepper extract also repels spider mites, *Tetranychus urticae* (Koch) (Antonious et al., 2007), and is highly toxic to the cabbage looper, *Trichopulsia ni* (Hübner), one of the most prominent insect pests of crucifers (Hines & Hutchison, 2001). According to Antonious et al. (2007), unidentified compounds from the pepper extracts were the causal agents of the repellency and death of the cabbage loopers.

Peppers may act as direct toxins or have synergistic effects (Antonious et al., 2007). Crude extracts from pepper fruits may be utilized as a natural insecticide, especially due to pepper's ability to deter oviposition (Antonious et al., 2007). Significant quantities of tannins are found in hot peppers (Malgorzata & Perucka, 2005, Antonious et al., 2006), which function in the defense systems of plants (Aharoni et al., 2003). The tannins in hot peppers function as deterrents and toxins especially to insects that are not adapted to diets heavy in tannins (Antonious et al., 1999). In addition, peppers contain stearic and oleic acid which also are the esters of natural waxes along with aliphatic alcohols.

2.3 Thiamethoxam

Thiamethoxam (Actara®, Syngenta Crop Protection, Inc., Greensboro, NC) is a conventional synthetic insecticide, but one of the most environmentally-friendly insecticides available (Lawson et al., 1999). Actara® contains the active ingredient thiamethoxam (25 WG) which is a second generation neonicotinoid insecticide. It has a re-entry interval of 12 hours and a pre-harvest interval of 7 days for leafy crucifer greens. For head and stem brassicas, cucurbit vegetables and fruiting vegetables, the pre-harvest interval is 0 days. These attributes make Actara® an insecticide of choice for vegetables and fruits that harbor insects even at maturity. Thiamethoxam is a systemic insecticide and acts both by ingestion or contact by binding to or interfering with nicotinic acetylcholine receptors as its primary mode of action (Maienfisch et al., 2001). Insects that are resistant to conventional pyrethroid, carbamate, and organophosphate insecticides are still affected by thiamethoxam (Maienfisch et al., 1999). In addition, this insecticide is not known to produce any mutagenic effects, and has demonstrated low toxicity to mammals and beneficial insects (Lawson et al., 1999).

2.4 The Diamondback Moth, *Plutella xylostella*

The diamondback moth may be found wherever crucifers are grown (Metcalf & Metcalf, 1993). DBM is believed to have spread with the cultivation of its host, the crucifers, and by its own abilities to migrate across the oceans (Chu, 1986). Occurring in over 128 regions around the world (Lim, 1986), DBM was first reported in North America before 1850 (Metcalf & Metcalf, 1993). DBM adults have a trademark row of diamond-shaped spots down their backs at the intersection of their wings. Their wings flare upward and outward toward their hind tip. They are approximately 8.5 mm long (Harcourt, 1957). Males live an average of 12 days, ranging from 3 to 58 days. Meanwhile, females live an average of 16 days, with a lifespan ranging from 7 to 47 days (Harcourt, 1960). DBM are weak flyers (Rosario & Cruz, 1986), usually staying within 1.5 m of the ground and flying short distances of about 3.5 m (Harcourt, 1957). However, they are readily carried by the wind (Rosario & Cruz, 1986). Adults rest during the day, remaining inactive on the bottom of host leaves (Harcourt, 1957). Around sunset, they become active and may move to flowering cruciferous weeds in order to feed (Rosario & Cruz, 1986).

Mating often occurs on the same day as the adult DBM emerges. Mating involves no courtship and lasts about one hour. Females mate once, while males are capable of mating three times. Just after dusk, females may begin oviposition, but most oviposition occurs two hours after dusk. The average fecundity is about 160 eggs, with a high of nearly 360 eggs per female.

Females lay eggs one at a time (Harcourt, 1960), but may group two or three eggs together (Harcourt, 1957). Oviposition often carries on for nine more nights (Harcourt, 1960).

The coloring of DBM eggs ranges from yellowish to greenish white, and they look like tiny scales (Marsh, 1917). A larva is visible coiled beneath the chorion as the egg darkens just before hatching. After gnawing an opening through an end of the chorion, the larva emerges (Hill & Foster, 2000). The larva then feeds underneath the outer leaves of crucifers (Liu et al., 2006). Larvae eat inside the leaves as shallow leaf miners during the first instar, leaving what appear to be groupings of white spots on the leaves. Next, they molt, receding to protected locations such as leaf curls and depressions. The larvae also spin a few strands of silk around themselves for added protection. Emerging as second instars, the larvae feed on the surface of leaves. However, the second and third instars may oftentimes be observed with their heads and thoraces stuck inside the leaves (Harcourt, 1957).

Most of the damage occurs during the fourth instar, while all of the damage occurs in the larval stages. The length of the matured larvae is 9 mm (Bhalla & Dubey, 1986). The fourth instar larvae spin a white cocoon often in a protected area of a leaf, such as the curl at the edge of a leaf or along the midrib. They undergo a prepupal stage involving one to two days of quiescence (Marsh, 1917). They pupate an average of 8.5 days, ranging from 5 to 15 days (Hill & Foster, 2000). As they pupate, larvae become slender, develop brown stripes, and often turn a yellowish color. Pupae are around 6.3 mm in length (Marsh, 1917).

The second to fourth instars feed by scarifying the leaf allowing the thin upper epidermis to remain intact along with the leaf veins (Hill & Foster, 2000). In this way, a skeletonized appearance is left behind on leaves indicating the presence of DBM larvae that have developed beyond the first instar. Moth larvae also attack young plants, eating the crowns and growing points and stunting their growth (Liu et al., 2006). Although damage by DBM is often overshadowed by the larger and more voracious cabbage looper, *Trichoplusia ni* (Hübner), and the imported cabbageworm, *Pieris rapae* (L.) (Bonnemaison, 1965), DBM has progressed from minor pest to major pest status. The main reason for its progression is DBM's striking capacity to swiftly gain resistance to all insecticides used to control it for a length of time. It can develop resistance to most classes of insecticides after a few applications (Yeh et al., 1986). Furthermore, multiple resistance and cross resistance are commonplace (Cheng, 1988). The ability of DBM to adapt to new insecticides and environments gives it the markings of a devastating pest. There has been a grim outlook for insecticide resistance management of DBM (Hill & Foster, 2000).

Control measures of the DBM populations worldwide have been principally achieved through the use of conventional insecticides and biorational insecticides such as *Bacillus thuringiensis* (Eltayeb et al, 2010). In North America, traditional farmers manage the DBM with synthetic insecticides. Conventional insecticides have been used indiscriminately and excessively to the point that the diamondback moth has developed resistance to many types of conventional insecticides (Hines & Hutchison, 2001; Liu et al., 2002). Development of resistance in diamondback moths to *Bacillus thuringiensis* subsp. *kursatki* (Btk) has been reported in various locations in the continental USA (Mahr et al., 1993; Shelton et al., 1993; Tang et al., 1997). Thailand, Malaysia, the Philippines, Japan and Central America have also detected resistance in field populations of diamondback moths (Talekar & Shelton, 1993; Rueda & Shelton, 1995; Tabashnik et al., 1997). Furthermore, diamondback moths in Malaysia have been reported to express resistance to spinosad (Sayyed et al., 2004; Maxwell & Fadamiro, 2006).

2.5 Crucifers (Brassicaceae)

Crucifers, *Brassica oleracea*, (also known as brassicas and cole crops), include plants that are grown for a variety of edible parts. Radishes and turnips are grown for their edible roots. Kohlrabi is cultivated for its edible stems. The flowering heads of broccoli and cauliflower are eaten. Mustard and canola are grown for their seeds. Small, leafy green buds are the usually eaten part of Brussels sprouts whilst, cabbage, kale and collard greens are cultivated for their edible leaves. Crucifers are the most important vegetables in Asia and are grown throughout most of the world (Eltayeb et al., 2010). Crucifers are rich in vitamins and minerals, such as Vitamins A, B and C, carotenes, iron, calcium, potassium and phosphorus. In addition, these crops have been shown to reduce the risk of heart disease and cancer (Liu et al., 2006). Cabbage is the only crucifer recorded in the 2011 North Carolina Agricultural Statistics. North Carolina had over 5000 acres planted in cabbage with a harvest worth over 14 million (US$) (Krueger, 2011).

Glucosinolates and their hydrolyzed derivatives characterize the secondary chemistry of crucifers (VanEtten et al., 1976). These compounds are usually toxic to insects that do not specialize in feeding on crucifers (Feeny, 1976). In addition, these compounds have an adverse effect on some mammals, fungi and bacteria (VanEtten & Tookey, 1979). On the other hand, specific glucosinolates attract insects that specialize in feeding on crucifers (Hillyer & Thornsteinson, 1969). DBM is such a specialist and has adapted to these glucosinolates. Glucocheirolin, sinalbin and sinigrin are specific glucosides that stimulate feeding for DBM. So far, 40 plant species containing at least one of these chemicals have been found to serve as hosts for DBM (Hill & Foster, 2000).

CHAPTER 3

Materials and Methods

3.1 Materials

3.1.1 Sources of commercial insecticides. Four insecticides were selected as representative insecticides for this study (Figure 1). Agroneem Plus® (Agrologistic Systems Inc., Diamond Bar, CA) performed better than other popular neem-based botanical insecticides in experiments conducted previously by the Integrated Pest Management (IPM) Research Group at NC A&T State University. A crude methanolic extract (1:2 w/v) of jalapeño pepper (Antonious et al., 2007) was made in the laboratory. We used jalapeño peppers from a local grower. Jalapeño pepper extract represents home-grown insecticides that could be readily made and used as enhancers by small growers. Spinosad (Monterey Garden Insect Spray, Lawn and Garden Products, Inc., Fresno, CA) was chosen based on results from a previous field trial where one application in the field was enough to manage pests on collard greens in the fall of 2010. Thiamethoxam, (Actara®, Syngenta Crop Protection, Inc., Greensboro, NC) was chosen as a reference synthetic insecticide to the natural-based insecticides. It replaced our previously field-tested neonicotinoid, Provado® containing imidacloprid, due to its post-harvest interval. Thiamethoxam is selective, has a short re-entry interval and low environmental impact, and therefore it may be considered a synthetic biorational insecticide under the loose definition. Filtered water was obtained from a water fountain at NC A&T State University.

Figure 1. Insecticides used.

3.1.2 Preparation of crude jalapeño pepper extract. Pepper was used to make a crude insecticide in reference to the protocol developed by Antonious et al. (2007). Jalapeño peppers were obtained from the Farmer's garden, Pine Hall, NC, USA. For the pepper extraction, we added 20 g frozen (-15-0˚C) jalapeño pepper fruit, *Capsicum annuum*, (unknown variety) to 40 mL methanol in a blender. This mixture was blended for about 1 minute, and the resultant slurry was immediately filtered into a 100 mL flask using a Buchner funnel and a filter paper (Whatman no. 1, 90 mm). This filtrate formed the crude methanolic extract. The 100 mL flask containing the pepper extract was kept in a freezer (-15-0˚C) to preserve its efficacy. An extract was prepared and used for all the preliminary experiments, within a 19 day period. A second extract was used for all the mixture experiments, which covered 158 days. All extracts were stored in a freezer (-15-0^{o}C) until needed.

3.1.3 Source of collard green leaves. Collard greens were chosen as the crop model as they are preferred by DBM, damage is easy to observe and they are traditionally grown by small growers in NC (Figure 2). Collard green seedlings were purchased in Greensboro, NC, USA, from J & S Farms, an organic grower. The collard green variety was Morris Heading. These seedlings were transplanted at the teaching and research farm at NC A&T State University farm in 2010. The plants were managed by normal agronomic practices with one insect control using spinosad in October 2010. This one time spray was the only insecticide treatment applied to the plants. Spinosad has a very low persistence. The leaves were harvested into clear plastic bags in December 2010 and stored in a cold room (just above 0^{o}C) until when needed as insect food or leaf discs for bioassays.

Figure 2. Damage on crucifer attributed to diamondback moth larvae.

3.1.4 Insect rearing. Larvae of diamondback moths were collected between late September and October 2010, from collard greens fields at NC A&T State University teaching and research farm. A culture was then maintained in clear plastic containers measuring 42.4 cm by 27.9 cm by 27.4 cm. The top of the container was sealed with netting glued to the perimeter by a glue gun. A flap was cut out of the center of the netting to serve as an opening, and a small piece of paper was placed under the flap to keep adults from escaping. The bottom of the container was lined with paper which was replaced when necessary to prevent fungal growth. During cleaning larvae were transferred as they hung onto camel hair brushes into new containers. Larvae were fed collard green leaves. Adults (Figure 3) were kept in separate containers for oviposition and were fed approximately 10% sugar solution in cotton balls placed in a petri dish. The cotton balls were replaced when dry or dirty.

Figure 3. Adult diamondback moth.

3.2 Experimental Design

We carried out 7 experiments to test the various hypotheses developed for each objective. A total of 11 different insecticide combinations served as the experimental treatments (Figure 4).

1) Agroneem Plus®	2) Pepper	3) Spinosad	4) Thiamethoxam
5) Agroneem Plus® + Pepper (AP)	6) Agroneem Plus® + Spinosad® (AS)	7) Agroneem Plus® + Thiamethoxam (AT)	8) Pepper + Spinosad® (PS)
9) Pepper + Thiamethoxam (PT)	10) Spinosad® + Thiamethoxam (ST)	11) Control: Water	

Figure 4. Insecticide combinations and their corresponding abbreviations.

3.2.1 Efficacy of three commercial insecticides and a crude methanolic extract of jalapeño pepper (*Capsicum annuum)* against diamondback moth on collard greens (Morris Heading variety). We used different concentrations of the four test insecticides, namely Agroneem Plus®, crude pepper extract, spinosad and thiamethoxam. These experiments were to provide preliminary information and compare the recommended rates of each insecticide with a higher or lower concentration against DBM larvae on collard greens under laboratory conditions. Also, when insecticides are mixed in the field, they often become diluted. This initial data was used to identify and select a concentration of each insecticide that was used in mixture experiments in search of interactions.

Leaf dip bioassays similar to that described by Shelton et al. (1993) with the four insecticides were used in bioassays using DBM larvae. Leaf dip bioassays are better known, more sensitive and easier to replicate than leaf spray and topical application bioassays (Immaraju et al., 1990). It has been the most common procedure for assessing DBM resistance to commercial formulations including Bt (Tabashnik et al., 1990; Shelton et al., 1993). In all experiments second to fourth instar larvae were fed treated collard green leaf discs. First instar larvae were not used as they are tiny, often still inside the leaves and difficult to handle. Each leaf disc was 5 cm^2. The collard green leaf discs were dipped into a specific chemical or chemical combination at a specific concentration of the test insecticides. Each leaf disc was presented to 5 larvae in a Petri dish. Experiments were replicated three times and ended before the 3^{rd} day. A preliminary experiment using untreated leaf discs indicated that 3 days was the maximum duration for a $5cm^2$ leaf dic to be consumed by five 4^{th} instar larvae.

3.2.1.1 Experiment 1: Efficacy of insecticides at recommended and half recommended rates on leaf damage and mortality of diamondback moth larvae in a laboratory bioassay. Two different concentrations of each insecticide were used, the full and half the recommended concentrations. The recommended field application rates of the insecticides are: 15 mL Agroneem Plus®/L (15,000 ppm), 16 mL spinosad/L (16,000 ppm) and 3 g thiamethoxam /L (3,000 ppm). Using Antonious et al. (2007), we set the standard rate of jalapeno pepper extract at 250 mL/L (250,000 ppm). An aliquot of 10 mL of each insecticide at the recommended rate into which the leaves were dipped will thus contain 150 uL Agroneem Plus®, 2.5 mL jalapeño pepper, 160 uL spinosad or 30 mg thiamethoxam respectively. Half the recommended concentrations were as follows: 75 uL Agroneem Plus®, 1.25 mL jalapeño pepper, 80 uL spinosad and 15 mg thiamethoxam.

Leaf discs were dipped into the 10 mL solutions contained in 50 mL plastic vials and held for approximately 3-6 seconds with the discs completely immersed in the solutions. The leaves were taken out and allowed to drip back into the vial for up to 3 seconds. Then the leaves were placed onto filter paper (Whatman no. 1, 90 mm) in Petri dishes. Importantly, leaves were not dried before placing them on the filter paper. Therefore, leaves may have been at different levels of wetness. The filter paper was kept moist throughout the experiment. Five test larvae were then individually transferred from the insect colony containers as they hung onto camel hair brushes and introduced into each Petri dish. The Petri dishes were stacked together on a table in the laboratory, arranged by treatment and replication and monitored. Leaf area damage and mortality were recorded 24 hours after the introduction of larvae. Leaf area damage was estimated by visual observation and recorded in 5% increments. An insect was considered dead when it did not respond after external probing with a blunt object.

3.2.1.2 Experiment 2: Efficacy of insecticides at double recommended, recommended and quarter recommended rates on leaf damage and mortality of diamondback moth larvae in a laboratory bioassay. We used the following proportions of the recommended application rate: quarter, full and double. We used a bioassay-guided approach in selecting the dilutions. The quarter recommended rate gave the following: 37.5 uL Agroneem Plus®, 625 uL jalapeño pepper, 40 uL spinosad and 7.5 mg thiamethoxam. These concentrations for a 10 mL solution were: 150 uL Agroneem Plus®, 2.5 mL jalapeño pepper, 160 uL spinosad and 30 mg thiamethoxam. The, double recommended concentrations were: 300 uL Agroneem Plus®, 5.0 mL jalapeño pepper, 320 uL spinosad and 60 mg thiamethoxam. These treatments were fed to DBM larvae as before. Larvae were monitored and leaf consumption and mortality were recorded at 24, 42 and 67 hours.

3.2.2 Efficacy of selected mixtures of biorational insecticides against DBM larval feeding using collard greens as the crop model in laboratory bioassays.

3.2.2.1 Experiment 3: Efficacy of insecticide mixtures at the ratios of 2:1, 1:1 and 1:2 on leaf damage and mortality of diamondback moth larvae in a laboratory bioassay. Insecticides mixtures in different proportions were used in bioassays to identify possible interactions. Initially, three different ratios were used: 1:2, 1:1 and 2:1. For the 1:2 ratio mixtures, 3.3 mL of one chemical at its recommended rate were mixed with 6.7 mL of a second chemical at its recommended rate and also the reverse proportions to obtain the 2:1 mixtues. For the 1:1 ratio mixture, 5 mL of one chemical at its recommended rate was mixed with 5 mL of a second chemical at its recommended rate. These experiments were conducted using all six possible combinations, AP, AS, PS AT, PT and ST (abbreviations recall Figure 1). DBM larvae were exposed to these treatments as previously. The Petri dishes were stacked randomly and kept in a plastic container in the laboratory. Larvae were monitored and leaf consumption and mortality were recorded at 24 and 62 hours.

3.2.2.2 Experiment 4: Efficacy of insecticide mixtures at the ratios of 4:1 and 1:4 on leaf damage and mortality of diamondback moth larvae in a laboratory bioassay. The six chemical combinations described in 3.2.2.1 were screened to four, namely AP, PS, AT and ST, based on their performance to this point. Additionally, these mixtures represented all four insecticides equally. These four mixtures underwent additional testing using other ratios including 1:4 and 4:1. For the 1:4 ratio mixtures, 2 mL of one chemical were mixed with 8 mL of the second chemical and vice versa for the 4:1 ratio. These treatments were fed to DBM larvae as before. Larvae were monitored, and leaf consumption and mortality were recorded at 24 and 67 hours.

3.2.2.3 Experiment 5: Efficacy of insecticide mixtures at the ratios of 8:1 and 1:8 on leaf damage and mortality of diamondback moth larvae in a laboratory bioassay. The four selected combinations were further diluted using two additional ratios: 1:8 and 8:1. For the 1:8 ratio mixtures, 1.1 mL of one chemical were mixed with 8.9 mL of the second chemical and vice versa for the 8:1 ratio. These treatments were fed to DBM larvae the same way as before. Larvae were monitored and leaf consumption and mortality were recorded at 24 and 67 hours.

3.2.3 Ovicidal activity and oviposition deterrence of the most effective mixture (from 3.2.2).

3.2.3.1 Experiment 6: Efficacy of insecticides at recommended rates on ovicidal activity and oviposition deterrence in a laboratory bioassay. An experiment using adult DBM was conducted to determine oviposition deterrence and ovicidal activity. The experiment was done using 500 mL clear plastic cups with perforated lids for ventilation. Collard green leaves were used as the substrate for oviposition. The leaves were placed in 50 mL plasic vials containing 10 mL of the test insecticides and gently swirled by hand for approximately 6 seconds. The swirling was done to ensure the leaves were thoroughly covered, as they were too large to be immersed. One leaf was placed into each ventilated plastic cup. These cups with the treated leaves were then allowed to dry in cold storage overnight. Five unsexed adult DBM were introduced into each cup. Adults were monitored, and deterrent effects and mortality were recorded at 24 hours as most eggs were expected to be laid by that time (Harcourt 1960). Larva emergence was monitored and recorded at 5 and 10 days.

3.2.3.2 Experiment 7: Efficacy of the most effective insecticide mixtures at the ratio of 1:1 on ovicidal activity and oviposition deterrence in a laboratory bioassay. A choice test was conducted to determine oviposition deterrence and ovicidal effects. The choice test involved 10 mL filtered water as a control, 160 uL spinosad/10 mL, 80 uL spinosad/5 mL in combination with 1.25 mL pepper/5 mL, and 80 uL spinosad/5 mL in combination with 15 mg thiamethoxam/5 mL. These four treatments were each applied to mature collard green leaves approximately the same age and cut to nearly the same size. The leaf petioles were wrapped in wet paper towels, placed in open plastic cups and labeled. Then four treated collard green leaves were all caged with newly emerged DBM adults.

3.3 Data Analysis

Data were analyzed using analysis of variance (ANOVA) (SAS 9.2, SAS Institute, 2002). Values were considered significantly different if 5% or less overlap occurred. Comparisons between values were made by Duncan's Multiple Range test (DMR). Abbott's Formula was used to correct mortality means (Abbott, 1925).

CHAPTER 4

Results and Discussion

4.1 Results

4.1.1 Efficacy of three commercial insecticides and a crude methanolic extract of jalapeño pepper (*Capsicum annuum)* against diamondback moth on collard greens (Morris Heading variety). The tested insecticides performed equally at either the recommended (full) or half the recommended rate in preventing damage by DBM larvae (Table 1). At both rates, spinosad and thiamethoxam gave significantly better protection than Agroneem Plus®, pepper or the control. Agroneem Plus® and pepper were however not significantly different from the control in preventing damage by DBM. These application rates did not show a clear or distinct superiority in any of the insecticide treatments, hence we could not screen them using only these two rates. We subsequently tested a double and a quarter recommended rate.

Table 1.

Effect of insecticides at recommended and half recommended rates on leaf damage and mortality of diamondback moth larvae at 24 hours in a laboratory bioassay.

	% Leaf area damage*		Mortality / 5 larvae*	
Insecticide	**(1x) Rec**	**(½x) Rec**	**(1x) Rec**	**(½x) Rec**
Agroneem Plus®	20.0^{bc}	28.3^{ab}	1.3^{cd}	2.3^{bcd}
Pepper	33.3^{a}	30.0^{ab}	1.7^{bcd}	1.0^{d}
Spinosad	5.0^{d}	6.7^{d}	5.0^{a}	4.7^{a}
Thiamethoxam	6.7^{d}	10.0^{cd}	3.7^{ab}	3.3^{abc}
Control	21.7^{abc}		1.0^{d}	

* = When comparing insecticide ratios within an insecticide type, or when comparing insecticide types within an insecticide ratio, means having the same letter in common are not significantly different at the 5% level of probability as indicated by Duncan's Multiple Range test.

The double recommended (2x) rates were not significantly different from the recommended (1x) or quarter (¼x) recommended rates in controlling DBM larvae (Table 2). An exception was the mortality of 1.7 caused by thiamethoxam(¼x), which was significantly lower than mortalities of 2.7-3.3 at the other rates. Thiamethoxam is a systemic neonicotinoid and from personal communication has minimal effect at field conditions on the chewing caterpillars on crucifers. Spinosad was very effective at all rates, rendering all larvae incapacitated within 24 hours. Thiamethoxam like spinosad acted as a good anitfeedant, but was significantly less effective than spinosad in causing larva mortality after 24 hours. Generally, Agroneem Plus® and pepper were as ineffective as the control in preventing leaf damage and causing larval mortality.

Table 2.

Effect of insecticides at double recommended, recommended and quarter recommended rates on leaf damage and mortality of diamondback moth larvae at 24 hours in a laboratory bioassay.

	% Leaf area damage*			**Mortality / 5 larvae***		
Insecticide	**(2x) Rec**	**(1x) Rec**	**(¼x) Rec**	**(2x) Rec**	**(1x) Rec**	**(¼x) Rec**
Agroneem®	8.3[cd]	11.7[bcd]	18.3[abc]	0[e]	0[e]	0[e]
Pepper	10.0[bcd]	20.0[abc]	21.7[ab]	1.0[de]	0[e]	0[e]
Spinosad	0[d]	0[d]	0[d]	5.0[a]	5.0[a]	5.0[a]
Thiamethoxam	0[d]	0[d]	5.0[d]	2.7[bc]	3.3[b]	1.7[cd]
Control		28.3[a]			0[e]	

* = When comparing insecticide ratios within an insecticide type, or when comparing insecticide types within an insecticide ratio, means having the same letter in common are not significantly different at the 5% level of probability as indicated by Duncan's Multiple Range test.

Treatments of Agroneem Plus® and pepper at double the recommended rates were more effective in reducing damage to leaves and only marginally effective in killing the larvae (Table 3). Other treatments continued to be as effective for both variables as they were at 24 hours. Agroneem Plus® at all the tested concentrations had significantly lower leaf damage than the control at 42 hours. Mortality of larvae due to the spinosad treatment was constant after 24 hours as all larvae were incapacitated or killed by then.

Table 3.

Effect of insecticides at double recommended, recommended and quarter recommended rates on leaf damage and mortality of diamondback moth larvae at 42 hours in a laboratory bioassay.

	% Leaf area damage*			Mortality / 5 larvae*		
Insecticide	**(2x) Rec**	**(1x) Rec**	**(¼x) Rec**	**(2x) Rec**	**(1x) Rec**	**(¼x) Rec**
Agroneem®	10.0[cd]	16.7[cd]	28.3[bc]	0.7[bc]	0.3[bc]	0[c]
Pepper	16.7[cd]	46.7[ab]	55.0[a]	1.3[bc]	0[c]	0[c]
Spinosad	0[d]	0[d]	0[d]	5.0[a]	5.0[a]	5.0[a]
Thiamethoxam	0[d]	0[d]	11.7[cd]	4.0[a]	4.0[a]	1.7[b]
Control		60.0[a]			0[c]	

* = When comparing insecticide ratios within an insecticide type, or when comparing insecticide types within an insecticide ratio, means having the same letter in common are not significantly different at the 5% level of probability as indicated by Duncan's Multiple Range test.

Agroneem Plus® was only slightly more effective at the double recommended rate than both the recommended and half the recommended rates (Table 4). Spinosad was effective in all the concentrations tested. Thiamethoxam was similarly effective like spinosad in most cases. Doubling the concentration of thiamethoxam only resulted in a negligible increase in mortality, whereas the 25% dilution of the recommended rate had a significant reduction in mortality which was in contrast to a treatment like spinosad. While the quarter recommended rate of pepper performed better than the recommended rate, it was an insignificant change.

Table 4.

Effect of insecticides at double recommended, recommended and quarter recommended rates on leaf damage and mortality of diamondback moth larvae at 67 hours in a laboratory bioassay.

	% Leaf area damage*			Mortality / 5 larvae*		
Insecticide	**(2x) Rec**	**(1x) Rec**	**(¼x) Rec**	**(2x) Rec**	**(1x) Rec**	**(¼x) Rec**
Agroneem®	10.0[bc]	23.3[bc]	36.7[b]	1.3[bc]	1.3[bc]	0.3[c]
Pepper	23.3[bc]	76.7[a]	66.7[a]	2.0[bc]	0.3[c]	0.7[c]
Spinosad	0[c]	0[c]	0[c]	5.0[a]	5.0[a]	5.0[a]
Thiamethoxam	0[c]	0[c]	20.0[bc]	5.0[a]	4.7[a]	2.7[b]
Control		80.0[a]			0.3[c]	

* = When comparing insecticide ratios within an insecticide type, or when comparing insecticide types within an insecticide ratio, means having the same letter in common are not significantly different at the 5% level of probability as indicated by Duncan's Multiple Range test.

Pepper was most effective at double the recommended rate. However, it still underperformed when compared to the commercial insecticides. The amount of pepper needed to achieve that 50% solution would be economically prohibitive. Furthermore, the double recommended rates for each insecticide did not improve their crop protection properties enough (Tables 2-4) to offset the cost and environmental risk. On the other hand, using the quarter recommended rates (Tables 2-4) may lead to swifter development of resistance by DBM as is the case with sub-lethal concentrations of most insecticides. Therefore, the recommended rate for each insecticide was chosen for use in subsequent experiments. Additionally, this would allow uniformity in the comparison of these different products based on the manufacturer-tested recommendations.

4.1.2 Efficacy of selected mixtures of biorational insecticides against DBM larval feeding using collard greens as the crop model in laboratory bioassays.

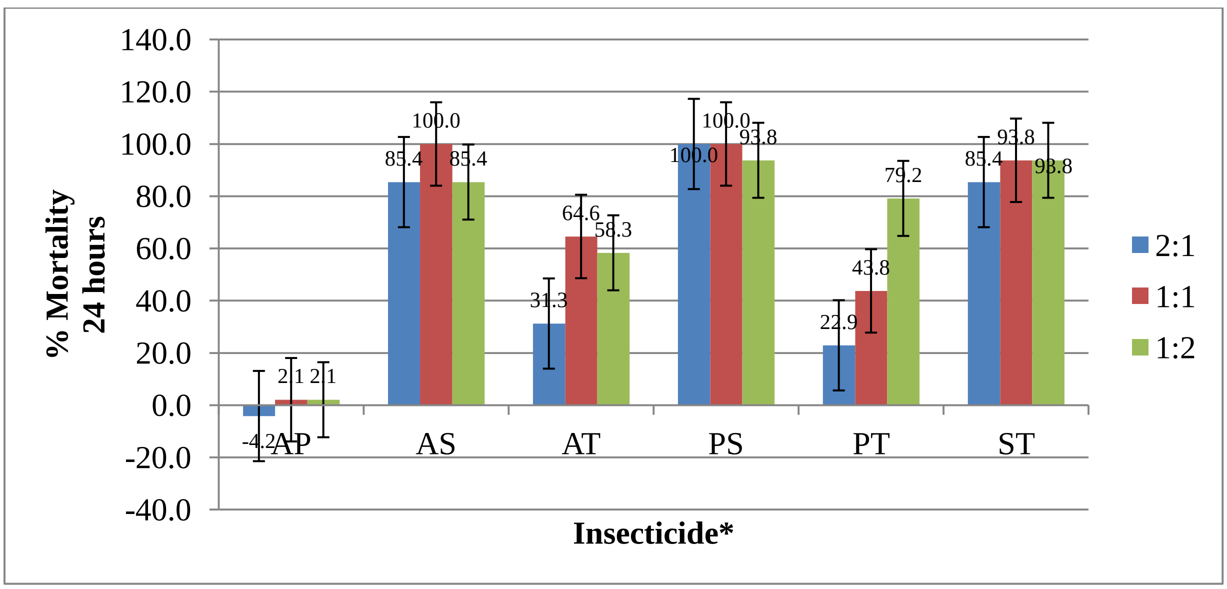
Spinosad performed extremely well in all mixtures or in any of the proportions for the two variables studied (Table 5, Figure 5). Thiamethoxam in 1:2 ratio combinations were comparable to spinosad, except for the AT(1:2) treatment where mortality was 3.0 while the least for any spinosad combination was 4.3. In 2:1 and 1:1 ratios, thiamethoxam had significantly lower mortality than spinosad when in combination with Agroneem Plus® and pepper. The AP combination was not different, in causing mortalities, from the control. The AP(1:2) even had 50% leaf damage compared to 21.1% in the control at 24 hours.

Table 5.

Effect of insecticide mixtures at the ratios of 2:1, 1:1 and 1:2 on leaf damage and mortality of diamondback moth larvae at 24 hours in a laboratory bioassay.

	% Leaf area damage**			**Mortality / 5 larvae****		
Insecticide*	**2:1**	**1:1**	**1:2**	**2:1**	**1:1**	**1:2**
AP	21.7^{b}	25.0^{b}	50.0^{a}	0^{g}	0.3^{fg}	0.3^{fg}
AS	0^{c}	0^{c}	0^{c}	4.3^{abc}	5.0^{a}	4.3^{abc}
AT	8.3^{c}	1.7^{c}	0^{c}	1.7^{e}	3.3^{bcd}	3.0^{cd}
PS	0^{c}	1.7^{c}	1.7^{c}	5.0^{a}	5.0^{a}	4.7^{ab}
PT	8.3^{c}	1.7^{c}	0^{c}	1.3^{ef}	2.3^{de}	4.0^{abc}
ST	0^{c}	1.7^{c}	0^{c}	4.3^{abc}	4.7^{ab}	4.7^{ab}
Control		21.1^{b}			0.2^{fg}	

* = AP – Agroneem Plus® and Pepper, AS – Agroneem Plus® and Spinosad, AT – Agroneem Plus® and Thiamethoxam, PS – Pepper and Spinosad, PT – Pepper and Thiamethoxam, ST – Spinosad and Thiamethoxam.
** = When comparing insecticide ratios within an insecticide type, or when comparing insecticide types within an insecticide ratio, means having the same letter in common are not significantly different at the 5% level of probability as indicated by Duncan's Multiple Range test.



* = AP – Agroneem Plus® and Pepper, AS – Agroneem Plus® and Spinosad, AT – Agroneem Plus® and Thiamethoxam, PS – Pepper and Spinosad, PT – Pepper and Thiamethoxam, ST – Spinosad and Thiamethoxam.

Figure 5. Effect of insecticide mixtures at the ratios of 2:1, 1:1 and 1:2 on mortality of diamondback moth larvae at 24 hours in a laboratory bioassay, corrected using Abbott's correction for mortality.

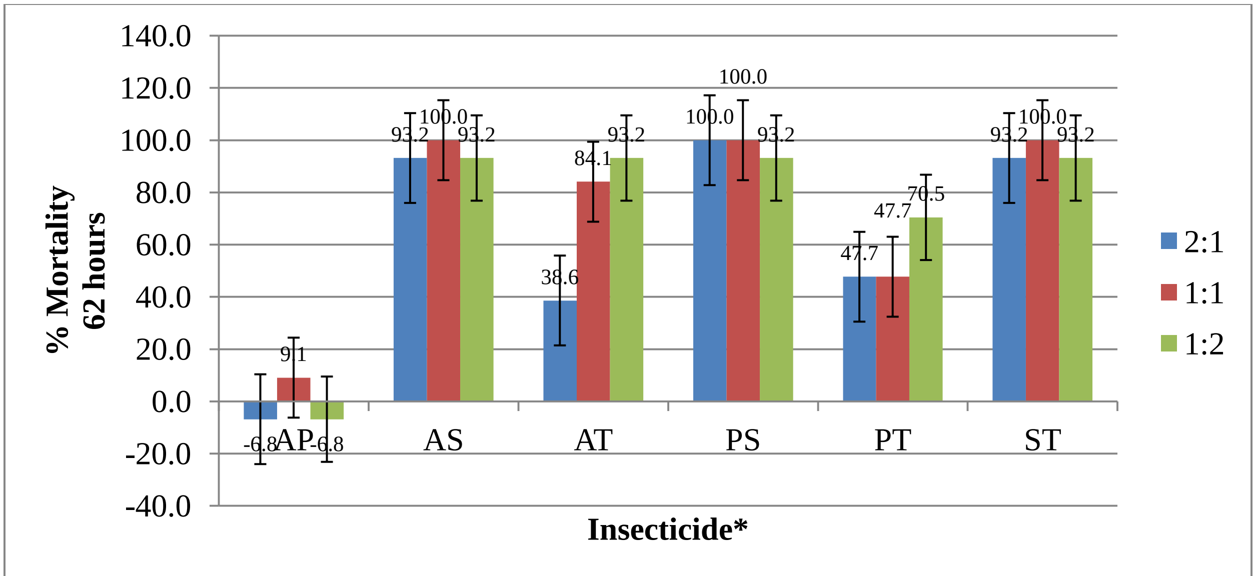
Spinosad caused a reduction in leaf damage and subsequent increased mortalities when combined with any of the tested insecticides at 62 hours exposure. This suggests that spinosad does not interact antagonistically with the other insecticides. The 2:1 and 1:1 thiamethoxam combinations improved the protection of collard leaves against DBM damage. The 1:1 and 1:2 AT combinations resulted in 1.7% leaf damage for both and 4.3 and 4.7 larvae mortality, respectively, which are equivalent to the results obtained from the spinosad combinations (Table 6, Figure 6). The AP combinations at 62 hours, as at 24 hours, offered the lowest protection, which was similar to the control treatment except for leaf damage at the 2:1 and 1:1 ratios.

Table 6.

Effect of insecticide mixtures at the ratios of 2:1, 1:1 and 1:2 on leaf damage and mortality of diamondback moth larvae at 62 hours in a laboratory bioassay.

	% Leaf area damage**			**Mortality / 5 larvae****		
Insecticide*	**2:1**	**1:1**	**1:2**	**2:1**	**1:1**	**1:2**
AP	28.3^{bc}	31.7^{b}	63.3^{a}	0.3^{e}	1.0^{e}	0.3^{e}
AS	0^{d}	0^{d}	0^{d}	4.7^{ab}	5.0^{a}	4.7^{ab}
AT	10.0^{bcd}	1.7^{d}	1.7^{d}	2.3^{d}	4.3^{ab}	4.7^{ab}
PS	0^{d}	1.7^{d}	1.7^{d}	5.0^{a}	5.0^{a}	4.7^{ab}
PT	15.0^{bcd}	8.3^{cd}	1.7^{d}	2.7^{cd}	2.7^{cd}	3.7^{bc}
ST	0^{d}	1.7^{d}	0^{d}	4.7^{ab}	5.0^{a}	4.7^{ab}
Control		68.3^{a}			0.6^{e}	

* = AP – Agroneem Plus® and Pepper, AS – Agroneem Plus® and Spinosad, AT – Agroneem Plus® and Thiamethoxam, PS – Pepper and Spinosad, PT – Pepper and Thiamethoxam, ST – Spinosad and Thiamethoxam.
** = When comparing insecticide ratios within an insecticide type, or when comparing insecticide types within an insecticide ratio, means having the same letter in common are not significantly different at the 5% level of probability as indicated by Duncan's Multiple Range test.



* = AP – Agroneem Plus® and Pepper, AS – Agroneem Plus® and Spinosad, AT – Agroneem Plus® and Thiamethoxam, PS – Pepper and Spinosad, PT – Pepper and Thiamethoxam, ST – Spinosad and Thiamethoxam.

Figure 6. Effect of insecticide mixtures at the ratios of 2:1, 1:1 and 1:2 on mortality of diamondback moth larvae at 62 hours in a laboratory bioassay, corrected using Abbott's correction for mortality.

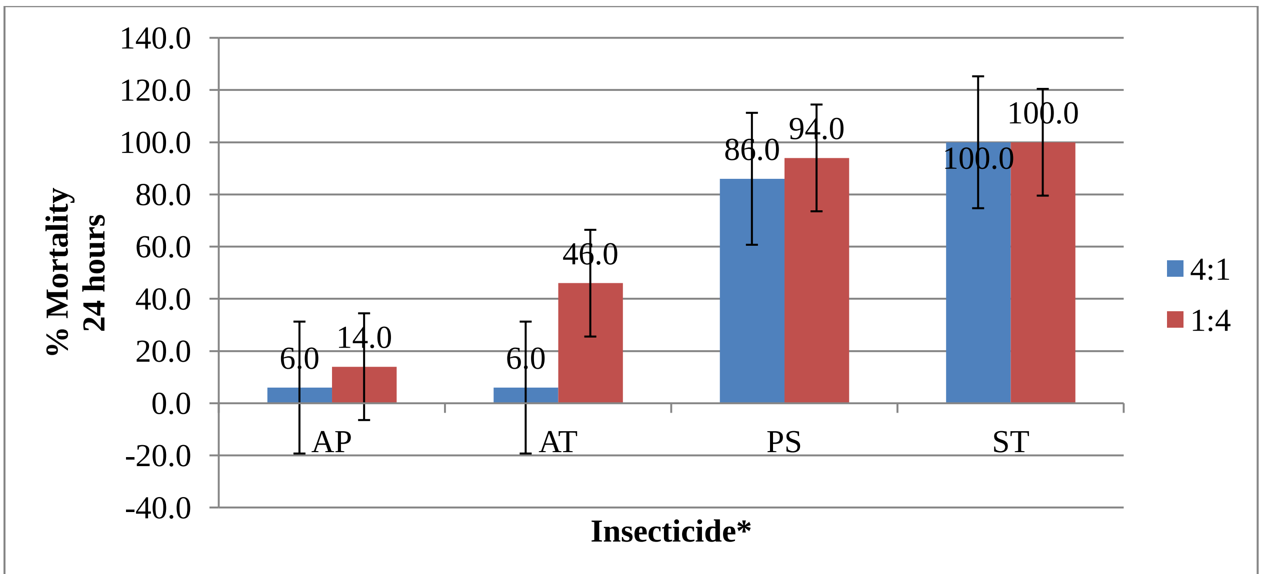
At the ratios 4:1 or 1:4 spinosad combinations resulted in the highest mortalities and reduction in leaf damage (Table 7, Figure 7). The mixture ST performed slightly better than PS. The AT mixture was statistically similar to the spinosad combinations in leaf damage, with only 0-5% damage. The AT combinations however caused significantly lower mortality when compared to the spinosad treatments. AP as in other ratios was the least potent in causing larva deaths or reducing leaf consumption, which was no different than the control. The combinations AP(4:1) and AT(4:1) resulted in equal mortalities which were not significantly different from the control. Therefore, Agroneem Plus® may interact antagonistically with thiamethoxam at this ratio.

Table 7.

Effect of insecticide mixtures at the ratios of 4:1 and 1:4 on leaf damage and mortality of diamondback moth larvae at 24 hours in a laboratory bioassay.

	% Leaf area damage**		Mortality / 5 larvae**	
Insecticide*	**4:1**	**1:4**	**4:1**	**1:4**
AP	33.3^{b}	46.7^{a}	0.3^{c}	0.7^{c}
AT	5.0^{c}	0^{c}	0.3^{c}	2.3^{b}
PS	0^{c}	0^{c}	4.3^{a}	4.7^{a}
ST	0^{c}	0^{c}	5.0^{a}	5.0^{a}
Control	43.3^{ab}		0^{c}	

* = AP – Agroneem Plus® and Pepper, AT – Agroneem Plus® and Thiamethoxam, PS – Pepper and Spinosad, ST – Spinosad and Thiamethoxam.
** = When comparing insecticide ratios within an insecticide type, or when comparing insecticide types within an insecticide ratio, means having the same letter in common are not significantly different at the 5% level of probability as indicated by Duncan's Multiple Range test.



* = AP – Agroneem Plus® and Pepper, AT – Agroneem Plus® and Thiamethoxam, PS – Pepper and Spinosad, ST – Spinosad and Thiamethoxam.

Figure 7. Effect of insecticide mixtures at the ratios of 4:1 and 1:4 on mortality of diamondback moth larvae at 24 hours in a laboratory bioassay, corrected using Abbott's correction for mortality.

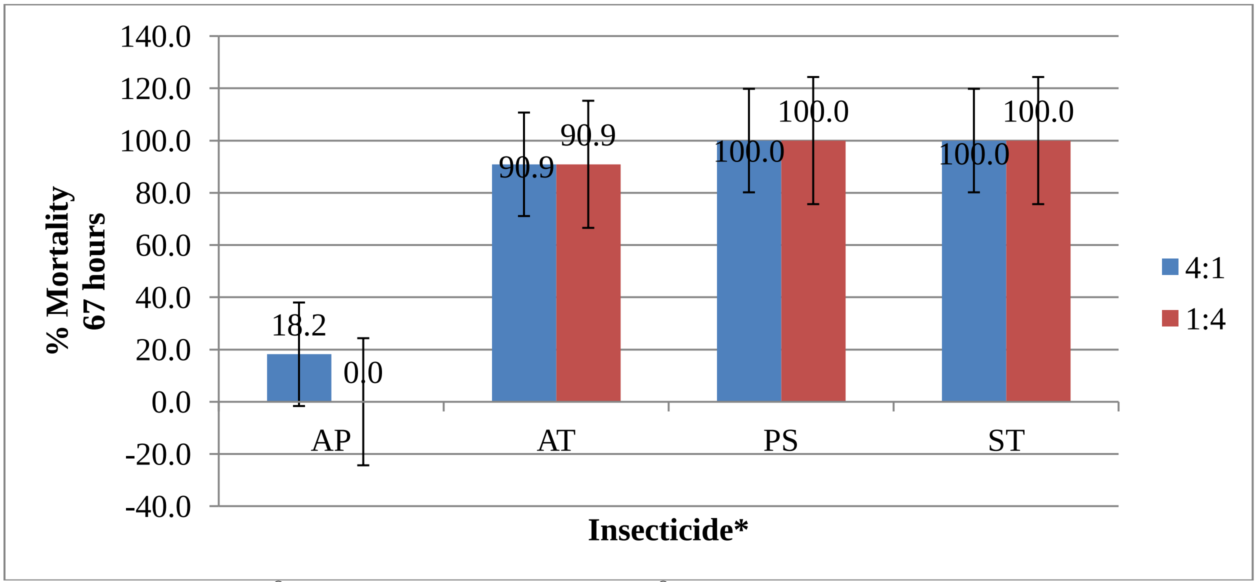
All the spinosad combination treatments reached the maximum effectiveness after 67 hours. The AT mixture caused mortalities and leaf damage similar to the spinosad combinations at the 67 hour (Table 8, Figure 8). The AP mixture resulted in mortality similar to the control treatment, but the consumption of leaves treated with AP (38-60%) was lower than the control (95%).

Table 8.

Effect of insecticide mixtures at the ratios of 4:1 and 1:4 on leaf damage and mortality of diamondback moth larvae at 67 hours in a laboratory bioassay.

	% Leaf area damage**		**Mortality / 5 larvae****	
Insecticide*	**4:1**	**1:4**	**4:1**	**1:4**
AP	38.3[c]	60.0[b]	2.3[b]	1.7[b]
AT	6.7[d]	0[d]	4.7[a]	4.7[a]
PS	0[d]	0[d]	5.0[a]	5.0[a]
ST	0[d]	0[d]	5.0[a]	5.0[a]
Control	95.0[a]		1.7[b]	

* = AP – Agroneem Plus® and Pepper, AT – Agroneem Plus® and Thiamethoxam, PS – Pepper and Spinosad, ST – Spinosad and Thiamethoxam.
** = When comparing insecticide ratios within an insecticide type, or when comparing insecticide types within an insecticide ratio, means having the same letter in common are not significantly different at the 5% level of probability as indicated by Duncan's Multiple Range test.



* = AP – Agroneem Plus® and Pepper, AT – Agroneem Plus® and Thiamethoxam, PS – Pepper and Spinosad, ST – Spinosad and Thiamethoxam.

Figure 8. Effect of insecticide mixtures at the ratios of 4:1 and 1:4 on mortality of diamondback moth larvae at 67 hours in a laboratory bioassay, corrected using Abbott's correction for mortality.

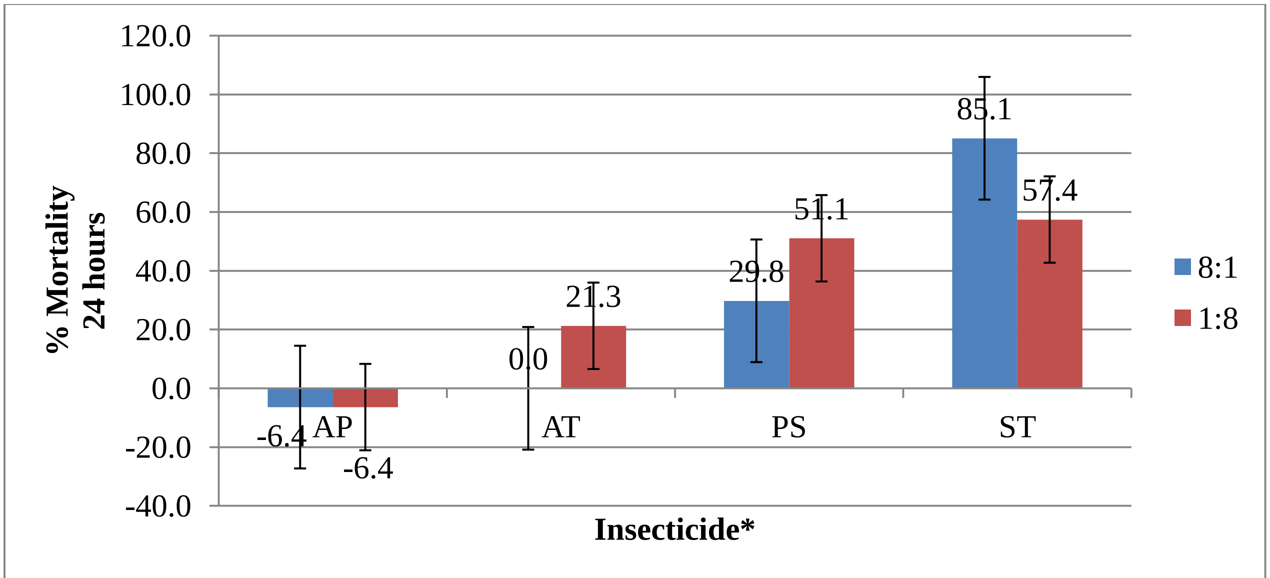
A significant insecticide x ratio interaction was found in the analysis of variance for leaf damage, but not for mortality (Table 9, Figure 9). Leaf damage differed between insecticide ratios for AP and AT but not for PS and ST. Relative to the control, all treatment combinations led to a reduction in leaf damage except AP(8:1). The most effective treatments were AT(1:8) and PS and ST at both the 8:1 and 1:8 ratios. Mortality trends were somewhat similar to those obtained with the leaf damage data. Treatments with the highest mortality relative to the control were PS at the 1:8 ratio and ST at both the 8:1 and 1:8 ratios. Based on the leaf damage and mortality results, these three treatment combinations seem to optimize damage control. The mortality rate of PS was significantly lower than ST at an 8:1 ratio. This proportion could be indicative of the limit of effectiveness or the sub-lethal concentration of spinosad. Notably, AP and AT at 8:1 ratios continued to demonstrate similar mortality, just as they had at 4:1 ratios. This lack of protection from a thiamethoxam combination offers further support to an antagonistic interaction occurring between Agroneem Plus® and thiamethoxam.

Table 9.

Effect of insecticide mixtures at the ratios of 8:1 and 1:8 on leaf damage and mortality of diamondback moth larvae after 24 hours in a laboratory bioassay.

	% Leaf area damage**		**Mortality / 5 larvae****	
Insecticide*	**8:1**	**1:8**	**8:1**	**1:8**
AP	68.3^{a}	43.3^{b}	0^{d}	0^{d}
AT	40.0^{b}	1.7^{c}	0.3^{d}	1.3^{cd}
PS	1.7^{c}	1.7^{c}	1.7^{bcd}	2.7^{bc}
ST	0^{c}	0^{c}	4.3^{a}	3.0^{ab}
Control	75.0^{a}		0.3^{d}	

* = AP – Agroneem Plus® and Pepper, AT – Agroneem Plus® and Thiamethoxam, PS – Pepper and Spinosad, ST – Spinosad and Thiamethoxam.
** = When comparing insecticide ratios within an insecticide type, or when comparing insecticide types within an insecticide ratio, means having the same letter in common are not significantly different at the 5% level of probability as indicated by Duncan’s Multiple Range test.



* = AP – Agroneem Plus® and Pepper, AT – Agroneem Plus® and Thiamethoxam, PS – Pepper and Spinosad, ST – Spinosad and Thiamethoxam.

Figure 9. Effect of insecticide mixtures at the ratios of 8:1 and 1:8 on mortality of diamondback moth larvae at 24 hours in a laboratory bioassay, corrected using Abbott's correction for mortality.

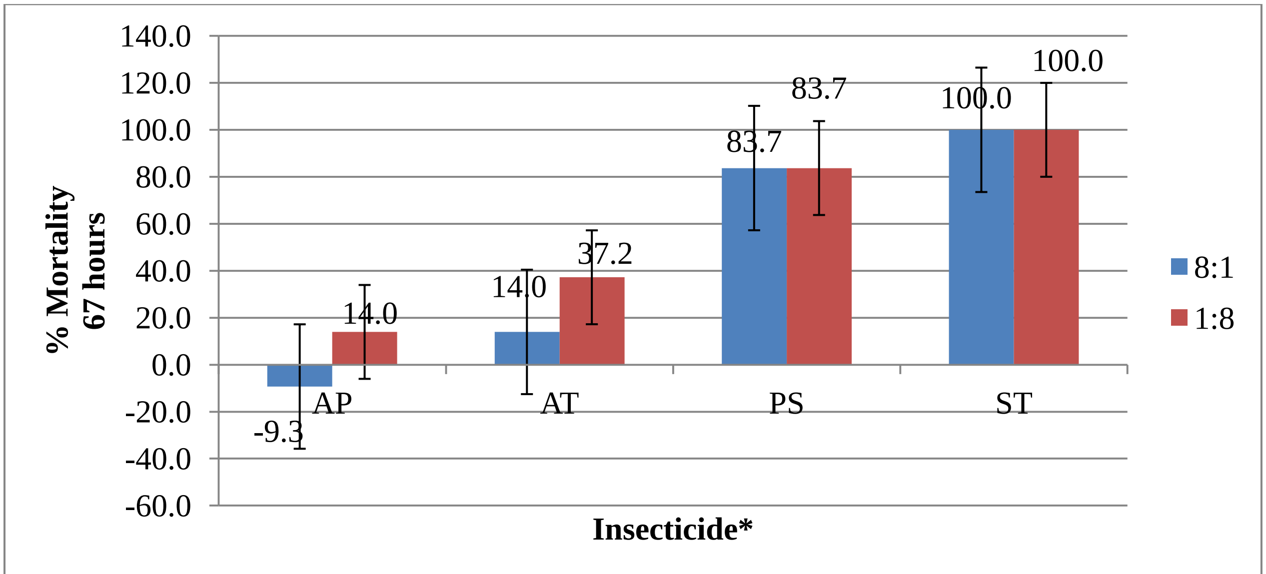
Analysis of variance found a significant insecticide x ratio interaction for leaf damage, but once again not for mortality (Table 10, Figure 10). For leaf damage, a ratio effect existed only for AT, while AP, PS and ST showed no ratio effect. AP insecticide at both 8:1 and 1:8 ratios resulted in the same leaf damage as the control. As previously, all other insecticides differed significantly from the control for leaf damage. AP and AT combinations performed similar to the control for mortality, as they had at 24 hours. The AT and AP combinations had similar mortalities at the 8:1 and 1:8 ratios respectively. PS and ST at both 8:1 and 1:8 ratios outperformed the control. Thus, the lethality of PS is the same as ST after 67 hours.

Table 10.

Effect of insecticide mixtures at the ratios of 8:1 and 1:8 on leaf damage and mortality of diamondback moth larvae at 67 hours in a laboratory bioassay.

	% Leaf area damage**		**Mortality / 5 larvae****	
Insecticide*	**8:1**	**1:8**	**8:1**	**1:8**
AP	76.7^{a}	83.3^{a}	0.3^{c}	1.3^{bc}
AT	43.3^{b}	1.7^{c}	1.3^{bc}	2.3^{b}
PS	1.7^{c}	3.3^{c}	4.3^{a}	4.3^{a}
ST	0^{c}	0^{c}	5.0^{a}	5.0^{a}
Control	96.7^{a}		0.7^{bc}	

* = AP – Agroneem Plus® and Pepper, AT – Agroneem Plus® and Thiamethoxam, PS – Pepper and Spinosad, ST – Spinosad and Thiamethoxam.
** = When comparing insecticide ratios within an insecticide type, or when comparing insecticide types within an insecticide ratio, means having the same letter in common are not significantly different at the 5% level of probability as indicated by Duncan's Multiple Range test.



* = AP – Agroneem Plus® and Pepper, AT – Agroneem Plus® and Thiamethoxam, PS – Pepper and Spinosad, ST – Spinosad and Thiamethoxam.

Figure 10. Effect of insecticide mixtures at the ratios of 8:1 and 1:8 on mortality of diamondback moth larvae at 67 hours in a laboratory bioassay, corrected using Abbott's correction for mortality.

4.1.3 Ovicidal activity and oviposition deterrence of the most effective mixture (from 4.1.2). In Experiment 6, the error was too high for the emergence data to be of value. Very few eggs were found, and even fewer lavae emerged from either the control or treated leaves.

Possible sources of error include the handling of the DBM adults, the confined space inside the 500 mL cups and mold growth on the collard green leaves. However, a significantly higher number of adults died after 24 hours of exposure to spinosad than the other insecticides at recommended rates. Choice tests using DBM adults to measure oviposition deterrence in subsequent experiments were not plausible as the highly mobile adults got in contact with all the treatments and died.

4.2 Discussion

Spinosad and the spinosad mixtures outperformed all other insecticides and mixtures. Spinosad at the recommended rate killed 100% of the exposed larvae (Table 2). Jalapeño pepper extract and spinosad mixture (PS) performed the same as spinosad and thiamethoxam mixture (ST) in causing mortality and reducing leaf damage. The ST mixture works faster than PS. These mixtures were comparatively similar to the sole use of spinosad. Since spinosad killed 100% in the bioassays, synergy cannot be explained where a mixture would have to cause greater than 100% mortality. Thus, it is unclear whether synergy exists in any of the spinosad mixtures. However, the results indicate that no antagonistic interactions occur between spinosad and other insecticides. The greater complexity of these mixtures compared to spinosad by itself may cause a delay in insect resistance (Wirth et al., 2004). Therefore, further evaluations of spinosad mixtures would be needed in order to determine their ability to delay resistance in diamondback moth populations. If the spinosad mixtures delay resistance longer than when spinosad is used on its own, we may then use resistance slowing as a parameter instead of mortality/damage as an indicator of synergy between spinosad and the other insecticides.

On the other hand, the Agroneem Plus® and thiamethoxam mixtures (AT) demonstrated the full spectrum of defined interactions, synergistic, additive and antagonistic (Working Group on Synergy in Complex Mixtures, 1986; Ahmad, 2004). AT(4:1) showed possible synergy as it was more effective than either thiamethoxam at ¼x the recommended rate or Agroneem Plus® at 2x the recommended rate (Tables 2-4, 7-8). Meanwhile, AT(1:8) gave an additive interaction as its results were better than Agroneem Plus® at the aforementioned rate but worse than thiamethoxam at the aforementioned rate. Furthermore, AT(8:1) displayed an antagonistic interaction as leaf damage was higher than either insecticide by itself at any rate coupled with a low mortality (Tables 2-4, 9-10). These interactions however need further clarification. The mixture of Agroneem Plus® and jalapeño pepper (AP) performed poorly at each of the ratios tested (Figure 11).

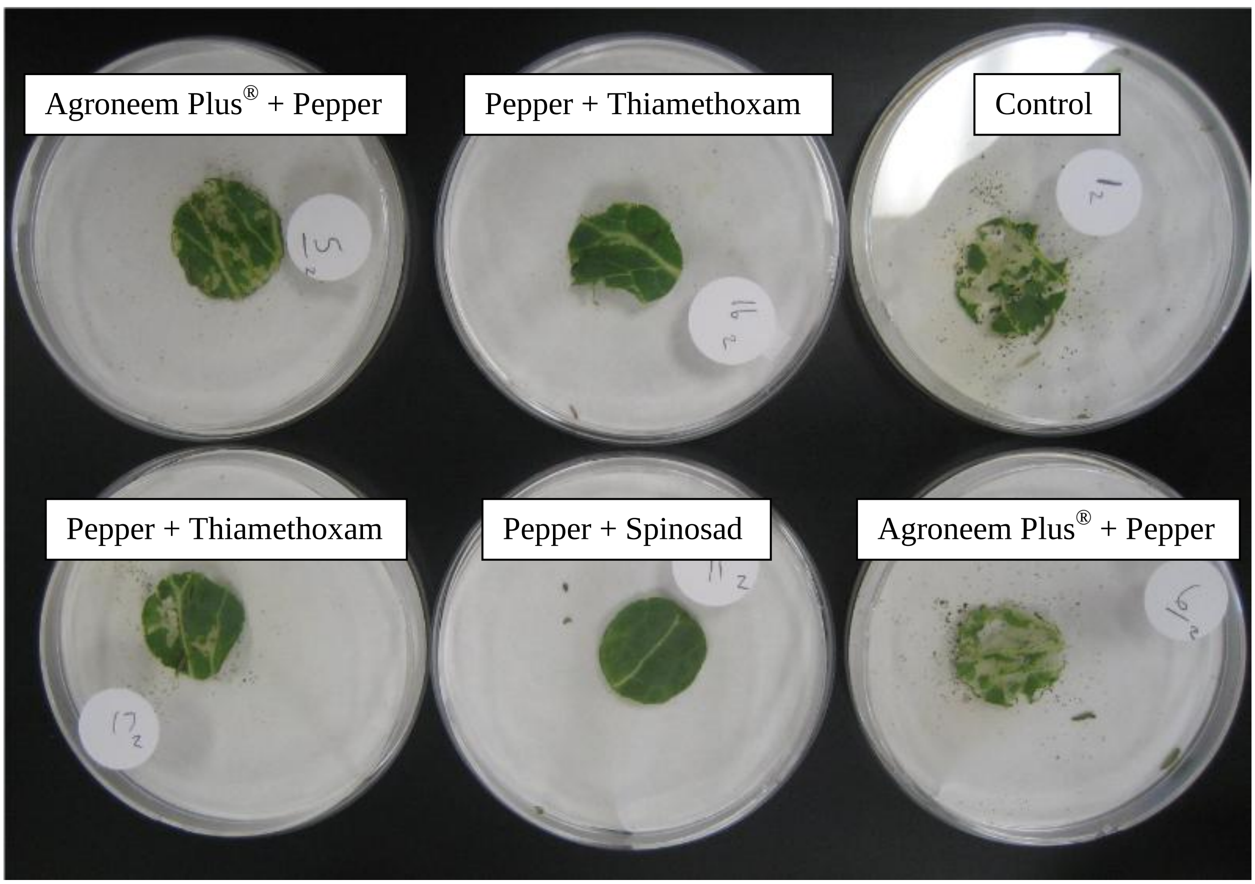


Figure 11. Composite leaf damage images of representative treatments.

CHAPTER 5

Conclusion

Synergistic mixtures have the potential be an economic and environmental boon by protecting crops better while reducing the number of sprays. Reducing the number of sprays reduces costs of insecticides and labor and the chemical residues in the environment. On the other hand, antagonistic mixtures would have the opposite effect. Antagonistic chemicals would have an unnecessary cost and residue impact on the environment. Further study must be carried out to discover what combinations and ratios have the greatest synergy. Research must be done to discern what mechanisms cause the synergy and antagonism at specific combinations and ratios for these insecticides as well. The environmental impact of these synergistic combinations must also be observed before they can be recommended for use. Toxicological data must be developed for these mixtures just as they are for their individual components. Moreover, the synergistic combinations of biorational insecticides must still work in conjunction with natural enemies and other integrated pest management strategies (Ahmad, 2004).

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