The Pennsylvania State University

The Graduate School

Department of Entomology

PLANT DEFENSE ELICITORS AS SEED TREATMENT AGAINST INSECTS AND IMPLICATIONS FOR SMALLHOLDER FARMERS.

A Thesis in

Entomology

by

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Submitted in Partial Fulfillment
of the Requirements
for the Degree of

Master of Science

December, 2013

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ABSTRACT

Seeds may be receptive to plant defense activators such as β-amino butyric acid (BABA) and jasmonic acid (JA), conferring protection to the subsequent plant against a wide spectrum of plant pathogens and insects. We examined the independent and interactive effects of methyl jasmonate (MeJA) seed treatment on tomato fruit worm (*Helicoverpa zea*) larval growth and the activity of the defensive protein, polyphenol oxidase (PPO), in leaves of tomato plants at three different plant stages. Additionally, we measured the dosage effects of MeJA seed treatment on several vegetative and reproductive traits. To assess the effectiveness against different insects, we tested MeJA seed treatment against the tomato leaf miner (*Tuta absoluta*) and tobacco caterpillar (*Spodoptera litura*), in Brazil and Bangladesh, respectively. Results suggest that seed treatment with defense elicitors will induce defenses in plants, which is correlated with increased PPO activity in leaves and reduction in larval growth. However, fitness costs in plants were observed with higher dosage of MeJA.

The magnitude of the increase in chemical pesticide use in recent times in developing countries like Bangladesh is alarming, threatening to human health and the environment. We investigated level of pesticide use and status of non-chemical integrated management approaches presently among the small holder farmers, and interpreted the result to suggest how small integrated pest management (IPM) technology such as ‘seed treatment’ could be efficiently exploited in Bangladesh context. Key reforms recommended include screening of the technology under different conditions, making the seed treating elicitors easily available with proper label and formulation and government providing farmers

with proper subsidies and training of know-how to encourage farmers who are interested toward non-chemical based pest management. If successfully integrated with other facets of an integrated pest management program, the use of MeJA as elicitors of plant defense could be an important tool in managing insect pests and contribute to a reduction in applications of chemical pesticides.

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ACKNOWLEDGEMENTS

I take this opportunity to express my deep sense of gratitude and indebtedness to my advisory committee for their regular intellectual guidance, incurring inspiration, and critical appraisal of manuscript. I am especially thankful to Professor Edwin G. Rajotte, for continuingly convincingly and conveying a spirit of adventure in regards to both my research and study. Without his guidance and persistent help, this thesis would not have been possible.

I would also like to thank Professor Gary Felton for providing me the opportunity to be a part of his lab and helping me with all the necessary facilities to carry out the research work. Also, the guidance and support received from all the members of Felton Lab, especially Michelle Peiffer and Donglan Tian, was vital for the success of my project. Special thank goes to Dr. Mary Barbercheck, who was willing to participate in my final defense committee at the last moment.

Furthermore, I would like to show my greatest appreciation to Dr. Paulo H.G. Zarbin and Dr. Syed Nurul Alam, for arranging all the necessary facilities to carry out my work in Brazil and Bangladesh respectively. I would also like to acknowledge the Integrated Pest Management Collaborative Research Support Program (IPM-CRSP) of USAID (United States Assistance for International Development), International Program-College of Agriculture Sciences, Penn State and Department of Entomology, for their generous support toward completing of this thesis.

A special gratitude and thanks to my parents and family for cheering me up and standing by me through the good and bad times. Last but not the least, I am grateful to all my friends, fellow graduate students and other PSU colleagues for their insights, encouragement and their support throughout my stay here at Penn State.

CHAPTER 1
INTRODUCTION/LITERATURE REVIEW

1.1 Introduction to plant defenses

Plants are regularly challenged by insects and diseases from the time of crop establishment to after harvest. If unable to resist damage, poor crop establishment or even complete loss may result. Herbivorous insects represent a significant component of life on earth, and thus can pose a considerable threat to plants (Karban and Baldwin, 1997). Depending on the kind of insect, various feeding strategies used to obtain nutrients from above- (vegetative) and below-ground plant parts can inflict mechanical damage on plant tissues. Leaf-feeding beetles (Coleoptera) and caterpillars (Lepidoptera) damage plants with their mouthparts designed for chewing, snipping, or tearing and are believed to account for approximately two thirds of all known herbivorous insects on earth (Schoonhoven et al., 1998; Howe and Jender, 2008). Plants thathave the ability to either escape from the herbivores or decrease herbivore fitness will be more likely to survive and reproduce compared with plants that are more susceptible to damage by herbivores. Therefore, plants are under selection pressure to continuously evolve traits that will help them survive attack by herbivores (Karban and Baldwin, 1997; Futuyma and Agrawal, 2009).

1.2 Constitutive and induced defenses

As plants are regularly exposed to attack from a wide array of herbivores, plants have acquired different defense mechanisms namely constitutive and induced resistance (Karban and Baldwin, 1997; Gatehouse, 2002; Kaplan et al., 2008). Constitutive defenses are always present within the plant, while induced defenses are produced or mobilized to the site only when needed. Constitutive defenses range from mechanical defenses to digestibility reducers and toxins that protect plants against the direct effects of biotic and abiotic factors. Many external mechanical defenses and extensive, structural defenses (e.g bark, cell walls, epidermal cuticles) are constitutive, as they require large amounts of resources to produce and are difficult to mobilize. Also, biochemical defenses such as anti-microbial peptides, proteins and secondary metabolites (alkaloids, terpenoid and phenolics) that are already present in the plants (large amounts in reproductive tissues), are also constitutive form of plant defenses (Gatehouse, 2002; Howe and Jander, 2008). The mechanisms of these defensive traits include antixenosis (negative impact on the insect's preferences such as host plant selection, oviposition and feeding behavior) and antibiosis (negative impact on the performance of insects).

In addition to the pre-formed barriers (constitutive defenses), most living plants have the ability to detect invading herbivores or pathogens and respond with inducible defenses thatmay include secondary metabolic products as well as morphological and physiological changes. Induced defensive traits minimize negative fitness consequences of the subsequent herbivore attacks on plants either by repelling herbivores or by attracting the natural enemies (predators and parasitoids) of the herbivores. The advantage of inducible, as opposed to constitutive defenses, is that as they are only produced when needed and are potentially less costly, especially when herbivore populations are variable (Karban and Myers, 1998; Karban and Baldwin 1997). Detection of attack by plants, signal transduction and the launch of specific chemical defenses are considered three major steps involved in inducible defense (Walling, 2000; Kessler and Baldwin, 2002; Ferry et al., 2004).

The phenomenon of induced resistance after attack by herbivores or pathogens was not noted until the early 1990s (Chester, 1933). One of the earlier studies demonstrated a reduction in virus symptoms in the leaves of *Dianthus barbatus* when challenged with previously-exposed carnation mosaic virus (Glipatrick and Weintraub, 1952). In another study, inoculation of the lower leaves of tobacco (*Nicotiana tabacum*) with tobacco mosaic virus (TMV) enhanced resistance to secondary infection in the upper leaves (Ross, 1961). The authors termed this phenomenon Systemic Acquired Resistance (SAR). Research since these early studies has provided advances in the field of induced resistance, from uncovering molecular mechanisms (Ferry et al., 2004) to building theoretical models (Poitrineau et al., 2004).

1.3 Direct and indirect induced plant defenses

Two types of inducible defense mechanisms against herbivore have been observed in plants: direct defenses and indirect defenses. Direct defenses involve the expression of biochemical and morphological traits that interfere with herbivore development or behavior (Chen, 2008; Chen et al., 2011), while indirect defenses facilitate the action of natural enemies of herbivores (DeMoraes et al., 1998; Walling, 2000; Heil et al., 2008). Direct defenses rely on morphological features such as thorns, spines and trichomes, epicuticular wax film and wax crystals, tissue toughness, as well as secretory structures such as lattices and resin-containing channels. Additionally, they include chemical defense compounds, secondary metabolites, digestibility-reducing proteins and anti-nutritive enzymes (Howe and Jander, 2008; Schaller, 2008). Indirect defenses include extra-floral nectaries. Herbivore feeding induces production of extra-floral nectar that can be exploited as an alternative food source by carnivorous arthropods (Karban and Baldwin, 1997). Herbivore-induced plant volatile production (VOCs) in response to stimuli can provide airborne signals to natural enemies of herbivores (DeMoraes et al., 1998; Walling, 2000; Heil, 2008; Schaller, 2008).

1.4 Benefits and costs of induced defenses

Induced defenses are considered to be advantageous over constitutive defenses for multiple reasons. First, it may reduce the possibility of development of counterproductive strategies by insect herbivores (Karban et al., 1997; Cipollini and Heil, 2010; Karban, 2011; Karban et al., 2013). Variation in the defense constituents of a plant created by induced defenses makes a plant more unpredictable environment for insect herbivores and thus may have an additional benefit to the plants under attack ((Futuyma and Agrawal, 2009; Karban, 2011). Second, continuingly producing and maintaining a high level of constitutive defenses requires high energy costs and nutrient requirements, which render costs in plants (Karban, 2011; Karban et al., 2013). Thus, induced defenses reduce the metabolic load on the plants in conditions where such defenses are not required. This is particularly the case where the presence of herbivore is not always predictable (Agrawal and Karban, 1999).

The resource allocation hypothesis suggests that plants can either invest their limited pool of resources on growth or in defense; each at the expense of other (Coley et al., 1985) assumes that defenses are costly. Therefore, if plants are going to defend themselves in the absence of herbivores they have to invest resources in defense rather than in growth or reproduction, sustaining costs or "trade-offs" (Strauss et al., 2002). While in the presence of herbivores, investing in defense should pay off due to benefits from increased growth and reproduction in the absence of herbivory (McKey 1974; Coley et al., 1985). The general understanding regarding inducible defenses is that a plant will only allocate more energy towards defense when the benefits of protection outweigh the costs, especially where herbivore pressure is high (Zangerl and Rutledge, 1995). Induced defenses in plants are believed to have evolved to save energy under enemy-free conditions; however, under unfavorable conditions when defenses are activated, costs are involved (Walters and Heli, 2007). Plants use different strategies, such as creating defensive structures and chemicals, which require resources that could be otherwise be used by plants to maximize growth and reproduction (van Hulten et al., 2006; Cipollini and Heil, 2010; Kempel et al., 2011).

The costs to induced defenses are quantified as the resource-based trade-off between resistance and fitness (allocation cost) or as the reduced fitness resulting from the interactions with other species or the environment (ecological costs) (Strauss et al., 2002; Heil, 2002; Cipollini and Heil, 2010). Allocation costs occurs when a large quantity of fitness-limited resources are diverted to defend plant instead for fitness-related processes such as growth and reproduction (Strauss et al., 2002; Kempel et.al., 2011; Karban, 2011). Meanwhile, ecological costs results from the disruption of many symbiotic relationships that a plant has with the environment (Heil, 2002). For example, consequences of induced defenses on fruit characteristics such as fewer fruits, longer ripening time, delayed fruit-set and fewer seeds per plant will affect the plant attractiveness toward seed dispersers, which will ultimately reduces the fitness costs (Redmann et al., 2002).

1.5 Polypheonol oxidase (PPO) and its role in anti-herbivore plant defenses

Polyphenol oxidases (PPO) are ubiquitous copper-containing enzymes which oxidizes *o*-dihydrophenolics such as cafeic acid and catechol to the corresponding *o*-quinones (Duffey and Felton, 1991). Rapid polymerization of *o*-quinones results in black, brown, or red pigments, which cause fruit browning and discoloration in damaged and diseased tissues (Constabel and Barbehenn, 2008). Role of PPO in plant defense against pests and pathogens has been extensively studied in several systems (Felton et al., 1989, 1992; Kowalski et al., 1992; Felton et al., 1992; Thipyapong and Steffens, 1997). Duffey and Felton (1989) first reported the inverse correlation of between *Helicoverpa zea* growth and PPO levels in tomato plants. PPO activity in tomato plants were also found to be strongly induced by methyl jasmonate (MeJA) and oligogalacturonic acid, major plant defense signaling compounds, which further supported the anti-herbivore role of PPO (Constable et al., 1995). Furthermore, induction of PPO activity in tomato following wounding, insect damage and exogenous application of MeJA is being demonstrated in several studies (Stout et al., 1998; Constable and Ryan, 1998; Thaler et al., 1996, Thaler, 2002). Regarding the role of PPO in defense against herbivores, three mechanisms have been proposed: 1) alkylation of amino acids/protein by *o*-quinones, decreasing the nutritive quality of foliage, 2) elevated oxidative stress in the gut lumen, and 3) absorption of phenolic oxidation products, thus resulting in toxic effects on herbivores (Constabel and Barbehenn, 2008).

1.6 The jasmonic acid (JA) pathway and induced plant resistance

Induced defenses require a signal to initial the defense condition. Signaling pathways in plants that are controlled by phyto-hormones such as jasmonic acid (JA), salicylic acid (SA) and ethylene are associated with attack by different organisms, such as insect herbivores, and biotrophic and necrotrophic pathogens (Howe, 2004; Pozo et al., 2005; Lorenzo and Solano, 2005; Grant and Lamb, 2006; Van Loon et al., 2006; Von Dahl and Baldwin, 2007). The plant hormone jasmonic acid (JA) and related signaling compounds referred to as jasmonates play a central role in regulating defense responses to a wide range of herbivores.

Over the past decade it has become increasingly clear that JA is also involved in plant responses against both biotic and abiotic stresses (Ballare, 2011). JA can be induced endogenously by several factors such as mechanical wounding, stress, herbivory or attack by certain pathogens. In addition, exogenous JA application can induce a similar, though not identical, set of defensive compounds compared with insect herbivory

(Thaler et al., 1996; Thaler, 1999a; Omer, 2000; Thaler et al., 2001; Lian-You et al., 2004). JA has also not been found to be directly toxic to herbivores (Avdiushko et al., 1997).

Induced resistance in plants against herbivory is largely dependent upon the JA pathway (Schaller and Stintzi, 2008). The jasmonate signaling or octadecanoid pathway regulates the expression of direct and indirect defenses against herbivores in plants (Howe, 2004). Recently, several studies have emphasized the role of the jasmonate pathway in regulating gene expression in response to both mechanical wounding and herbivore damage (Reymond et al., 2000; Dicke et al., 2004; De Vos et al., 2005; Devoto et al., 2005; Giri et al., 2006 ; Major et al., 2006 ; Lippert et al., 2007). There are several other roles of jasmonates that have been identified, such as in regulation of tritrophic interactions (Engelberth et al., 2004; Thaler, 1999b), host plant resistance to phloem-feeding insects (Zavala et al., 2004; Mewis et al., 2005; Gao et al., 2007), trichome-based defenses (Boughton et al., 2005; Peiffer et al., 2009), priming of direct and indirect defenses (Engelberth et al., 2004 ; Ton et al., 2007 ; Worall et al., 2012), pathogen resistance and systemic transmission of defense signals (Howe, 2004). Jasmonates are also associated with several aspects of plant development promoting defensive and reproductive processes while inhibiting the growth and photosynthetic output of vegetative tissues (Devoto, 2005; Giri et al., 2006). Jasmonate-signaled defenses are also believed to provide protection against a wide range of herbivores and are relatively nonspecific in nature (Howe and Jander, 2008). This suggests that jasmonates plays a crucial role in plant defense against rapidly changing and hostile environments.

Biosynthesis of JA from fatty acid precursors occurs very rapidly following herbivore attack. For the past few decades, efforts have been made to identify the central components of JA signaling cascade, which is initiated by the interaction of JA-lle with the E-3 ubiquitin ligase SCFCOl1 complex resulting in the degradation of the jasmonate ZIM-domain (JAZ) transcription factors that repress expression of JA-responsive genes (Schaller, 2001; Schaller and Stintzi, 2008; Chini et al., 2009).

1.7 Induction of plant defenses using plant defense elicitors

In natural systems, plant defenses (direct and indirect) are not always expressed to their maximum potential, but rather require certain kinds of stimuli to induce them to higher levels (Karban and Badwin, 1997; Agrawal et al., 1999; Baldwin and Preston, 1999; Walling, 2000). Crop plants are frequently grown in pest environments to which they are not adapted and are mostly bred or cultivated with no regard for their inducible responses. Hence, it is very likely that the plants do not express defenses to their potential and thus inducible responses of plants are generally under-utilized (Stout et al., 2002).

Recent improvements in the understanding of induction of plant defenses has led to the discovery of different kinds of natural and synthetic chemicals, called elicitors, which are believed to induce plant defenses similarly to plant herbivores (Karban and Kuc. 1999). These discoveries have led to interest in manipulating the host plant defenses for crop protection. A variety of environmental signals such as different plant defense elicitors, volatile organic compounds (VOCs) and several pathogens are found to induce defenses in plants (Conrath et al., 2006; Beckers and Conrath, 2007; Frost et al., 2008; Kim and Felton, 2013). Plant hormones and several chemical signals have also been identified as effective elicitors of plant defense (Inbar et al., 1998; Thaler et al., 1999; Stout et al., 2002; Conrath et al., 2006; Boughton et al., 2006; Ton et al., 2009; Stout et al., 2002). Elicitors are believed to be safe to both humans and the environment and are compatible with biological control by natural enemies. Methyl jasmonate (MeJA), the SA mimic benzothiadiazole (BTH), ethepon, and the bacterial protein 'harpin' are the commonly used plant defense elicitors. JA or its methyl ester, MeJA has been shown to induce resistance to arthropods in various agricultural crops (Avdiushko et al., 1997; Havill and Raffa, 1999; Cipollini and Redman, 1999; Black et al., 2003; El-Wakeil et al, 2010; Worall et al., 2012).

Even though several elicitors have been tested to enhance plant resistance against insect pests, JA remains the well-studied one (Stout et al., 2002). Exogenous application of JA or MeJA to tomato induces several classes of defense-related compounds that result in enhanced plant resistance against insects such as flea beetles, aphids, caterpillars and thrips, both in field and greenhouse (Farmer et al., 1992; Thaler et al., 1996). An increase in the activity of defense-related proteins such as proteinase inhibitors, peroxidase and polyphenol oxidase followed the exogenous application of JA or MeJA to plants, similar to the response induced by *Helicoverpa zea* feeding (Felton et al., 1989; Duffey and felton, 1991; Thaler et al., 2001).

However, use of elicitors to induce plant resistance cannot be considered as an isolated strategy or an alternative to the chemical pesticides, but rather as a potentially important component of an integrated pest management approach (Stout et al., 2002).

Elicitors are likely to be compatible with other management strategies, such as cultural methods and may provide protection to the plants against herbivores in many cropping systems (Kogan, 1998; Way and van Emden, 2000).

1.7 Induction of plant defense stimulated by seed treatment against insects

Recently, Worall et al. (2012) demonstrated that seeds are receptive to plant defense elicitors such as β-amino butyric acid (BABA) and JA that confer protection to the subsequent plant against a wide spectrum of plant pathogens and insects (Worall et al., 2012). Whilst the mechanism underlying such response still remains unclear, it is assumed to be due to epigenetic modifications of JA- and BABA-responsive genes in embryonic tissues during imbibition resulting in more responsive JA and SA-dependent signaling pathways during attack (Worall et al., 2012). Also, while biotic agents such as *Trichoderma spp* and *Pseudomonasspp* when applied with seeds have been found to induce plant defenses effectively against pathogens (Abuamsha et al., 2011; Nagaraju et al., 2012), very little is known regarding their effect on inducing plant resistance against insects.

Seed treatment with chemical elicitors offers a simple, easily accessible and cost-effective means of pest management and holds considerable promise for sustainable pest management (Worall et al., 2012). The simplicity of the method, which requires dipping seeds in elicitor solutions, makes it convenient for farmers and is also industrially applicable (Worall etal., 2012). Furthermore, as reported by Worall et al., (2012), it overcomes the antagonistic effects frequently observed between JA-dependent plant defenses against insect herbivores and SA-dependent plant defenses against bio-trophic pathogens. Despite the potential, very limited efforts have been made to exploit seed-applied, plant defense elicitors at a large scale.

1.8 Costs of chemical induction

Because chemical induction of plant defense is easy to use and has potential benefits in crop protection, there have been several studies that measure the plant-based costs of chemical elicitor treatment. Wheat (*Triticum aestivum*) treated with benzothiadiazole (BTH), a chemical analogue of salicylic acid achieved comparatively lower biomass and developed fewer shoots, heads, and seeds in comparison with control plants (Heil et al., 2000). These results paralleled findings of fitness costs of SA treatment in *Arabidopsis* (Cipollini, 2002). Similar results have been observed in several other systems using BTH (Csinos et al., 2001; Buzi et al., 2004; Faessel et al., 2008) and JA (Redman et al., 2001; Heijari et al., 2005). However, Worall et al. (2012), demonstrated that plants chemically primed with JA and BABA did not suffer any long-term effects on vegetative and reproductive growth (Worall et.al., 2012). For plants primed by seed treatments, little is known about the balance between the dosage of seed treatment agents and subsequent resistance levels, and fitness costs. Therefore, it is important to examine the potential for negative effects on plant growth and fitness following seed treatment with defense elicitors to use this technology efficiently in an agricultural context.

1.9 Bangladesh and the small holder farmers

Bangladesh, a developing South Asian country, relies heavily on agriculture employing about 45% of the total labor force. The agriculture sector contributes 18.5% of the total Gross Domestic Product (GDP), making it an important contributor to the economy (**https://www.cia.gov/library/publications/the-world-factbook/geos/bg.html**). Various factors such as rapid population growth, decline in cultivable land, subsistence farming, lack of farmer awareness and pest problems have directly contributed to agriculture underdevelopment (Robanni et al., 2007).

Agriculture in Bangladesh is characterized by smallholder farmers cultivating small plots of land. The average size of landholdings is only 0.5 hectares, whereas their share in total cultivated land is around 69%. Due to inappropriate land reform policies and rapid urbanization, the farm size is regularly declining; the average farm size of 1.4ha in 1977 to 0.6 ha in 1996 land (Thapa and Gaiha, 2011). However, small farms play an indispensable role in global food security, particularly in developing countries. In fact, four-fifths of the developing world's food comes from small-sized farms and they make 85% of world's farmers (FAO, 2013). Therefore supporting small holder farmers, which are often cut off from access to training and sophisticated tools and techniques, is believed to be the most effective way of stimulating economic development and reducing poverty (IFAD, 2013)

1.10 Synthesis of current knowledge and research questions

Exogenous application of a variety of plant hormones and synthetic chemicals have been used to induce defenses in plants (Stout et al., 2002; Conrath et al., 2006; Heil and Ton, 2008; Ton et al., 2009). Recently, Worall et al. (2012) demonstrated seeds are also receptive to plant defense activators like β-amino butyric acid (BABA) and JA, conferring protection to the subsequent plant against a wide spectrum of plant pathogens and insects. Seed treatment, which offers a simple, easily accessible and cost-effective means of pest management, holds considerable promise for sustainable pest management. Despite this potential, very limited research efforts have been made to exploit seed treatment using plant defense elicitors any further.

It is important to investigate and examine the consistency of induced defenses stimulated by seed treatment during various plant growth stages, and the fitness costs associated, to better understand its effectiveness against insect pests. The present study examines the independent and interactive effects of MeJA seed treatment on *H.zea* caterpillar growth and the activity of PPO in leaves of tomato plants at three different plant stages. We also measured the dosage effects of MeJA seed treatment on several traits associated with vegetative and reproductive fitness in tomato plants: germination success, plant height, fruit yield and the ripening time. We further expanded our research to Brazil and Bangladesh to evaluate the seed treatment effect of MeJA on two economically important insects; tomato leaf miner, *Tuta .absoluta, and* the tobacco caterpillar *(Spodoptera.litura),* respectively. These studies will contribute to further understanding regarding the efficacy of induced defenses in protecting plants from herbivore attack following seed treatment and the fitness costs associated with these defenses.

We also investigated the present pest management strategies and the level of pesticide use among small holder farmers in Bangladesh, and then interpreting the results to suggest how small integrated pest management (IPM) technology such as ‘seed treatment using defense elicitors’, which has a great appeal to the small holder farmers, could be efficiently exploited in Bangladesh.

This thesis has four chapters; the first chapter is an introduction and an overview of induced plant defenses and anti-herbivore defense using defense elicitors. The second chapter describes the long lasting plant resistance against various insects following MeJA seed treatment and the fitness costs. The third chapter describes the present status of pest management in Bangladesh and how technology like ‘seed treatment using defense elicitors’ might be used in Bangladesh. The fourth chapter is an overall summary and conclusion.

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CHAPTER 2
TOMATO SEED TREATMENT WITH PLANT DEFENSE ELICITORS DECREASES FITNESS OF SEVERAL HERBIVORES

2.1 Introduction and Background

Plants are regularly exposed to attacks from a wide array of herbivores, and in response they have acquired different defense mechanism to protect themselves, including constitutive and induced defenses (Karban and Baldwin, 1997; Kaplan et al., 2008; Arimura et al., 2011). Constitutive defenses are always present and are expressed under the regular developmental program within the plant, while induced defenses are produced or mobilized in response to herbivory as needed (Karban and Mayers, 1989). Inducible defenses put the plant into a state of induced resistance only when necessary and are advantageous in that they conserve energy in plants when pest attack is variable (Karban and Myers, 1989; Karban and Baldwin 1997; Karban et al., 1997). There are two different types of inducible defense mechanisms against herbivory: direct and indirect. Direct induced defenses include repellent, anti-nutritive or toxic compounds (Chen, 2008) as well as physical defenses such as leaf toughness, spines, and trichomes (Peiffer et al., 2009; Chen et al., 2012). Indirect induced defenses include the production of herbivore-induced plant volatiles (HIPVs) and/or extra-floral nectar that attract natural enemies in response to stimuli (Karban and Baldwin, 1997; Haruta et.al., 2001; Arimura et al., 2005; Miranda et.al., 2007). Insect herbivores may attack different plants and plant tissues, so the ability of the plant to respond variably and on demand may be favored by selection (Karban et al., 1997; Agrawal and Karban, 1999). Induced resistance in plants against herbivory is largely dependent upon the Jasmonic Acid (JA) pathway (Schaller and Stinzi, 2008).

Recent improvements in the understanding of induction of plant defenses has led to the discovery of different kinds of natural and synthetic chemicals called elicitors, which are believed to induce plant defenses similarly to plant herbivores (Karban and Kuc. 1999). A variety of plant hormones (both naturally occurring and synthetic) and pathogens have been found to activate plant defense responses (Stout et al., 2002; Conrath et al., 2006; Frost et al., 2008; Ton et al., 2009). Elicitors are believed to be safe to both humans and the environment and are compatible with biological control by natural enemies. Methyl jasmonate (MeJA), the SA mimic benzothiadiazole (BTH), ethepon, and the bacterial protein 'harpin' are the commonly used plant defense elicitors (Stout et al., 2002).

Jasmonic acid (JA) or its methyl ester, MeJA has been widely demonstrated to induce resistance to arthropods in various agricultural crops (Avdiushko et al., 1997; Havill and Raffa, 1999; Cipollini and Redman, 1999; Black et al., 2003; El-Wakeil et al, 2010; Worall et al., 2012). Exogenous application of JA or MeJA to tomato induces several classes of defense-related compounds that result in enhanced plant resistance against insects such as flea beetles, aphids, caterpillars and thrips, both in field and greenhouse (Farmer et al., 1992; Thaler et al., 1996). An increase in the activity of defensive proteins such as proteinase inhibitors, peroxidase and polyphenol oxidase followed the exogenous application of JA or MeJA to plants, similar to the response induced by*Helicoverpa zea* feeding (Felton et al., 1989; Duffey and Felton, 1991; Thaler et al., 2001). Meanwhile, inverse correlations between larval growth and polyphenol oxidase (PPO) levels in tomato plants have also been demonstrated (Felton and Duffey, 1991).

Recently, Worall et al. (2012) demonstrated that seeds are also receptive to plant defense activators like β-amino butyric acid (BABA) and jasmonic acid (JA), conferring protection to the subsequent plant against a wide spectrum of plant pathogens and insects. Also, biotic agents such as *Trichoderma spp* and *Pseudomonasspp* when applied to seeds induced plant defenses effectively against pathogens (Abuamsha et al., 2011; Nagaraju et al., 2012), however very little is known regarding their effect against insects. Seed treatment using defense elicitors, which offers a simple, easily accessible and cost-effective means of pest management, holds considerable promise for sustainable pest management in agriculture. The simplicity of the method, which requires dipping of seeds in the elicitor solutions, makes it convenient for individual farmers and is also industrially applicable (Worall et al., 2012). Recently, JA seed treatment technology was licensed for use by the US agricultural company Becker Underwood in collaboration with Plant Bioscience Limited, UK (http://www.pbltechnology.com/cms.php?pageid=296). However, examination of the consistency of plants defense stimulated by seed treatment during the various plant growth stages and the associated fitness costs is needed to better understand its effectiveness against insect pests.

The effectiveness of induced resistance may depend upon plant stage or development. Induced plant responses can differ depending on plant tissue type and even different stages of development in the same tissues producing variation even within individual plants (Baldwin and Karb, 1995; Zangerl and Rutledge, 1996). Young plants or tissues are more responsive to defense elicitors than older plants or tissues (Cipollini and Redman, 1999): however there are some instances where the inverse is true (Karban and Myers, 1989; Faeth, 1993; Thaler et al., 1996). One tomato study demonstrated a significant decrease with plant age in induction of proteinase inhibitor activity following wounding (Wolfson and Murdock, 1990). Similarly, following wounding, mRNA of polyphenol oxidase F, which is the most wound-responsive polyphenol oxidase gene family member, was found to be more strongly induced in younger tomato leaves than older ones (Thipyapong and Steffens, 1997). Bruce and West (1989) suggested that the change in some constitutive defense-related enzymes such as peroxidases also varied with age in non-induced plants as well. Thus, induced defenses stimulated by seed treatment might differ with age and plant stage, affecting the defense induction and pest resistance.

Induced defenses may have evolved to conserve resources during enemy-free conditions, but during herbivore attack, when defenses are activated, resource and fitness costs may be incurred (Heil, 2002; Cipollini et al., 2003). Plants use different defensive strategies, which require resources that could otherwise be used by plants to maximize growth and reproduction (van Hulten et al., 2006; Cipollini and Heil, 2010; Kempel et al., 2011). However, Worall et al. (2012) demonstrated that the plants chemically induced with a very low level of JA and BABA did not show any long-term effects on vegetative and reproductive growth. For plants induced by seed treatments, little is known about the balance between the resistance levels, the amount of seed treatment agents, and the resulting fitness costs and benefits. In this paper, we will examine the hypothesis that treatment of tomato seeds by MeJA induces plants for defense, but results in negative effects on plant growth and fitness. We tested our hypothesis in three different host plant and pest combinations in the United States, Brazil and Bangladesh.

In the United States we conducted greenhouse trials to examine the independent and interactive effects of MeJA seed treatment on the growth of the tomato fruit worm (*Helicoverpa zea*) and the activity of polyphenol oxidase (PPO) in leaves of tomato plants at three different plant stages. We also measured the dosage effects of MeJA seed treatment on several traits associated with vegetative and reproductive fitness in tomato plants: germination success, plant height, numbers of fruit harvested and ripening time. We further expanded our research to Brazil and Bangladesh to evaluate the effectiveness MeJA seed treatment on two economically important insects; tomato leaf miner *(T.absoluta)* andtobacco caterpillar *(S.litura)* respectively. These experiments improved our understanding regarding induced defenses following seed treatment and the fitness costs of these defenses.

Brazil is one of the top ten countries producing large volumes of tomatoes in the world today (FAO, 2009). However, they are also one of the most noted crops for pesticide use in the country (Moreira et al., 2002). The tomato leaf miner, *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae), which is one of the most serious pests of tomatoes, invaded Europe and is spreading throughout the Mediterranean basin and Africa (Garcia and Vercher, 2010; Desneux et al., 2010). Its larvae mine into the leaves and also bore inside fruits and stems (http://www.tutaabsoluta.com/). At high densities damage can result in a loss of up to80% of fruit yield (Picanco et al., 2011). In favorable weather conditions, ten to twelve generations can occur in a single year (www.tutaabsoluta.com/tuta-absoluta). Multiple applications of chemical pesticides (10-30 application/cycle) are used to manage the *T. absoluta* in absence of any current effective alternative strategies (Siqueira et al 2000).

Similarly, the cabbage or tobacco caterpillar, *Spodoptera litura* (Lepidoptera: Noctuidae) is a serious threat in vegetables, such as tomato, cabbage and several others in Bangladesh. It destroys plant leaves by making holes and greatly reducing the market value of produce (especially where cabbage heads are damaged) (Butani and Jotwani, 1984). Crop losses up to 50% have been noted (Bhat et al., 1994). Farmers rely solely on frequent applications of chemical insecticides to control *S. litura* (Dasgupta et al., 2004). Although there have been several investigations on how to exploit plants resistance for control, they have focused more on genotype selection against tomato leaf miner (Maluf et al., 1997; Tadeu et al., 2006; Oliveira et al., 2012) and tobacco caterpillar (Ahmed et al., 2013; Bhat and Singh, 1994).

2.2 Materials and Methods

2.2.1 Seed treatment

For the experiments in the United States we treated, 50-60 seeds with various dosages (05mM, 0.1mM, 0.2mM, 0.4mM or 1mM) of MeJA (Bedoukian Research, Inc. CT, USA). Before treatment, MeJA was dissolved in a small amount (0.8%) of ethanol and made up to a desired concentration with distilled water. Treated seeds were kept atroom temperature for 24 hours before sowing. Following the treatment, seeds were washed twice in distilled water. A similar lot of seeds wassoaked only in distilled water to represent controls.

Similarly, 50-60 seeds were treated with a single dose (0.2mM) of MeJA in Brazil (MeJA from Sigma Aldrich-Brazil Ltd, Product code: 101186144) and Bangladesh (MeJA from Bedoukian Research, Inc, CT, USA). Additionally in Bangladesh, *Pseudomonas fluorescens* (Strain: IPL/PS-01, International Panacea ltd., India) @5g/kg seeds wastested as a seed treatment agent in a separate experiment.

2.2.2 Plant material

Tomato (*Solanum lycopersicum* cv Micro-Tom) plants were used in all of the experiments at Pennsylvania State University. Seeds were originally purchased from Tomato Growers Supply (Fort Myers, FL 33906). Seedlings were grown in Metromix 400 potting mix (Premier Horticulture, Quakertown, PA, USA) in a greenhouse. Three separate plantings staggered in 25 day intervals produced plants that were 25, 50 and 75 days old at the start of the experiment. Leaves from three different plant stages (25DAS, 50DAS and 75DAS) were used for insect feeding and chemical analysis. All the greenhouses were maintained on a 16-h/8-h light/dark photoperiod and temperature ranging from 25 to 30°C. Seedlings were regularly watered every 4-5 days as needed.

In Brazil, tomato (*Solanum lycopersicum* cv Santa Clara I-5300) plants were used. Seeds were purchased from *Conheca O Mix de Produtos das Marcas ISLA Pro eISIA* and the seedlings were grown in potting mix (Linha Ornamental, Brazil) in a greenhouse at Federal University of Parana (UFPR). Similarly in Bangladesh, tomato plants (*Solanum* *lycopersicum* L., cv BARI Hybrid Tomato 4) bought from Kashim Seed Company, Siddique Bazar, Dhaka were used for the experiments at Bangladesh Agriculture Research Institute (BARI).

2.2.3 Herbivore Bioassay

For the U.S. experiments, eggs of *Helicoverpa zea*, were obtained from BioServ (Frenchtown, NJ, USA) and neonates were used for feeding bioassays. Neonates were transferred to caterpillar cups (30ml) containing excised leaves and allowed to feed for 72 hours at 25°C. Feeding was measured as average larval weight gain (AWG) per day calculated by dividing the entire weight gain with number of days of feeding and compared with the control to document the difference in larval growth. No larva consumed an entire leaflet during the course of the bioassay, indicating they were not resource limited.

In Brazil, a population of *Tuta absoluta* was maintained in a colony at UFPR, Brazil. Newly hatched *T.absoluta* larvae were placed in a plastic cup (30ml) with leaves. The petioles of the leaves were wrapped in cotton cloth to maintain turgidity. Every three days, a new set of excised leaves from one-month-old plants were presented to the insects to ensure freshness and to avoid desiccated leaves. The containers were observed daily by noting the occurrence of pupae, which were weighed. The parameters evaluated were duration of larval development and pupal weight.

Similarly in Bangladesh, leaves from one-month-old seedlings were used, where neonates of *S. litura* (from Rangpur Research Farm, Rangpur) were exposed to leaves from different treatments in caterpillar cups for feeding bioassays. The larval weights were taken after 72 hrs and compared with controls. Average weight gain (AWG) per day was calculated by dividing the weight by the number of days of feeding.

2.2.4 Polyphenol Oxidase (PPO) Activity

The detached leaves from both treated and untreated plants were weighed before being flash frozen in liquid nitrogen and stored at -80°C until analysis for PPO activity. 50 mg samples from each leaf were ground in liquid nitrogen. Immediately, 1.25 ml buffer (0.1M potassium phosphate buffer, pH 7.0) containing 5% insoluble polyvinylpolypyrrolidone (PVP) was added. The samples were centrifuged at 11,000 g for 10 min at 4°C. Five µl of the supernatant was mixed with 200µl of 3mM caffeic acid (Sigma, Cat. No. C0625), and absorbance at 450nm was tracked for 5 min. PPO activity was expressed as change in absorbance/min/mg tissue (Felton et al., 1989).

2.2.5 Germination, plant growth and harvesting

Percentage of seeds germinated (green sprouts coming out from the soil) was monitored for each treatment in space of four days until 28 days after sowing. Similarly, plant height (inches) was measured in two different plant stages: 25 Days after sowing and 50 days after sowing. Also, plants were inspected daily for fruits thatwere harvested at ripening(first appeared red). Harvested tomatoes were numbered, dated and weighed.

2.2.6 Statistical Analysis

Plants were randomly allocated to treatments and random placementamong greenhouse benches allowed the use of a completely randomized design. All of the bioassays and chemical data were analyzed using ANOVA. Two-way ANOVA was used with the main effects being plant age (25, 50, 75 days old) and MeJA treatment plus all interaction terms. Means were separated with Tukey's HSD. All data (from all the experiments) were checked for normality and analyzed using 'Minitab 16.0' software.

2.3 Results

2.3.1 Long lasting plant's resistance stimulated by seed treatment

Seed treatment with MeJA enhances plant resistance against insects and defenses are consistent throughout all the different stages of plant development.

To determine the influence of seed treatment on insect resistance, we examined the effects of MeJA seed treatment on 25 day-old-plants against *H.zea*. The average weight gain (AWG) of *H.zea* on leaflets of tomato was greatly reduced with various concentration (0.05mM, 0.1mM, 0.2mM) of MeJA seed treatment in comparison to the control (MeJA: $F= 29.41$, $df=4, 60$: $p=0.00$) (Fig. 2.1). In a subsequent set of experiments, we compared the effect of 0.1mM MeJA seed treatment on plant resistance against *H.zea* at three different time periods (25DAS, 50DAS and 75DAS to test whether the induction of plant defenses remains throughout all the plant development stages. The average weight gain (AWG) of *H.zea* on leaflets of tomato was reduced by MeJA treatment (F= 19.16, df=1, 24: p=0.00) (Fig 2.2) in all three plant stages, and the effect of plant stage alone was not significant (F= 0.62, df=2, 24: p=0.540). The effect of MeJA was not dependent on plant age (MeJA * Age; F =2.71, df=2, 24; P=0.070). In almost all plant stages, MeJA reduced AWG similarly compared with the controls (Fig. 2.2). In accordance with the bioassay results, polyphenol oxidase activity (PPO) of tomato leaves was enhanced in MeJA seed treated plants (F= 210.48, df=1, 24: p=0.00) (Fig. 2.3), in a similar pattern in all ages of plant. Even though PPO activity was not significantly different in 75 day-old plants, the overall inductive effect of MeJA on plant was significant (Age; F =2.71, df=14.67; P=0.000).

2.3.2 Associated costs with seed treatment

Increased plant resistance with higher dosages of MeJA areassociated with costs to plant performance.

In the second experiment in the U.S. with higher MeJA dosages there was noticeable difference in AWG of larvae; *H. zea* grown on plants treated with higher concentration of MeJA were significantly reduced compared to those at the lower concentrations (F= 313.02, df=3, 60: p=0.00) (Fig 2.4). We also assessed the difference in PPO activity in tomato leaves takenfrom the plants treated with higher MeJA concentration. PPO activity also corresponded with the results from bioassays showing that the levels of PPO increase with increasing concentration of MeJA (F= 67.78, df=3, 24: p=0.00) (Fig. 2.5).

We observed differences inboth the vegetative and reproductive performanceof plants with increasing amount of MeJA in the high rate experiment. With the increasing amount of MeJA (0.2mM, 0.4mM, 1mM) treatments, germination % between the treated and non-treated plants was significantly different; ranging from 74% in the control to 43% in 1mM 28 days after sowing (DAS) (Fig.2.6). Significant growth reduction of seedlings (25 and 50 days old) for the 0.2mM, 0.4mM and 1mM treatments were also detected in comparison to the control, (25DAS: F= 106.69, df=1, 24: p=0.00 and 50DAS:F= 143.30, df=1, 24: p=0.00) (Fig. 2.7 and 2.8). We also detected decreasing differences in plant height among the treatments as the plants aged (Fig 2.7 and 2.8). Additionally, we observed that average fruit weight per plant was significantly lower in plants from seeds treated with higher concentration of MeJA than those in the other treatment groups (F=390.81, df=3, 12: p=0.00) (Fig. 2.9). Similarly, on average, fruits from plants treated with 1mM reached ripeness at a significantly later date (83.56 days after showing) than those from controls (74.91 days) or 0.1mM (75.81 days) (F=113.84 , df=3, 24: p=0.00) (Fig. 2.10).

2.3.3 Effectiveness of seed treatment in Brazil and Bangladesh

Plants' resistance against tomato leaf miner (T.absoluta) and tobacco caterpillar (S.litura)was enhanced, in Brazil and Bangladesh respectively.

MeJA seed treatment was effective in Brazil and Bangladesh against *T.absoluta* and *S.litura*, respectively. *T.absoluta* larvae reared on leaves from the treated plants showed significantly lower pupal weight in comparison with those reared on the leaves from untreated plants (Fig.2.11) (MeJA: F=341, df=1:p=0.00). Similarly, duration of the larval stage of *T.absoluta* was significantly lengthened when feeding on seed-treated tomato plants in comparison with the control (Fig.2.12) (MeJA: F=229.61, df=1 :p=0.00).

On average, larvae feeding on the leaves from tomato plants required an additional 1.3 days to pupate in comparison with those fed on control plants. We observed similar effects of MeJA seed treatment with *S.litura:* as the average weight gain (AWG) of *S. litura* on tomato leaflets was greatly reduced by MeJA seed treatment in comparison with the control (Fig 2.13) ($F=4.61$, $df=2, 20$: $p=0.014$). However, we did not find any significant effect of *Pseudomonas fluorescens* treatment on *S. litura* growth (fig.2.14). Combining JA with *P. fluorescens*had a significant and detrimental impact on the germination percentage of plants, as only 10% of the seeds germinated one month after sowing compared with 85 % in the control (Fig. 2.14).

2.4 Discussion

Activation of plant defenses can provide plants with broad-spectrum enhanced resistance against pests and may be effective in the field and greenhouse (van Hulten et al., 2006; Beckers and Conrath, 2007; Walters et al., 2008; Worall et al., 2012). However, the resistance can vary with the treatment agent concentration, herbivore species, and antagonism with other treatments. In the present work, seed treatments with MeJA were effective elicitors of plant defenses, indicating that seeds are receptive to these phytohormones and establish a primed state against insects. There was a significant decrease in weight gain of *H.zea* larvae on plants grown from MeJa-treated seeds, and this was correlated with increased polyphenol oxidase activity in leaves. These findings are in general accordance with other studies (e.g Thaler et al., 1996; Avdiushko et al., 1997; Havill and Raffa, 1999; Thaler et al., 2001; Black et al., 2003; Lian-You et al., 2004; Boughton et al. 2006; Worall et.al., 2012) where plants were treated with MeJA or JA.

Surprisingly, plant age did not affect induction of plant defenses following MeJA seed treatment. Others demonstrated variation in induced plant responses within different stages of plant development (Baldwin and Karb, 1995; Zangerl and Rutledge, 1996), and often the induction declines with age (Karban and Mayers, 1989; Cipollini and Redman, 1999). Here we demonstrated that the induction of plant defense following seed treatment remains relatively consistent throughout all the plant stages. As suggested by Worall et al. (2012), this kind of long lasting plant resistance is suggestive of a priming response rather than constitutive activation of defenses. Although MeJA treatment reduced AWG of *H.zea* in almost all the plant stages, treatment effects were more pronounced in 25 and 50 day old plants where AWG was significant reduced in comparison with the control. PPO activity was also significantly induced with the seed treated plants during various stages, corresponding with previous studies on MeJA inducing PPO activity (Constable et al., 1995) and reducing the larval growth (Felton and Duffey, 1991). In contrast, 75 day-old-plants had the weakest induction effect both in terms of AWG of *H.zea* and activity of polyphenol oxidase, which is in general accordance with other studies (e.g., Thipyapong and Steffens, 1997). It is believed that during the reproductive phase, resources are invested more toward the reproductive parts rather than on plant defense (research allocation hypothesis) (Coley et al., 1985). Nevertheless, the fact that MeJA seed treatement was considerably long lasting during plant development enhances the appeal of this method for integration into pest management practices.

Plant fitness costs were correlated with some seed treatments. Three different concentrations (0.1mM, 0.4uM and 1mM) of MeJA showed that the mean germination percentage and first seedling emergence of treated seeds decreased as the MeJA dosage was increased, an affect noted by Norastehnia et al., (2007) with MeJA. Given that sensitization of defenses can minimize fitness costs while improving future resistance to attack (van Hulten et al., 2006), we saw that as the concentration of treating agent was increased, there was significant reduction in plant germination, seedling growth and other reproductive traits (ripening time, fruit quantity) as suggested by some other studies (e.g Redman et.al., 2001). Thus, a balance must be struck between resistance level induced by the seed treatments, the projected amount of herbivory expected and the resulting fitness costs or benefits experienced by plants. While there will be low costs associated with low-level induction in the absence of pests (Thaler et al., 1996), the benefits of such defenses under even moderate herbivory in the field should be balanced and vice-versa. Thus, care should be taken in implementing this technology. The formulation of MeJA should minimize the chances of using the wrong dose. Too little and pest resistance will be too weak. Too much and seed germination and plant growth will be affected.

Seed treatment was alsoeffective against *T.absoluta* and *S.litura*, demonstrating the conferral of protection to the plants against a wide spectrum of insect herbivores. In two different environments (Brazil and Bangladesh), plants grown from seed treated with MeJA hinder the development of *T.absoluta* and *S.litura*, lengthen the larval development time and reduce final pupal weight gain, complementing earlier results (Worall et al., 2012). Feeding on leaves from the seed-treated plants prolonged larval development time, potentially reducing the number of insect generations per year, stemming exponential insect population growth and consequently reducing the overall damage from the pest. Interestingly when MeJA and *Psuedomonas*were combined ina single treatment in Bangladesh, we observed significant reduction inseed germination. Further investigations are needed, but an antagonistic behavior of SA- and JA-mediated defense responses (Thaler et al., 2002; Koornneef and Pieterse, 2008), could explainour results.

In countries like Brazil and Bangladesh, where vegetables are extensively sprayed for crop protection in absence of alternatives (Moreira et al., 2002; Dasgupta et al., 2010), exploiting seed treatment could potentially prove a valuable tool for insect pest management. Despite several previous efforts to utilize host-plant resistance in Brazil (Maluf et al., 1997; Tadeu et al., 2006; Oliveira et al., 2012) and in Bangladesh (Ahmed et al., 2013; Bhat and Singh, 1994) against various insect pests, using elicitors of plants' defense has apparently not been exploited. Thus as we proceed with research, our results offer potential for the management of insects beyond reliance on chemical pesticides and traditional plant resistance via genotype selection.

A simple, environmental friendly tool that is capable of augmenting plant resistance makes seed treatment an attractive possibility for insect pest management. Unlike other induced defenses, here we observed that induction of defenses stimulated by seed treatment remained relatively constant during the various plant stages and the costs are mostly dependent on the dosage of the elicitor, which could be of additional value to the farmers. Seed treatments as opposed to scheduled foliar treatments require relatively small amounts of the treating agent, in this case MeJA. This makes MeJA seed treatment a considerably more attractive tool for pest management compared to foliar applications of defense activators. Furthermore, for those resource-poor farmers who grow annual crops from seed, treating the seeds with elicitors is a safe, effective and easily adopted technology thathas the potential to benefit such farmers in many ways. . However, sensitization of plant defenses with elicitors against pests should not be considered as a silver bullet strategy or an alternative to chemical pesticides, but rather as a potentially important component of an integrated program as suggested by Stout et al. (2002). Considering its apparent compatibility with other management tactics such as pheromones and cultural practices, integrating effectively with other facets of a management program would have a greater chance of significant benefit under field conditions (Kogan 1998; Way and van Emden, 2000). Despite this obvious potential, very limited efforts have been made to exploit elicitors at a large scale.

To further exploit this technology in the context of sustainable agriculture, more studies incorporating the effectiveness of seed treatment under normal agricultural practices and their economic analysis should be conducted. There is also a need to improve our mechanistic understanding of the molecular regulation of these kind of induced defense and examining biotic agents such as *Trichoderma spp* and *Pseudomonasspp* as treating agents against insects as they are found with plant diseases (Abuamsha et al., 2011; Nagaraju et al., 2012).

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Figures:

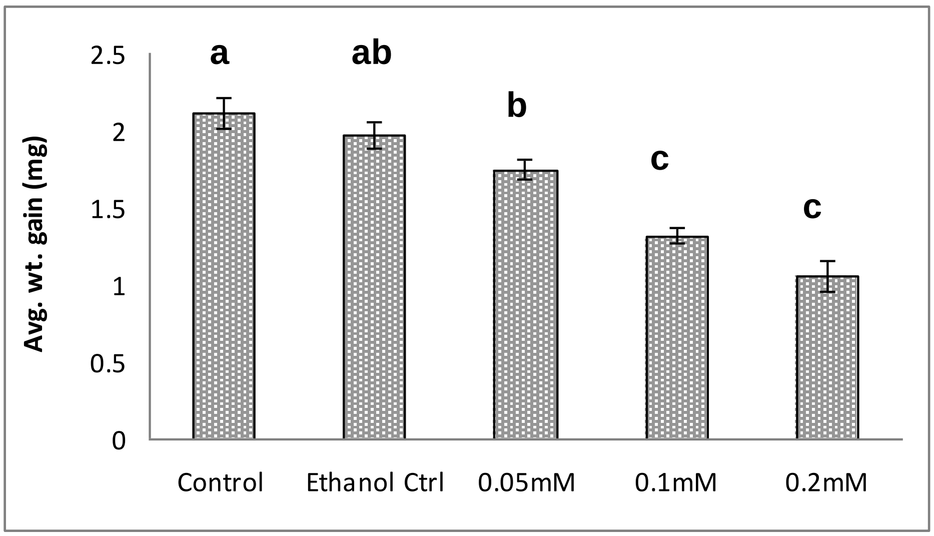

Figure 2-1: Average weight gain (AVG) of *H.zea* neonates on 25 day-old-tomato leaves grown from seed treated with three concentration of MeJA (0.05mM, 0.1mM and 0.2mM) for 72 hours. Within each figure, bars are $\bar{x}$ ± SEM, and the letters above bars indicate statistically different means as determined by a Tukey HSD means comparison test

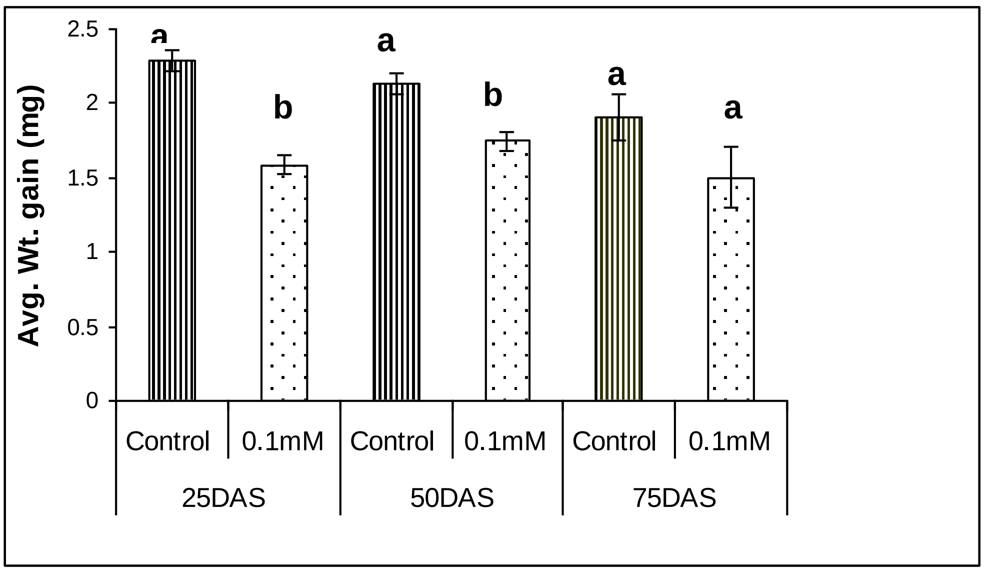

Figure 2-2: Average weight gain (± SEM) of *H.zea* neonate larvae on leaves of 25, 50 and 75 days old plants grown from seeds treated with 0.1mM MeJA, in 72 hr detached-leaf bioassay. Within figure, bars are $\bar{x}$ ± SEM, and the letters above bars indicate statistically different means as determined by a Tukey HSD means comparison test

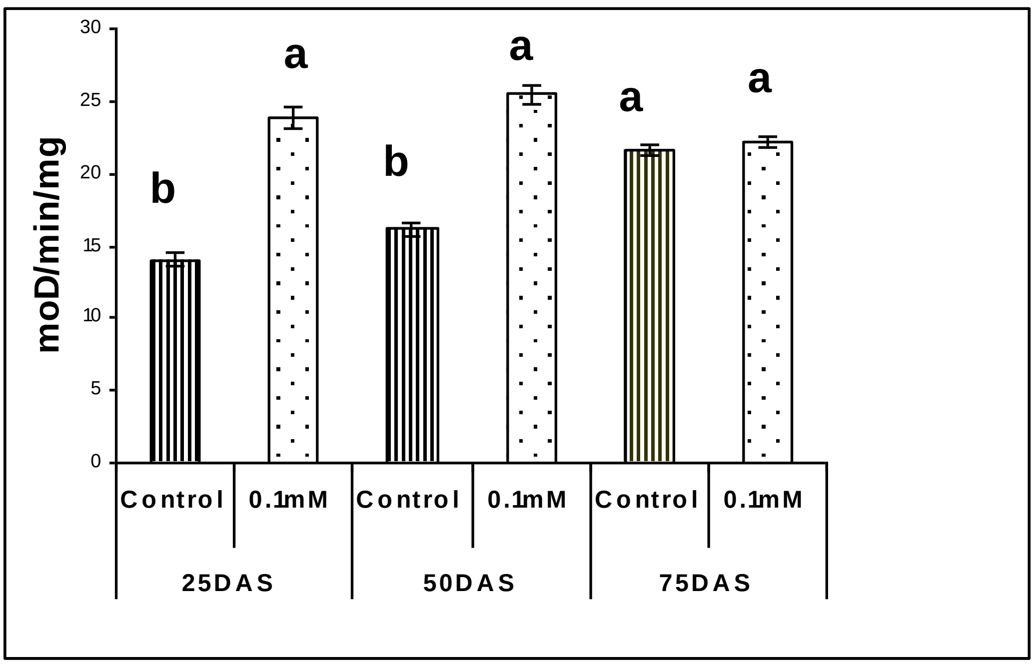

Figure 2-3: Polyphenol activity (± SEM) in leaves of 25, 50 and 75 days old plants grow from seeds treated with 0.1mM MeJA. Within each figure, bars are $\bar{x}$ ± SEM, and the letters above bars indicate statistically different means as determined by a Tukey HSD means comparison test

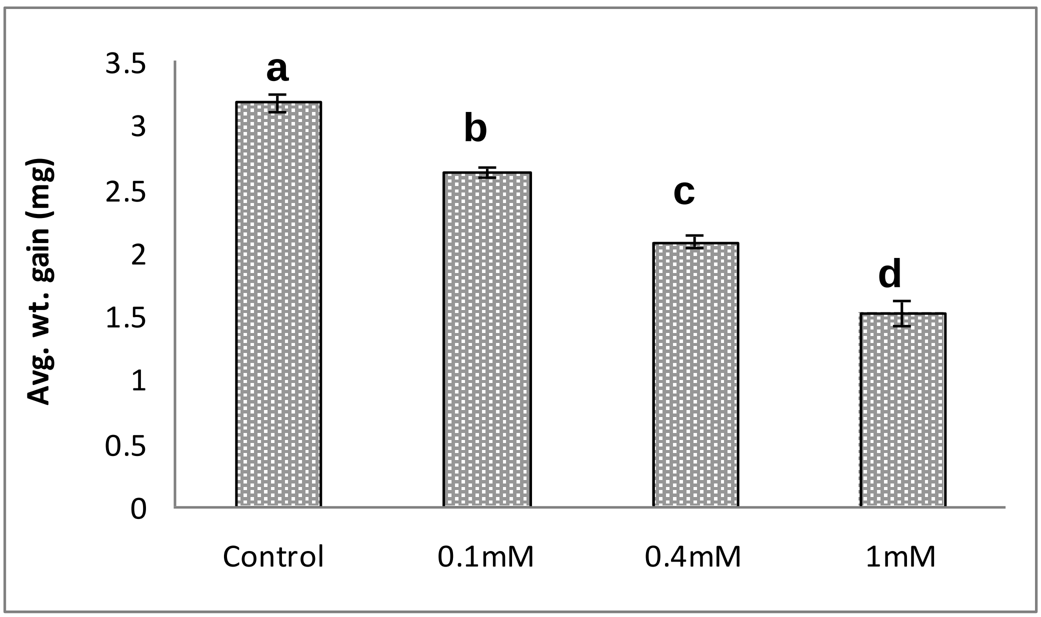

Figure 2-4: Bioassay results with *H.zea* neonates in tomato on leaves of 25-day-old tomato plants grown from seed previously treated with three concentration (0.1mM, 0.4mM and 1mM) of methyl jasmonate (MeJA) for 72 hours. Within figure, bars are $\bar{x}$ ± SEM, and the letters above bars indicate statistically different means as determined by a Tukey HSD mean separation test

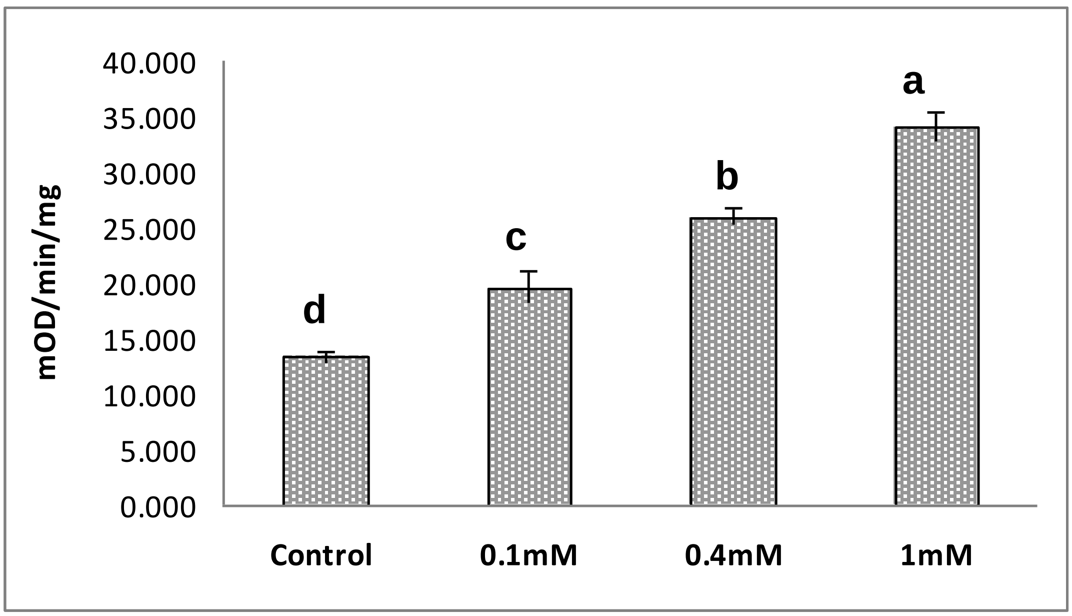

Figure 2-5: Polyphenol activity on seed-treated tomato leaves with three different concentration of MeJA (0.1mM, 0.4mM and 1mM). Within each figure, bars are $\bar{x}$ ± SEM, and the letters above bars indicate statistically different means as determined by a Tukey HSD means comparison test

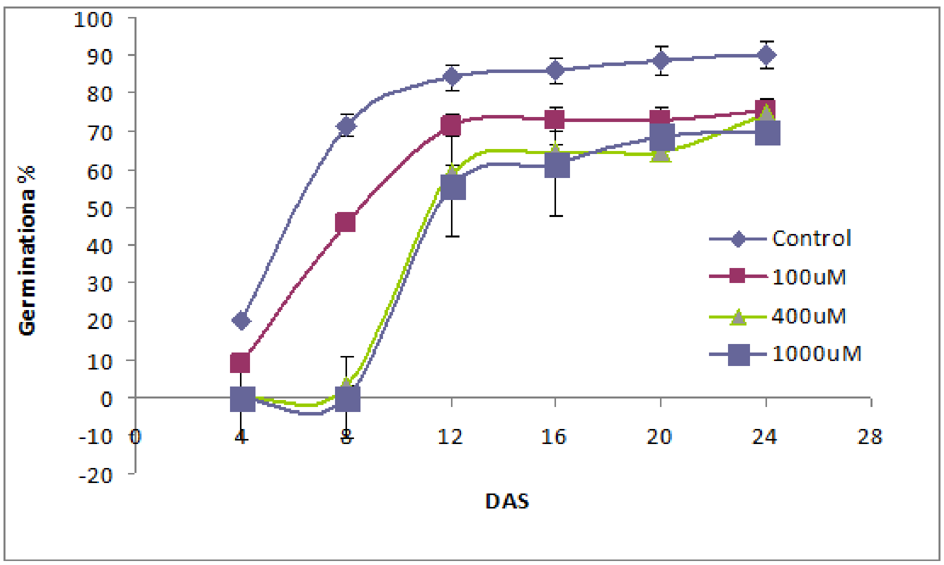

Figure 2-6: Percentage germination of the seeds treated with three doses of MeJA (0.1mM, 0.4mM and 1mM) and monitored for four weeks. Within figure, bars are $\bar{x}$ ± SEM.

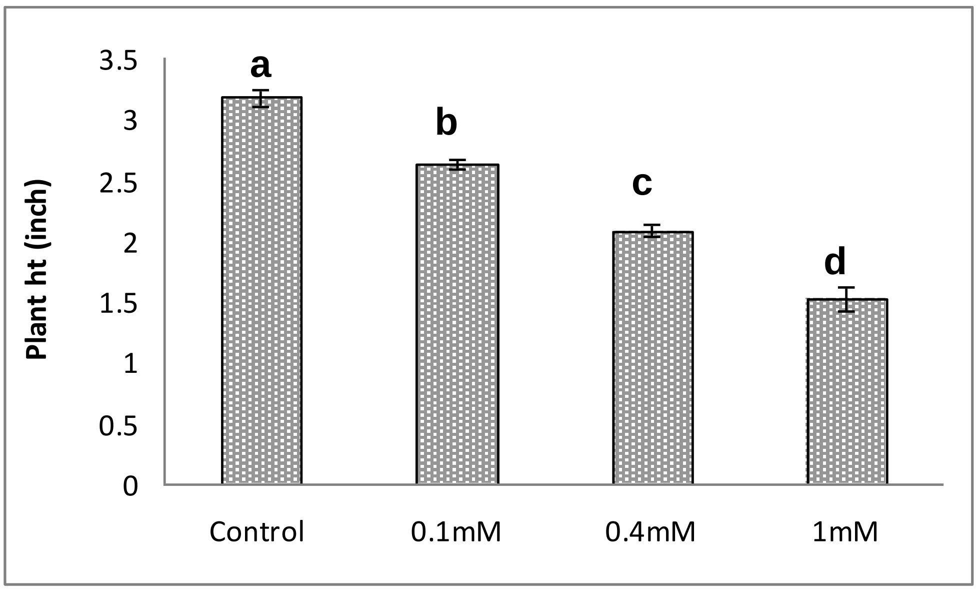

Figure 2-7: Height of tomato plants previously seed treated with different concentration of MeJA (0.1mM, 0.4mM and 1mM) after 25 days of sowing. Within each figure, bars are $\bar{x}$ ± SEM, and the letters above bars indicate statistically different means as determined by a Tukey HSD means comparison test

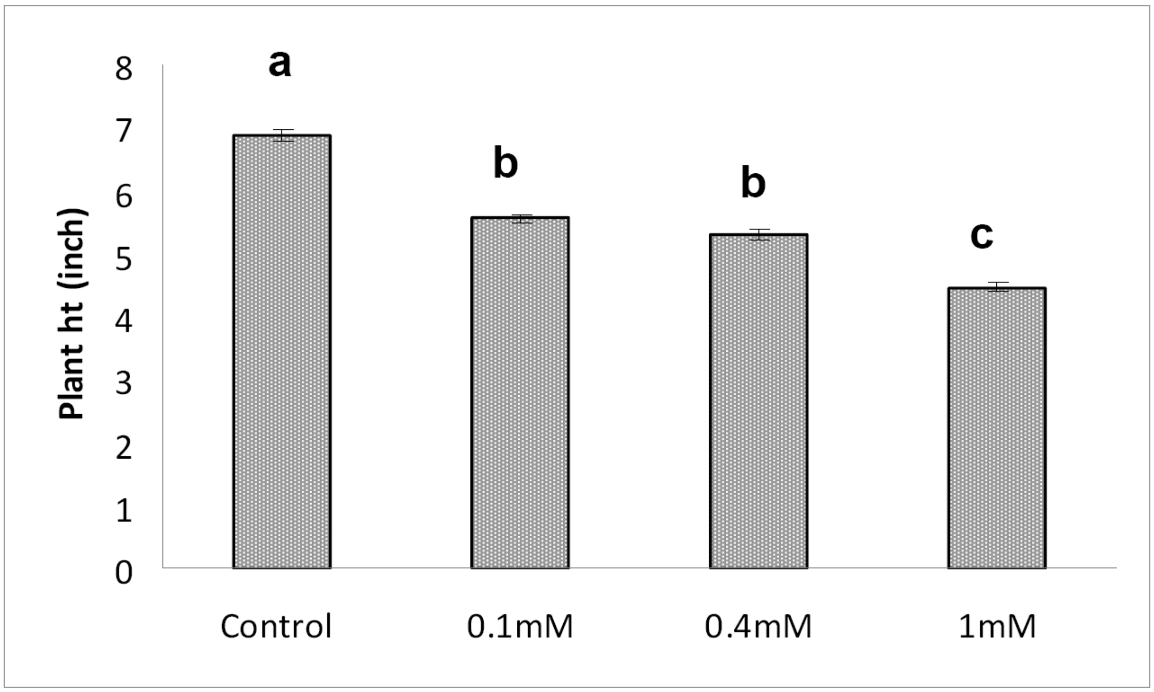

Figure 2-8: Height of plants previously seed treated with different concentration of MeJA (0.1mM, 0.4mM and 1mM) after 50 days of sowing. Within each figure, bars are $\bar{x}$ ± SEM, and the letters above bars indicate statistically different means as determined by a Tukey HSD means comparison test

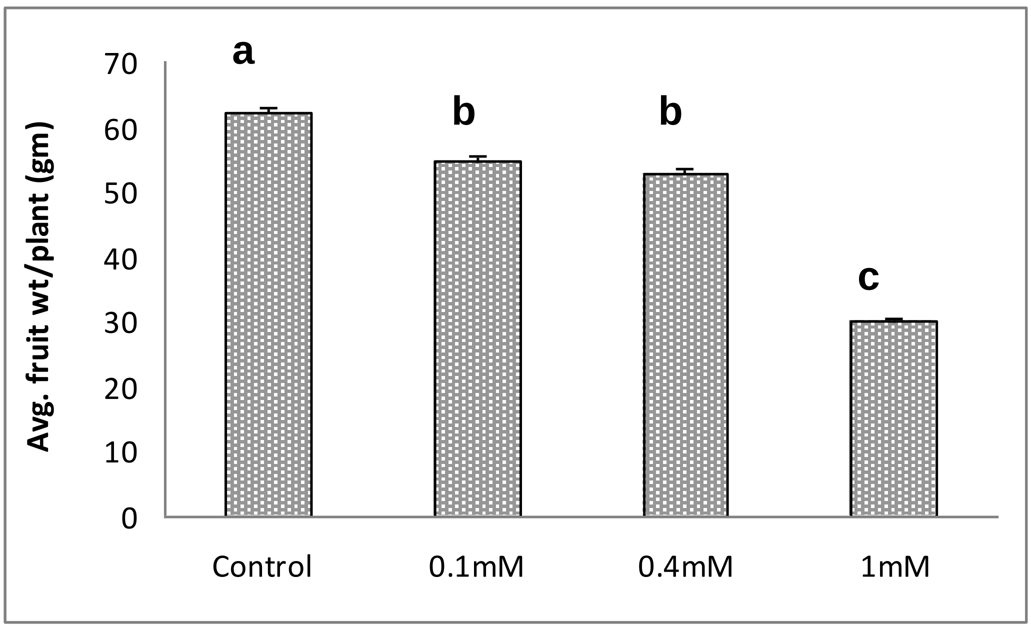

Figure 2-9: Average weights of fruit yield per plant from the plants previously seed treated with three different (0.1mM, 0.4mM and 1mM) concentrations of MeJA. Within each figure, bars are $\bar{x}$ ± SEM, and the letters above bars indicate statistically different means as determined by a Tukey HSD means comparison test

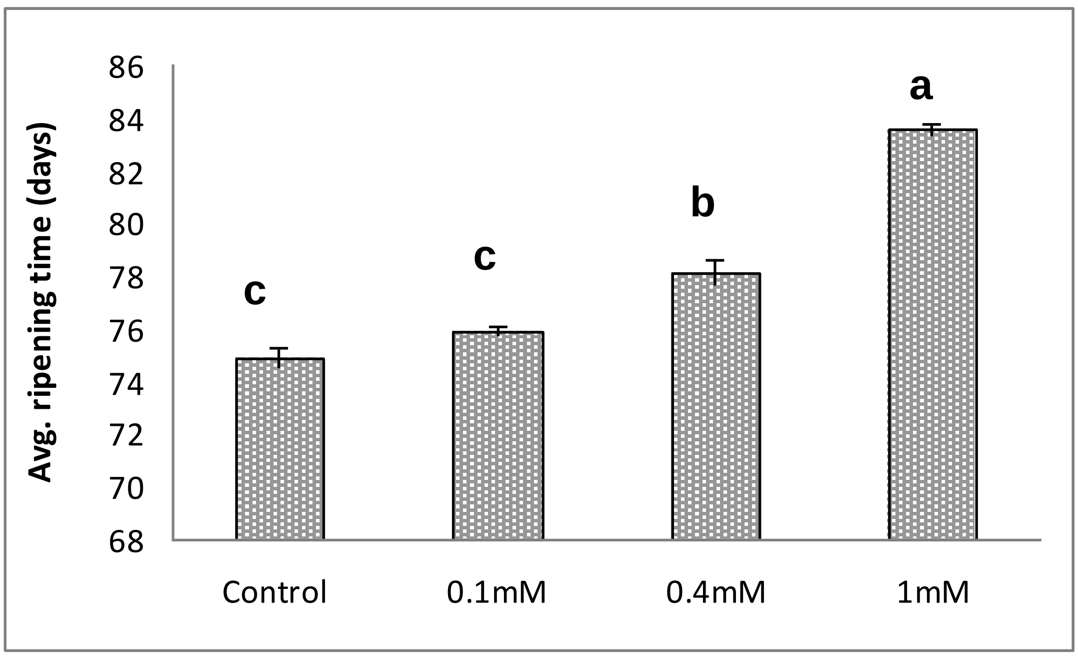

Figure 2-10: Average ripening time of the plant previously seed treated with three different (0.1mM, 0.4mM and 1mM) concentrations of MeJA. Within each figure, bars are $\bar{x}$ ± SEM, and the letters above bars indicate statistically different means as determined by a Tukey HSD means comparison test

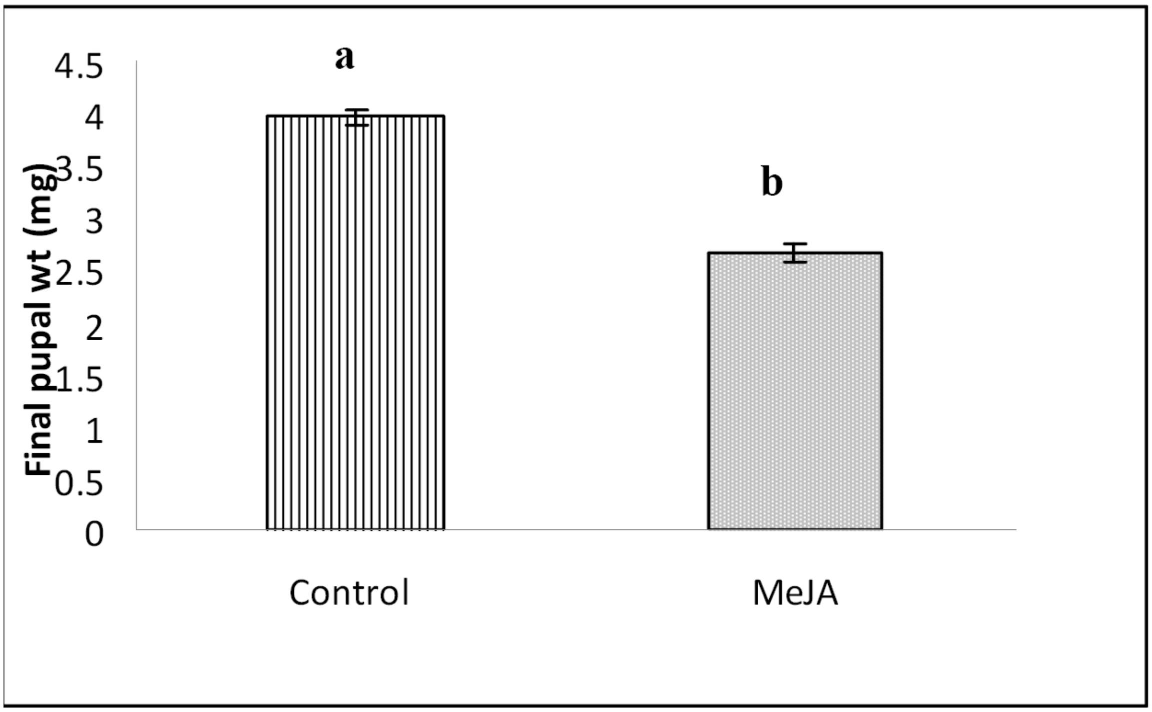

Figure 2-11: Average pupal weight (mg) from feeding bioassays with *Tuta absoluta* larvae fed on the leaves at UFPR, Brazil. Within each figure, bars are $\bar{x}$ ± SEM, and the letters above bars indicate statistically different means as determined by a Tukey HSD means comparison test

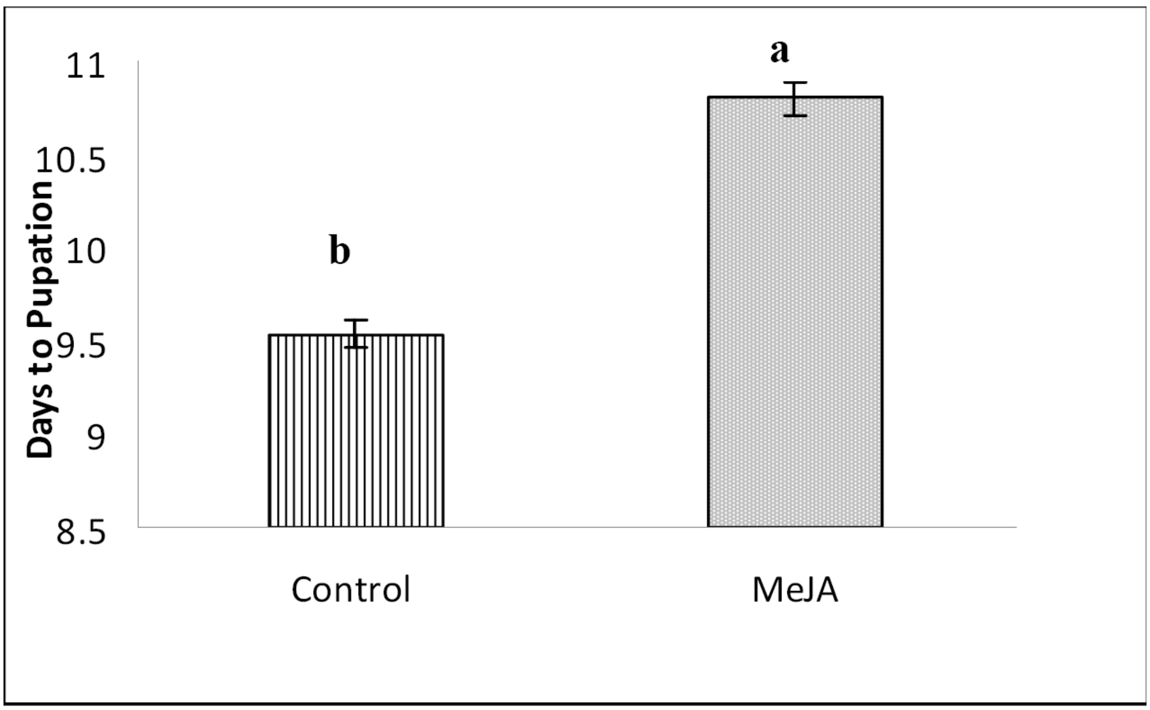

Figure 2-12: Average length of *T.absoluta* development (days) from larvae to pupae at UFPR, Brazil. Within each figure, bars are $\bar{x}$ ± SEM, and the letters above bars indicate statistically different means as determined by a Tukey HSD means comparison test

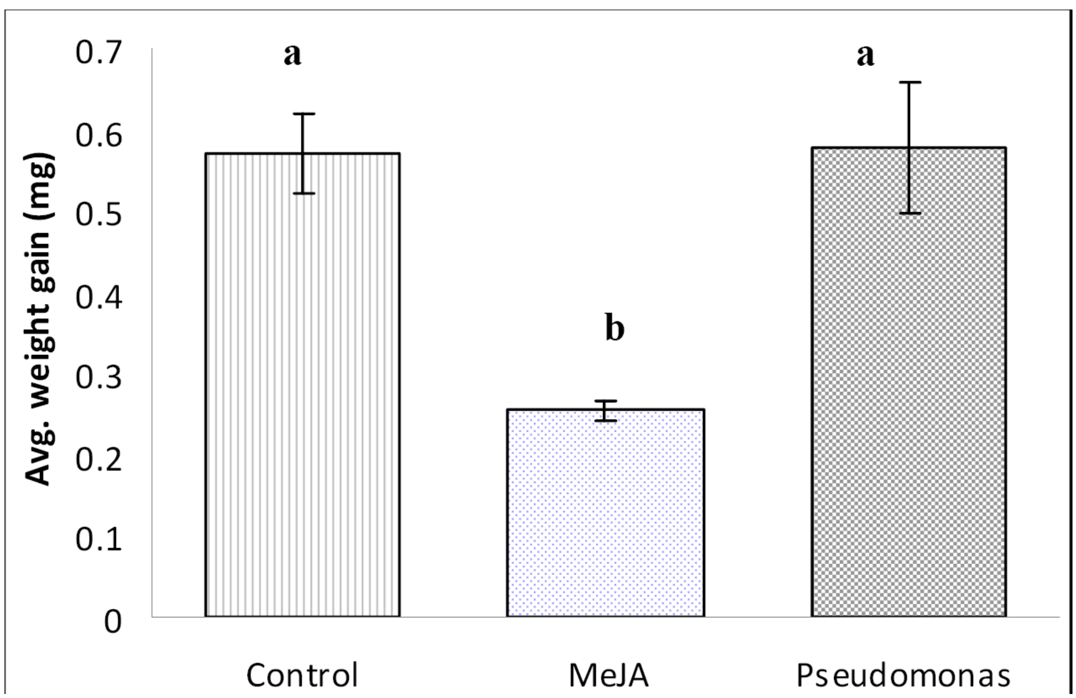

Figure 2-13: Feeding bio-assay results with *Spodoptera* neonates on tomato at BARI, Bangladesh. Within each figure, bars are $\bar{x}$ ± SEM, and the letters above bars indicate statistically different means as determined by a Tukey HSD means comparison test

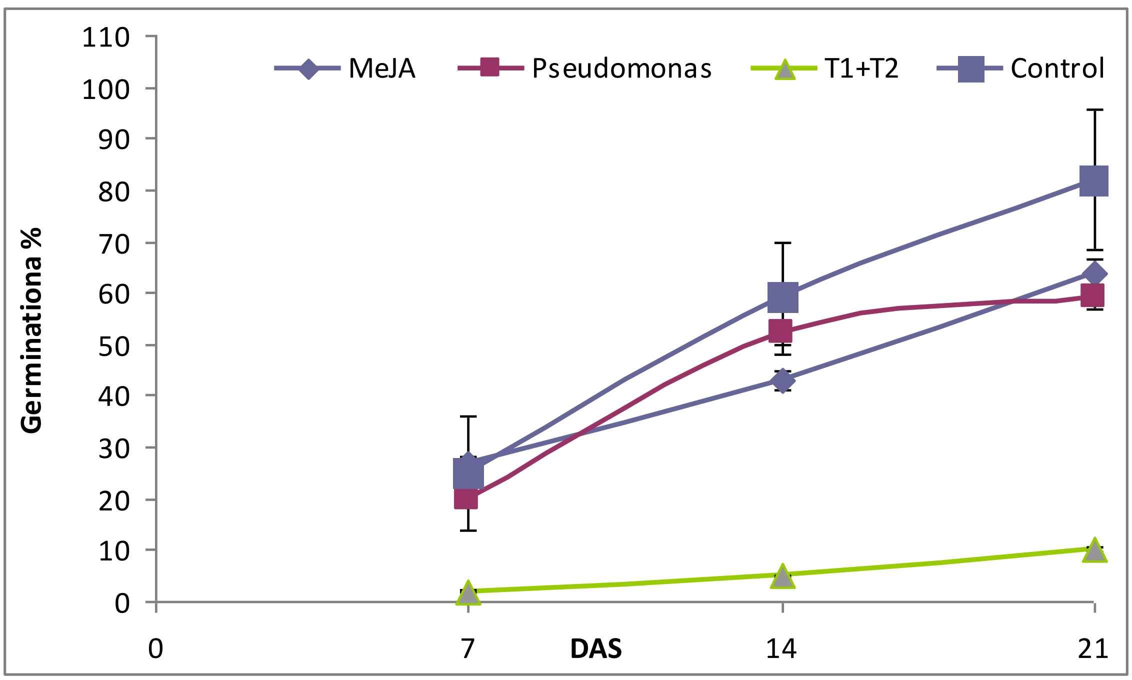

Figure 2-14: Percentage germination of tomato seeds among seed treatments, monitored for 3 weeks after sowing at BARI, Bangladesh. Within figure, bars are $\bar{x}$ ± SEM

CHAPTER 3
PRESENT PEST MANAGEMENT PRACTIVES AND POTENTIAL OF SEED TREATMENT TECHNOLOGY AMONG THE SMALL HOLDER FARMERS IN BANGLADESH

3.1 Introduction

Increasing population pressures and widespread food deficits in tropical, developing countries have compelled national and international programs to place a high priority on improving agricultural productivity and the economic wellbeing of small farmers. Intensive farming and mass monoculture has resulted in injudicious use of chemical pesticides to control insects and pests (World Resources, 1998-1999). Inadequate product labeling and farmers' lack of information are often the primary causes (Dung & Dung, 1999; Dung et al., 1998; Huan and Le Van Thiet, 2000). This is in spite of efforts to seek suitable agriculture production methods that balance improving land productivity while conserving the environment (Pretty, 1999).

Bangladesh, officially the People's Republic of Bangladesh, is a country in South Asia, whose economy is largely dominated by agriculture. Agriculture is the single largest producing economy since it comprises about 18.6% of the country's GDP and employs about 45% of the total labor force (https://www.cia.gov/library/publications/the-world-factbook/geos/bg.html). Various factors such as rapid population growth, decline in cultivable land, subsistence farming, lack of farmer awareness and pest problems have directly contributed to agriculture underdevelopment (Robanni et al., 2007).

Insect pests' infestation has hampered the agriculture growth in Bangladesh for a long time, and it still remains one of the major constraints of production, especially in vegetables (Robbani et al., 2007). An overall annual crop loss of 10 to 15 % has been reported due to insects and diseases without any intervention (Meisner, 2004), which however goes up to 30-40 % with vegetables, thus greatly hampering agriculture transformation (Rahman, 2007). Therefore, to protect their crops, vegetable farmers are applying pesticides up to the time of harvest, and the crop is shipped directly to market with no waiting period. Therefore, farmers are continually exposed to high health risks in the absence of proper pesticide regulations and policies (Meisner, 2004), emphasizing the need for sustainable pest management. Thus, the impact of current trends of pesticide use on future agricultural productivity, human health and environmental sustainability can, therefore, no longer be overlooked.

Since smallholder farmers produce most of the vegetables, pest management needs to be economical and easily accessible at the local level. Integrated pest management in vegetable agriculture requires multiple tactics and technologies to protect the crop from insects, diseases, weeds and other pests throughout the growing season. For smallholder farmers atechnology like 'seed treatment against insects', which simply requires dipping of seeds into the plant hormone solutions like Methyl Jasmonate (MeJA), is a simple, cost-effective, environmentally friendly and low-tech approach, which holds considerable potential to benefit a large number of farmers from Bangladesh and surrounding nations. Methyl Jasmonate (MeJA) is a volatile organic compound of Jasmonic acid, which is a naturally produced, non-toxic compound that, when applied to seeds provide season long resistance to pests of the plant. MeJA makes up2-3% of jasmine oil, one of the main odor components of jasmine flower (*Jasminus officinale*), which is widely cultivated in the tropics.

Utilization of the plants' own defense mechanism by treating the seeds with plant growth hormones (PGR) such as MeJA to induce resistance is a technology that has proved effective in the laboratory and greenhouse (Worrall et.al., 2012,). Our research also indicated that the treated seeds produce resistance against several insects like *Helicoverpa zea, Spodoptera litura and Tuta absoluta* (Paudel et al., unpublished data). Furthermore, we found that the seed treated plants exposed to herbivores are more resistant to herbivores than those of undamaged naïve plants, and these induced plants' resistance is relatively consistent throughout the all the plant ages (Paudel et al., unpublished data). This technology can be easily and economically exploited to produce seeds for crops with enhanced protection to insect pests.

In this paper we summarize the present pest management strategies and the level of pesticide use among small holder farmers in Bangladesh.Wethen interpret the results to suggest how integrated pest management (IPM) technology such as 'seed treatment against insects' which has a great appeal to the smallholder farmers, could be efficiently exploited in Bangladesh.

3.2 Methodology

This study was conducted in Bangladesh during the summer of 2012. We supplemented a literature search with personal observation made during several field trips to vegetable farms across the country. We assessed how anIPM technology like ‘seed treatment using defense elicitors’ could fit into the national Integrated Pest Management program. We also consulted withgovernmental officials, non-governmental organization staff, colleague scientists as well as observing farmers on their farms. Meanwhile, secondary information was obtained from library, newspaper archives, government, university and non-governmental organization (NGO) websites.

3.3 Results

3.3.1 Present status of vegetable production in Bangladesh

Agriculture in Bangladesh is characterized by smallholder farmers cultivating small plots of land. The average size of landholdings is only 0.5 hectares, whereas their share in total cultivated land is around 69%. Due to inappropriate land reform policies and rapid urbanization, the farm size is regularly declining; the average farm size of 1.4ha in 1977 to 0.6 ha in 1996 land (Thapa and Gaiha, 2011). Rice and jute are the primary crops in terms of production, however in the last few years, maize and vegetables are gaining popularity among farmers because of their financial and economic returns (Sahabuddin et al., 2002). As a result, the government has called for a shift from “rice-led” growth to a more diversified production system that includes vegetable crops (Hoque, 2000; Karim et al., 2005). Vegetables are also increasingly exported to European markets. In the year 2003-04, Bangladesh exported vegetables worth US$ 24.7 million, which constitutes 60.08 percent of the earnings from agriculture products (Karim et al., 2005). Vegetable production in Bangladesh increased after the 1980s, with an average annual growth rate of 2.8%, (AVRDC, 2003). Eggplant, various cucurbits, cowpea etc. are predominantly grown summer vegetables, whereas winter vegetables include tomato, cabbage, cauliflower, eggplant etc.

3.3.2 Excessive application of chemical pesticides

Pesticide use in Bangladesh has increased almost four foldduring the past two decades; 11224.89 MT in 1996 to 42240.63 MT in 2010 (BBS, 2010), resulting in increasing health and environmental hazards. Vegetable farmers, to protect their crops from insect pests are applying pesticides up to the time of harvest, and the crop is shipped directly to market with no waiting period. Moreover, many vegetables are consumed whole, resulting in maximum exposure to residues (Dey, 2010). Eggplant (*Solanum melongena*) and tomato (*Solanum lycopersicum*), two of the important vegetables, are sprayed frequently in comparison with other vegetable crops (Karim, 2004). More than 60% of the farmers apply insecticides to eggplant 140 times or more in a growing period of six to seven months and insecticides comprise approximately 32% of the total vegetable production costs (Karim, 2004).

From 2008 to 2009, 7,438 pesticide-related deaths have been reported nationwide amongst men and women aged 15-49 (IRIN, 2010). The extensive use of chemicals in vegetables was a major factor in the pesticide-related health issues as farmers apply pesticides on their crops without taking proper protective measures and thus exposing themselves to highly poisonous pesticides. Thus, they inhale substantial amounts of the pesticides they spray to kill insects or pest in their crops. In a country where estimated 56 million (75% of the civilian labor force) people are directly or indirectly engaged in the agriculture, this serious condition that cannot be overlooked (IRIN, 2010). The current trends in insecticide use are not only unsustainable but, if continued, have the potential to adversely affect productivity and environmental quality (AVRDC, 2003).

3.3.3 Weak governmental policies

The production and distribution of pesticides is growing dramatically and, in the absence of proper pesticide regulations and policies, farmers continue to be exposed to high health risks (Meisner, 2004). Several studies clearly document the fact that farmers' lack of awareness and inadequate labeling are one of the prime causes of the excessive increase of pesticide application in Bangladesh (Jackson, 1991 and Ramaswamy, 1992). This is one of the biggest challenges for a country whose adult literacy rate is only 56.8 % (World Bank, 2010). Policy regarding procedures for registration, import, manufacture, sale, packaging and advertisement was drafted as 'The Pesticide Rules, 1985' (http://www.dae.gov.bd/pdf/PESTICIDE-RULES-19851.pdf). Despite the rules and regulations, lack of proper enforcement and monitoring has contributed to several malpractices, including product adulteration and selling banned products. Many pesticides are sold in the market without names or under false labels, and with no clear warning or instructions to farmers, contravening the law.

Due to concerns regarding the environmental and health effects of pesticide use, high rates of pesticide use in vegetable production have emerged as a policy concern in Bangladesh (Hossain et al., 1999). In response, integrated pest management (IPM) policies are being developed to ensure a sustainable agriculture that includes reducing pesticide use (http://www.moa.gov.bd/policy/IPM.pdf). While the IPM approach often requires much more prudent use of pesticides than the methods they seek to replace, IPM still remains very unfamiliar to farmers and is erroneously considered too labor intensive and possibly prone to failure to protect fully against pest attacks. Therefore, the rate of adoption of IPM technologies by the smallholder farmers still remains very low (Dasgupta et al., 2004).). In addition, the weak extension system, lack of co-ordination among research and extension organizations and very few, often inadequately trained extension officers are considered important barriers to the adoption of IPM technologies in the farmers' fields (Uddin et al., 2008). Moreover, the weak public sector extension system is pitted against the well-funded marketing efforts of multinational agricultural firms.

3.4 Discussions and recommendations

Despite widespread concerns about the sustainability of conventional agriculture based on agro-chemicals, use of chemical pesticides continues to grow at an alarming rate.

Smallholder farmers in Bangladesh, who are largely illiterate and uninformed about alternative practices, are thus relying heavily on intensive use of pesticides as a sole pest management practice, representing risks to themselves and the environment. Therefore sustainable agricultural development is needed to address current agriculture concerns, which requires multi-dimensional approaches and introducing non-chemical IPM is certainly an important dimension. While, the need to strengthen pesticide regulations and promotion of IPM approaches are recognized in various governmental programs and policies, very little action has been taking to address these needs. Below is a summary of recommendations which we believe would help to promote and encourage IPM activities in the country and thus reduce the pesticide use:

- As the majorities of farmers are uneducated and are uninformed about current improved practices of farming, there is a strong need to raise farmers' awareness of the impacts of pest management decision-making, and the alternatives to the current chemical-based approach. Therefore to significantly check the pesticide flow in the agriculture crops, it is really important to make farmers and general public aware of pesticide poisoning and alternatives to chemicals. Farmers rarely have access to training in IPM methods and thus, without proper knowledge of natural enemies, pest pressure, re-entry period, pre-harvest interval (PHI) etc. are forced to assume that the only solution to pest problems is to apply insecticides more frequently. Hence, educational and training programs about the safe and appropriate use of pesticides and potential alternatives focusing on use by smallholder farmers should be implemented (Dasgupta et al., 2010). Effective use of mass media like television, radio or national newspaper could be one of the potential ways.

- Research on appropriate chemical-based pest control strategies (user-friendly formulations) with active participation of farmers, which can be integrated with more selective biological activity at lower rates of applications, should be prioritized in national research organizations like BARI, and agriculture universities. Integrated approaches based on expanding theoretical, methodological and empirically based studies, which is currently lacking, will produce solutions that are more effective, sustainable and competitive against current pest problems.
- Pesticide policies have to be improved and strongly implemented. Governments should formulate strict regulations regarding registration of pesticide traders, small sales kiosks and importation of pesticide products. Packaging and labeling should be standardized, coupled with strong monitoring mechanism.
- National IPM policy needs to be revisited emphasizing more community IPM activities such as farmer-farmer training. Also, to extend IPM coverage (e.g current IPM Farmer Field School programs) to all the corners of the country, adequate government and donor funding should be made available.
- The government should build a mechanism (e.g high level co-ordination committee) to improve the institutional capacity of current extension offices forging strong linkages among various players in the extension systembesides governmental extension departments, such as industry, private consultants, NGOs, universities, farmers' associations, research institutes, and others.

With the advent of modern agriculture, more sophisticated tools and techniques have been developed as alternatives to agro-chemicals. Unfortunately for farmers in developing countries like Bangladesh, these kinds of technologies are either not available for the crop of interest, or they are too costly and thus farmers are obliged to use chemical pesticides. Efficient use of simple and efficient IPM techniques such as ‘seed treatment’ could potentially revolutionize crop protection strategies in Bangladesh and surrounding nations. Classical plant breeding normally requires years/decade to produce resistant cultivars, but by using the seed treatment approach, seed could be produced with enhanced pest resistance in a very short period of time (24 hours treatment). Also, these kinds of techniques are entirely compatible with classical breeding programs and the integration of other pest management strategies (e.g., cultural practices).So, here we present a few possible steps to implement ‘seed treatment’ program in Bangladesh:

a) Screening of the technology under a range of agro-ecological zones (multi-location trials) to evaluate the suitability for poor and resource limited farmers. Economic analyses of benefits and should be established to encourage farmers with benefits.
b) Rather than considering the technology as an alternative to the chemicals, it needs to be disseminated as one of the technologies under the IPM package: a set of well tested and formulated activities from the seedling development to harvest, so that farmers are highly benefited.
c) The next important thing will be to make the seed treating agents readily available, so that farmer can have an easy access. Methly Jasmonate, which makes up 2-3% of jasmine oil, is one of the main odor components of jasmine flower (*Jasminus*

officinale), which is widely cultivated in Bangladesh. Therefore initiatives from governmental or any public organization to extract MeJA from the flowers would make the substance locally available and will reduce the costs too.

d) As a very little amount of treating agent is required, the feasible option will be to provide farmers with a ready-made solution of MeJA, so that a farmer can directly use a solutionto treat their seeds rather than the farmercalculating the amount of MeJA to add to the water before treatment (for e.g 50ml solution bottle of 1 mili mole MeJA to treat 100 seeds)

e) Government should encourage farmers who are using (or willing to use) non-chemical based pest management techniques ontheir farms to gainproper training andknow-how.

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CHAPTER 4
CONCLUSIONS AND FUTURE RESEARCH

Our study clearly demonstrates that the plants grown from seed treatment with the plant defense elicitor, MeJA hinders larval development and reduces overall damage to the plants. The preceding chapters have described the potential of defense elicitor to establish a long-lasting plants' resistance against multiple species of insects. We also document several fitness costs to plants associated with induction of plant defenses stimulated by seed priming. Furthermore, we conducted study on present pest management practices of resource-limited farmers in Bangladesh and presented few recommendations about the way technologies like 'seed treatment' could be implemented in the country. However, there are still several questions that remain unanswered in this thesis, which will require further research. In the following section, major findings of this dissertation research along with the potential future research are summarized.

3.1 Effectiveness of seed priming in real agriculture context

Data presented in Chapter 2 indicates that under greenhouse conditions, treating the seeds with elicitors results in enhancement of plant resistance against insect herbivory. However, very few studies have focused on the assessment of elicitor induction under field conditions and commercial success has been very limited (Stout et al., 2002; Worall et al., 2012). Underwood (1999) suggested that elicitors might not be effective in open field conditions in comparison greenhouse conditions because plants in the field continuously encounter many potential inducing factors and therefore are normally in an induced state. Therefore, further research to assess effectiveness under field conditions may have useful applications in agriculture. Identifying the systems where priming treatments will be most efficacious and profitable and exploring seed priming effects on indirect plant defenses need to be studied. Once efficacy is established in several crops, the next step will be to devise strategies for use that are economically feasible in different systems. Cultural practices (such as fertilization), which has been largely ignored in elicitor studies so far, could have an important role to play on the final outcome by accentuating the responses of plants to elicitors or minimizing the costs of induction (Stout et al., 2002).

3.2 Molecular regulation of seed-primed defense

Although research on anti-herbivore defense using elicitors has undergone exciting developments over the past decade, knowledge about the regulatory mechanisms underlying the stimulated long-lasting and durable plant defenses still remain sparse (Kim and Felton, 2013). Therefore, understanding the epigenetic regulatory mechanisms will be one critical step forward to further exploit the potential in agriculture. Regulatory genes can be used in marker-assisted breeding programs, or can be exploited in transgenic approaches to engineer second-generation GM crops that require less chemical agents to control diseases and insects.

3.3 Potential of using biotic agents to induce plant defenses against insects

Even though a variety of environmental signals such as plant defense elicitors, volatile organic compounds (VOCs) and several pathogens, have been found to trigger plant defenses (Conrath et al., 2006; Frost et al., 2008; Kim and Felton, 2013), commercial plant hormones and chemical signals are mostly used (Stout et al., 2002; Conrath et al., 2006; Ton et al., 2009). Recent reports have demonstrated that biotic agents such as plant growth-promoting rhizobacteria (PGPR), including *Trichoderma spp* and *Pseudomonasspp.,* when applied to seeds induce plant defenses conferringresistance against various pathogens (Wei et al., 1991; van Peer et al., 1991; van Loon et al., 1998; Abuamsha et al., 2011; Nagaraju et al., 2012), along with improving plant physiological characters (Mastouri et al., 2010). These kinds of biological agents may have substantial commercial potential beyond current uses and provideadvantages such as: effectiveness against various pests, stability due to the action of different mechanisms of resistance, systemicity, energy economy, and metabolic utilization of genetic potential for resistance in all susceptible plants (Bonaldo et al., 2005). However, testing these organisms in context of anti-herbivore defense is almost non-existent. Therefore, future evaluation of these biological agents is needed to further strengthen the current knowledge.

3.4 Amount of seed priming agents, resistance levels and fitness costs

The results of this study (chapter 2) suggest that seeds treated with higher dosages of elicitor result in increasedplant resistance against insects and isaccompanied by higher levels of PPO activity in leaves. Subsequently, we also observed that with higher dosages, fitness costs included a significant reduction of the percentage of plant germination, seedling growth and affected other reproductive traits (ripening time, fruit quantity). However, we did not measure other variables such as flower number and the dry weight of plants, which may have influenced some of our parameters. Furthermore, the extent to which the plants can be induced and whether there exists any boundary beyond which they cannot be induced, are topics that need further exploration.

3.5 Seed priming and opportunity for the small-holder farmers

As discussed earlier (chapter 3), use of chemical pesticides in developing countries is increasing at alarming rates. Small-holder farmers, who are largely illiterate and uninformed about alternative practices, rely on intensive use of pesticides as a sole pest management practice, representing risks to themselves and the environment. Thus, utilization of plant defense mechanisms by application of plant defense elicitors may contribute to the mitigation of the growing problem of unregulated pesticide use. Furthermore, for those resource-poor farmers who grow annual crops from seed, seed treatment, which is a safe, effective and easily adopted technology, has the potential to benefit such farmers in many ways if integrated well with other facets of pest management (e.g., cultural practices). As, very little research has been undertaken using host plant resistance in developing countries, utilization of induced plant defense against insect pests needs to receive attention. More studies exploring the potential of these small IPM technologies in the farmers' field with proper demonstration of techniques should be conducted to encourage farmers. Furthermore, proper analysis of benefits and costs of using the technologies over the existing ones needs to be established for a farmer to feel safe and convinced.

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