Report on,

"Evaluating the effect of seed priming against Tomato leafminer, Tuta absoluta (Lepidoptera: Gelechiidae) in tomato crop in Curitiba, Brazil."

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1. Executive Summary:

Following our successful research trials with seed priming against economically important pests like Tomato fruit borer, *Helicoverpa zea &* Tobacco Caterpillar, *Spodoptera litura* at Penn State and Bangladesh respectively, the current project was designed to explore the potential and effectiveness of seed priming in a varied agro-ecological zone against one of the serious South American pests on Tomato, *Tuta absoluta*. In particular, we assessed the effectiveness of Methyl Jasmonate (MeJA) seed treatment on enhancing the plants' resistance against South American tomato moth (*T.absoluta*), in the southern part of Brazil. All the experiments were conducted inside greenhouse during March-April, 2013 at University of Federal Parana, Curitiba-Brazil. Our final result suggested that larvae fed on the plants treated with MeJA affects the life-cycle of the insects; lengthening larval developmental phase and reducing the final pupal weight, thus greatly reducing the overall damage from the pest. Thus, if successfully integrated with other facets of integrated pest management program, the use of MeJA as elicitors of plants' defense could prove to be an important tool in managing *Tuta absoluta*, without relying heavily on chemical pesticides.

2. Introduction and Objectives:

Brazil, one of the rising economic powers of the world today, has drastically improved its agriculture production in the past few decades. In less than 30 years Brazil has turned itself from food importer into one of the world's great breadbaskets (Brazilian Ministry of Agriculture, 2010). With its intensively developed agriculture, widespread and growing trend of pesticide use in agriculture is increasing in alarming rate to manage several kinds of insect pests, posing a serious threat to environment and health (Bellotti et al., 1990; Dasgupta, 2000; Siqueira et al., 2010). That comprise the accumulation of various biotic and abiotic factor in the segments of ecosystems (biota, water, air, soil, sediments etc.) and also several cases of diseases and health problems have also been reported in human populations (Caldas et al., 2008).

Brazil is one of the top ten countries producing large volume of tomatoes in the world (FAO, 2009). Most part of the production comes from southeast region of the country representing one of the main poles for the vegetable production region (USDA Report, 2009). Tomatoes are one of the most noted pesticide used crop which are extensively sprayed for crop protection: reaching 56.5 kg/employee/year, a value five times greater than the average in the southeastern region; the region characterized with many small holder farmers and intensive poly-culture farming (Moreira et al., 2002).

The tomato leaf miner or tomato pinworm, *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) is native to South America (Giordano & Silva, 1999). But, recently it has been found to invade Europe and North America (Garcia & Vercher, 2010; Desneux et al., 2010). This is one of the most serious pest of Tomatoes and other solanaceous crops such as potato, eggplant etc. in Brazil, as its larvae mine into the leaves and also bores inside fruits and stems (http://www.tutaabsoluta.com/). It is serious pest of both outdoor and greenhouse tomatoes. The insect deposits eggs usually on the underside of the leave, stems and to a lesser extent on fruits.

After hatching, young larvae bore into fruits, leaves on which they feed and develop creating mines and galleries. On leaves, larvae feed on mesophyll leaving the epidermis intact. Infestation can be at any developmental stage, from seedling to mature stage. At high densities, damage could be really serious resulting in a loss of almost 80% (Picanco et al., 1998). The adult moth has a wingspan around one centimeter. 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) is mostly what are used to manage the *T. absoluta,* in absence of any effective alternative strategies (Siqueira et al 2000). Besides being increasing the production costs, there is continuous danger of environmental hazards, affecting natural enemies (El-wakeil et al., 2008) and resistance development with continuous overuse of chemicals (Siqueira et al., 2000; Silva et al., 2011). Variety of other tactics such as pheromones, use of plants' resistance and bio control agents have been tested against the insect but with limited commercial success (Braham & Hajji, 2011). Thus, utilization of plants' own defense mechanism by seed priming to induce resistance against insect pests may play an important role in reducing the pest population without any negative affect on environment and human health.

Plants are induced for defenses when they are attacked, but the lag in response may allow herbivore sufficient time to damage the plants. Thus, as an alternative strategy that allows quicker defensive response without incurring the full metabolic cost of maintaining a high level of defense is to prime the defenses. Priming reduces the fitness cost associated with induced defenses, in which the plants are capable of responding more rapidly toward any kind of future stress (Kuc, 1987; Conrath et al., 2002, 2006; Conrath and Gollner, 2008). A variety of environmental signals such as different plant defense elicitors, volatile organic compounds (VOCs) & several pathogens, have been found to trigger priming of plant defenses (Stout et al., 2002; Conrath et al., 2006; Frost et al., 2008). Varieties of plant hormones and other chemical signals have also been identified to activate priming responses (Stout et al., 2002; Conrath et al., 2006). Recently, we (Paudel et al., unpublished data) and few others (Worall et al., 2012) demonstrated that seeds are also receptive to plant defense activators like B-Amin), Jasmonic Acid (JA) and Methyl Jasmonate (MeJA).

In our experiments conducted at Penn State and Bangladesh, plant defenses are found to be successfully induced following seed treatment with MeJA (Paudel et al., unpublished data). MeJA is a volatile organic compound of Jasmonic Acid (JA), which is a naturally occurring, non-toxic compound which is believed to play an important role in insect and plant resistance (Lian-You et al, 2004). From the bioassays we performed with neonate larvae on plant leaves from different developmental plant stages, larval weights were found to be significantly reduced with seed primed plants as compared to the control plants. Subsequently, polyphenol oxidase (PPO) activity, which is considered to play an important role in plants' induced resistance, was found elevated with seed primed plants across all the developmental stages.

There have been several research done previously to exploit plants' resistance against Tomato leaf miner (*Tuta absoluta*), but are mostly focused on genotypes selection only (Maluf et al., 1997; Gilardon et al., 2001; Tadeu et al., 2006; Oliveira et al., 2012). Exploring the potential of plant defense elicitors in enhancing plants' resistance against *T. absoluta,* to our knowledge has never been explored before. Thus, here we examine the hypothesis that treatment of tomato seeds with MeJA primes plant for defense against *Tuta absoluta,* reducing the overall damage.

3. Materials and Methods:

Initial visit to University of Federal Parana (UFPR) was made during first week of March, 2013. Lab and greenhouse under the facilities of Department of Chemistry, UFPR was being used for all the experiments.

Seed Treatment: 50-60 seeds were treated with 0.2mM of Methyl Jasmonate concentration (Sigma Aldrich Brazil ltd). Methyl Jasmonate was dissolved in a small amount (0.25%) of ethanol and made up to a desired concentration with distilled water. Following the treatment, seeds were washed twice in distilled water before sowing. A similar lot of seeds were soaked only in distilled water for 24 hours before sowing representing controls.

Plant Material: All the experiments were conducted inside the greenhouse of University of Federal Parana (UFPR). Tomato (*Solanum lycopersicum* cv Santa Clara I-5300) plants were used in all of the experiments. Seeds were originally purchased from Tomato Growers Supply and the seedlings were grown in potting mix (Linha Ornamental, Brazil) in a greenhouse at UFPR. The greenhouse was maintained on a 16-h/8-h light/dark photoperiod and temperature ranging from 25 to 30°C. Seedlings were regularly watered in every 3-4 days. Leaves from one month old plants were used for insect feeding.

Herbivore Bioassay: Population of *Tuta absoluta* was maintained in one of the laboratory inside UFPR. Newly hatched *T.absoluta* larvae were placed in a caterpillar cup with leaves. The petioles of the leaves were wrapped in cotton cloth to maintain turgidity. The experimental design was completely randomized with two treatments (Control and MeJA treatment), 60 replications and two larvae per replications. In every three days, new set of excised leaves were presented to the insects to make sure that the leaves are fresh and are not withered. The containers were observed daily by noting the occurrence of pupae and finally weighed. The parameters evaluated were: duration of larval development and pupal weight.

Statistical Analysis: Plants were randomly allocated to treatment which allowed us to use of completely randomized design. All the bioassays and other data were analyzed using ANOVA and the means were separated with Tukey's test. All data were checked for normality and were analyzed using 'Minitab 16.0' software.

4. Results and Discussion:

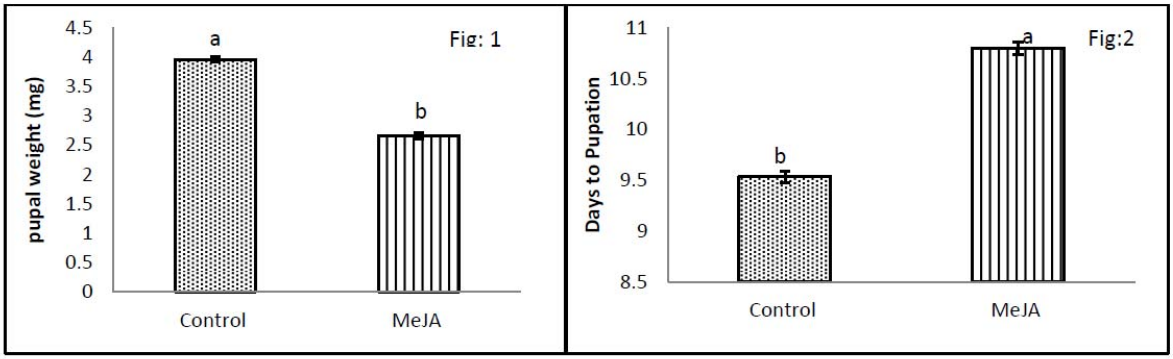

Fig.1: Average pupal weight (mg) from feeding bioassay with *Tuta aboluta* larvae fed on the leaves **Fig.2:** Average length of development from larvae to pupae (days). Within each figure, bars are $\bar{x} \pm$ SEM, and the letters above bars indicate statistically different means as determined by a Tukey test.

Duration of the larval stage of *T.absolata* was found significantly lengthened with seed primed tomato plants in comparison with the control (Fig.2) (MeJA: F=229.61, df=1 :p=0.00). On an average, larvae feeding on the leaves from tomato plants took 1.3 days more to pupate in comparison with those fed on control plants (Fig.2). Similarly, larvae reared on leaves from the treated plants showed significantly lower pupal weight in comparison with those reared on the leaves from untreated ones (Fig.1) (MeJA: F=341, df=1 :p=0.00).. But, we couldn't found any significant difference regarding larval survivorship; in both of them 72% of larvae were found to survive.

Thus, we found out that the plants grown from seed treated with MeJA hinders the development of *T.absoluta*, lengthening the larval development and also reducing the final pupal weight gain, which confirms its effectiveness in enhancing the plants' resistance against insect complementing our previous results (Paudel et.al, unpublished data) and others too(Worall et al., 2012). As the larva grown on leaves from the plants treated with MeJA successfully prolonged the larval development, it would certainly play a role in reducing the number of generations per year and consequently reducing the overall damage from the pest.

A simple, environmental friendly tool which is capable of augmenting plants' resistance makes seed priming potentially valuable tools of insect pest management. There have been several efforts to utilize host-plant resistance in Brasil against Tuta absoluta, but focus has been on genotype selection mostly (Maluf et al., 1997; Gilardon et al., 2001; Tadeu et al., 2006; Oliveira et al., 2012). Thus as we proceed with the work, our research results will certainly help others to look the management of *T. absoluta* beyond chemical pesticides and genotype selection, encouraging them on testing the potential of seed priming along with other non-chemical approach as an alternative pest management tool.

Very few researches to this date are found to explore the potential of these kinds of plant defense elicitors on real field conditions. Considering its compatibility with other management tactics, integrating it with other facets of management program such as pheromones and cultural practices would produce a good result in the field conditions too(Kogan 1998; Way & van Emden, 2000). As suggested by Stout et al (2002), priming plants with elicitors against pests cannot be considered as an isolated strategy or an alternative to the chemical pesticides, but rather as a potentially important component of an integrated program.

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