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INDUCTION OF RESISTANCE IN COTTON (*GOSSYPIUM HIRSUTUM*) AGAINST *HELICOVERPA ARMIGERA* AND *EARIAS VITELLA* BY ENVIRONMENTALLY SAFE CHEMICALS

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Abstract

Induced resistance was studied in cotton against bollworm (*Helicoverpa armigera* Hub.) and spotted bollworm (*Earias vitella* Fab.) by treating the plants with Salicylic acid, Bion (Benzo (1,2,3) thiadizole-7-carbothioc acid (S) methyl ester) and Jasmonic acid. At flowering stage, a set of plants were sprayed with different concentrations of Salicylic acid (1.0, 1.5 & 2.0 mM) and Bion (0.4, 0.8 & 1.2 mM). The mortality of *H. armigera* was measured by placing the third instar larva in Petri dishes containing leaves (old & young) from each treatment. At boll formation stage another set of plants were sprayed with Salicylic acid (1.5mM), Bion (0.8mM) and Jasmonic acid (0.1mM) for induced resistance against spotted bollworm (*E. vitella*). Control plants were sprayed with water only. *E. vitella* were released on the plants three days after induction treatments and percentage infestation was recorded. Results indicated that Bion and Salicylic acid- induction on *H. armigera* was systemic (young leaves) and local (old leaves) in cotton plant. Little decrease in spotted bollworm was observed with all the chemicals tested, while decrease on Jasmonic acid treated plants was higher than other treatments. There is need for detailed studies with biochemical analysis of defense compounds accumulated or produced after induction treatments.

Introduction

Cotton (*Gossypium hirsutum*) is the backbone of Pakistan's economy but it is attacked by a number of insects and diseases from seedling to fruiting stage. Many pesticides have been used so far for controlling the insect and disease problems but their indiscriminate use resulted into the development of insect pest resistance and disturbance of agro-ecosystem.

The ability of plants to actively defend themselves with inducible and constitutive mechanisms has been extensively demonstrated in many systems (Karban & Baldwin, 1997; Sarwar *et al.*, 2005). Host plant resistance, an important component of integrated pest management, which can be triggered by biotic (pathogen, non-pathogen) or abiotic elicitors (like simple chemicals), can be used very effectively when combined with selective pesticides and induced resistance technique. Inbar *et al.*, (1998) reported mild and inconsistent effect of Benzo (1,2,3) thiadiazole-7-carbothoic acid (S) methyl ester (BTH/Bion) on insect herbivores. BTH reduced the density of the leaf miner (*Liriomyza trifolii*) in tomatoes but not larval survival. Induction of systemic acquired resistance studied in cotton to phytophagous insect by BTH (Inbar *et al.*, 2001) indicated highest levels of systemic induction in young leaves. However, this induction had little effect on the insect herbivores tested (*Remisia tabaci* and *H. armigera*) except for a local effect on whitefly oviposition. Inducible defenses play a major role in conferring disease resistance against plant pathogens (Maleck & Dietrich, 1999) and their effects on phytophagous insects can include increased toxicity, delay of larval development or increased attack by insect parasitoids (Baldwin & Preston, 1999). Inducible defenses are thought to compromise plant fitness less and may be more durable than constitutive defense mechanisms (Agrawal, 1998). This paper reports that induction of resistance in cotton by treating the plants with Salicylic acid, Bion and Jasmonic acid had little effect on *H. armigera* and *E. vitella* survival. These observations led to investigate this technique in cotton in detail.

Materials and Methods

Plant material and induction treatments: Cotton plants (*Gossypium hirstum*) of variety NIAB-86 were grown in earthen pots of 12" diameter under natural environmental conditions. Plants were arranged in eleven groups, each set consisted of three pots and one plant was maintained in each pot. Di-Ammonium Phosphate (fertilizer) was applied once at 6-week age. No pesticide was used throughout the experiment. At flowering stage, three sets of the plants were sprayed with different concentrations (1.0, 1.5 & 2.0 mM) of Salicylic acid (SA) and other 3 sets were sprayed with Bion (0.4, 0.8 & 1.2 mM) for induced resistance against *Helicoverpa armigera*. One set served as control was sprayed with water only. At boll formation stage, 3 sets of the plants were sprayed with 1.5 mM SA, 0.8 mM Bion and 0.1 mM Jasmonic acid (JA) for induced resistance against spotted bollworm (*Earias vitella*). One set which served as control was sprayed with water.

Test insects, rearing and performance

***H. armigera*:** Cotton bollworm *Helicoverpa armigera* was collected from cotton field at NIAB and reared on artificial diet containing chickpea powder as main ingredient under controlled laboratory conditions (Temp. 27±2ºC, RH 65±5% and L14:D10 h) as described by Ahmed & McCaffery (1991). A homogenous stock of third instar larvae was obtained from F1 generation for testing. Three lower (old) leaves and three upper (young) leaves were cut from control and induced plants, one week after induction treatments and placed individually in Petri dishes. The young leaves grew after the induction treatments. A single third instar larva of *H. armigera* was placed in each Petri dish. The source of larvae was a colony reared on artificial diet at constant temperature of 27±2ºC. Plates were kept at room temperature for 96 hours and mortality was noted after every 24 hours.

***E. vitella*:** Spotted bollworm, *Earias vitella* collected from cotton field was fed on okra fruit to get F1 generation under laboratory conditions similar to *H. armigera*. Third instar larvae were collected for testing. Spotted bollworms were released on all the treated plants, induced at boll formation stage, three days after induction treatments. Plants were placed in a cage, covered with net to keep the insects inside. Infested and total bolls were counted on each plant 10 days after releasing the insects and percentage infestation was calculated.

Data were analyzed statistically by applying completely randomize design CRD.

Results

Mortality of *H. armigera* was observed at 24, 48, 72 and 96 h after feeding on old leaves (treated leaves) of induced plants. The results showed that all the induction treatments significantly affected the survival of *H. armigera* in Petri dishes except highest concentration of SA (Fig. 1). Lowest concentration of SA (1.0 mM) and highest concentration of Bion (1.2 mM) showed 67% mortality of bollworm, *H. armigera* after 24 h., followed by 33% mortality with 1.5 mM SA, 0.8 mM Bion and control plants. However, 67% mortality of the bollworm was observed with 0.8 mM Bion after 48 h and from leaves of control plants same percentage of mortality was recorded after 72 h., while 100% mortality was observed after 96 h with only two concentrations of SA (1.0 & 1.5 mM). The highest concentration of SA had no effect on insects. Induction treatments had significant effect on mortality of *H. armigera* when fed on young leaves of induced plants (Fig. 2). At 24 h, 67% mortality of the insects was observed with lowest concentration of Bion that increased to 100% at 48 h. After 96 h, 100% mortality was recorded on young leaves from treated plants with 1.0 and 1.5 mM SA and 1.2 mM. Bollworm fed on leaves of control and treated plants with SA (2.0 mM) and Bion (0.8 mM) showed 33% mortality at the end of the experiment.

Spotted bollworms (*E. vitella*) were released on SA, JA and Bion treated plants to study the induced resistance in cotton. Ten days after releasing the insects, infested and total bolls were counted, percent infestation was calculated (Fig. 3). Upto 46% bolls were infested on plants treated with JA while plants treated with SA and Bion showed 51 and 52% bolls infestation, respectively. The effect of induction treatments was statistically non-significant, but little decrease in boll infestation was observed with all the treatments, which was 20, 14 and 10% with JA, SA and Bion, respectively.

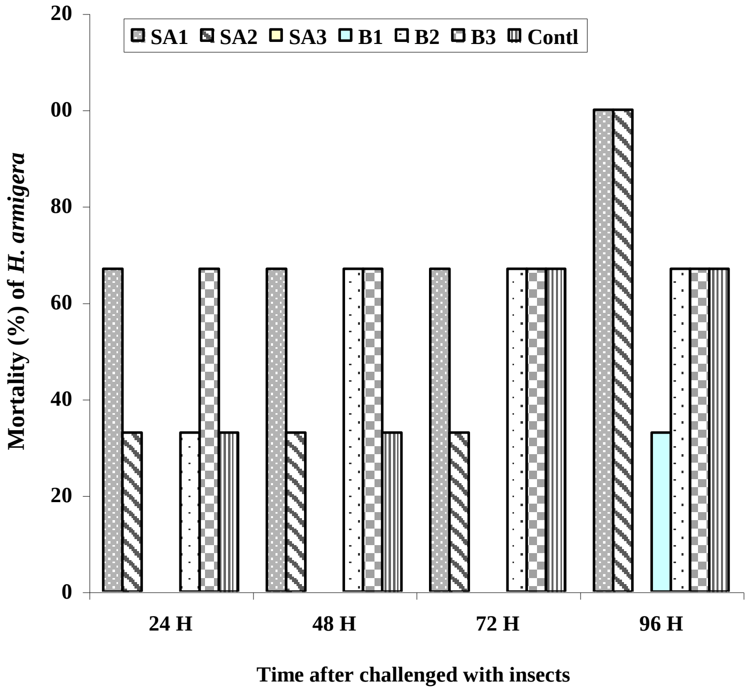

Fig. 1. Effect of different treatments on mortality (%) of *Helicoverpa armigera* fed on old leaves (treated leaves) of cotton in Petri dishes, at various time intervals.

SA1: 1.0 mM Salicylic acid; SA2: 1.5 mM Salicylic acid; SA3: 2.0 mM Salicylic Acid, B1: 0.4 mM Bion; B2: 0.8 mM Bion B3: 1.2 mM Bion, Contl: Control

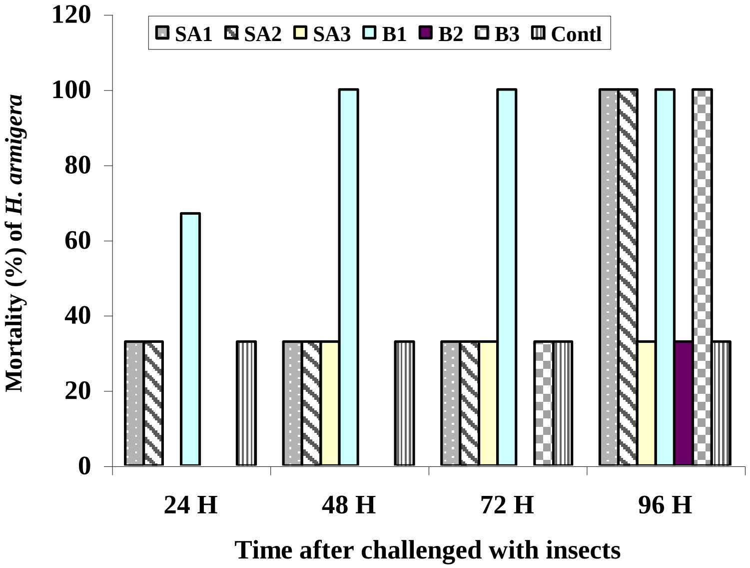

Fig. 2. Effect of different treatments on mortality % of *Helicoverpa armigera* fed on young leaves of cotton in Petri dishes, at various time intervals.

SA1: 1.0 mM Salicylic acid; SA2: 1.5 mM Salicylic acid; SA3: 2.0 mM Salicylic Acid, B1: 0.4 mM Bion; B2: 0.8 mM Bion B3: 1.2 mM Bion, Contl: Control

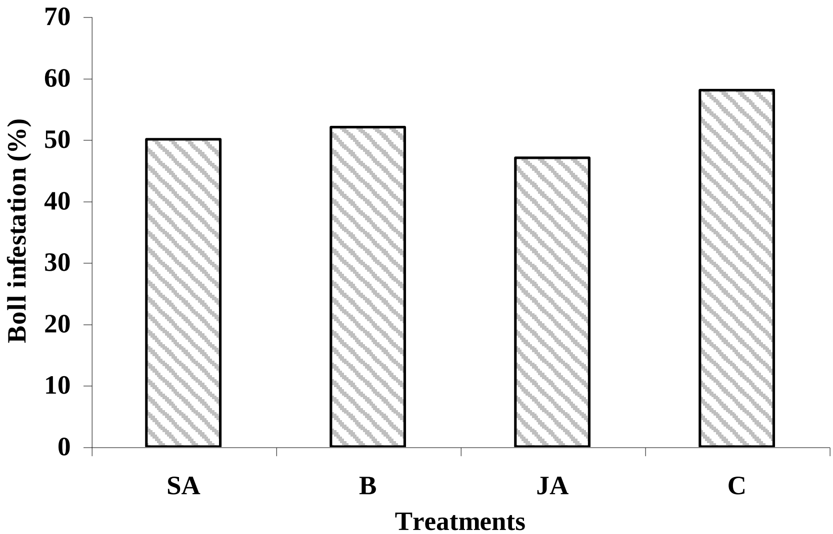

Fig. 3. Effect of different treatments, used for induction of resistance in cotton on spotted bollworm, *Earias vitella*.

SA: Salicylic acid (1.5 mM); B: Bion (0.8 mM); JA: Jasmonic acid (0.1 mM); C: Control

Discussion

Induced resistance has been considered a potential strategy for disease/insect pest control in plants (Agrawal, 1998, Allen, 2001). A number of studies on induced systemic resistance in plants against pathogens were reported (Karban & Baldwin, 1997) but little work has been done on induced resistance against insects. In the present studies 100% mortality of *H. armigera* when fed on old leaves of 1.0 & 1.5 mM SA induced plants indicated that SA induced resistance locally. Bollworm mortality was 67% on old leaves of plants induced with 1.0 mM SA and 1.2 mM Bion after 24 h while same mortality was observed with 0.8 mM Bion induced plants after 48 h. These findings indicated that defense related compounds might be produced more in plants induced with lowest concentration of SA and highest concentration of Bion as compared to other concentrations of both the chemicals (Agrawal, 1998; Heil & Bostock, 2002). Upto 67% mortality was observed on leaves of control plants after 72 h, which indicated that some defense compounds also produced in control plants due to environmental stress as the experiment was conducted in pots placed outside under natural environmental conditions. Upto 100% mortality was observed at 96 h on young leaves of treated plants with SA (1.0 & 1.5 mM) and Bion (1.2 mM), while lowest concentration of Bion (0.4 mM) showed 100% mortality just after 48 h. These results indicated that effect of SA and Bion–induction on *H. armigera* was systemic (young leaves grew after induction treatments) and stronger with lowest concentration of Bion as compared to other ones.

Present studies indicated that effect of Bion and SA induction on *H. armigera* was systemic in young leaves and local in old leaves in cotton plant. However, earlier studies reported that the effect of BTH (Bion) –induction on herbivores was found locally only in old cotton leaves (Inbar *et al.*, 2001). The difference may be due to some environmental stresses in present studies, since the experiments were conducted in pots under natural conditions, which may activate different defense pathways. It was also reported that mild and inconsistent effects of BTH –induced changes on insect herbivores, reduction in the density of leaf miner adults (*L. trifolii*) in tomatoes but not larval survival (Inbar *et al.*, 1998). Treatment with BTH had no effect on the population of the silver leaf whitefly (*B. argentifolli*) in tomato fields. Taking in account that the different pathways may not be similarly regulated in all systems (Maleck & Dietrich, 1999), it seems that the effect of ISR by Bion and SA on insect herbivores would vary among plant and insect species. The decrease in spotted bollworm population on JA treated plants was higher than other treatments, this may be explained as JA serves as a signal for expression of a number of defense-related compounds, such as proteinase inhibitors, oxidative enzymes and numerous phenolics (Heil & Bostock, 2002), against insects and pathogens.

Present studies showed some positive effects of induction treatments on the mortality of *H. armigera* and *E. vitella.* Further studied with other insects are needed to know the effectiveness of induced resistance technique in cotton against insect/pests. Biochemical analyses of induced plants for defense related compounds accumulated or produced after induction treatments are required to support the data, which is in progress.

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