

**EFFECTS OF PLANT DEFENSE ELICITORS ON SOYBEAN (*GLYCINE MAX L.*) GROWTH, PHOTOSYNTHETIC PIGMENTS, OSMOLYTES AND LIPID COMPONENTS IN RESPONSE TO COTTON WORM (*SPODOPTERA LITTORALIS*) INFESTATION**

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**Abstract**

To evaluate the effect of two elicitors, methyl jasmonate (20  $\mu$ M) and sodium nitroprusside (500  $\mu$ M), on six soybean genotypes and to enhance the ability of susceptible genotypes to resist cotton leaf worm (*Spodoptera littoralis*) was carried out. Results showed that Giza 82 and 22 were susceptible genotypes, Giza 83 and 21 were moderate resistant genotypes and Giza 35 and 111 were resistant genotypes. Both treatments, methyl jasmonate and sodium nitroprusside, positively affected the morphological criteria, photosynthetic pigments, soluble protein, amino acids, glycolipids and phospholipids contents in shoots of all soybean genotypes. Lipid peroxidation and H<sub>2</sub>O<sub>2</sub> were significantly decreased in response to both treatments. Treatment with methyl jasmonate was found to be more effective than sodium nitroprusside and enhanced the resistance of the susceptible genotypes.

**Introduction**

Egyptian cotton leaf worm, *Spodoptera littoralis* (Lepidoptera: Noctuidae) is a serious pest of a large variety of crops in many parts of the world. It is mainly found in Africa, the Middle East and Southern Europe (Azab *et al.* 2001). Larvae of this pest can feed on more than 90 economically important plant crops belonging to 40 families such as cotton, soybeans and other vegetables (Sakthivelu *et al.* 2008). In Egypt, the insects are one of the most destructive pests of cotton- most valuable crop in the country. Over the past 25 years, the intensive use of broad-spectrum insecticides against *S. littoralis* has led the development of insect resistance to many registered pesticides (Aydin and Gurkan 2006).

Several environmental manipulations can be attained by employing a number of control measures like the use of chemical insecticides and cultural and physical control methods. Chemical pesticides are effectively used against storage insect pests but are inseparably associated with a number of drawbacks including high costs and concerns about environmental pollution and food safety (Mohamed and Abd-El Hameed 2014). For these reason, plants can be treated with elicitors to induce resistance to herbivores (Thaler *et al.* 2001). Several signaling pathways, including jasmonic acid (JA), salicylic acid (SA), ethylene, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and nitric oxide (NO) orchestrate the induction of defenses (Reymond and Farmer 1998).

JA is an endogenous signaling molecule implicated in regulation of plant resistance to herbivores and pathogens (Creelman and Mullet 1997). In addition, NO is a lipophilic gas and a small signaling molecule. NO induces changes in the expression of defense genes, which lead to metabolic changes enhancing plant defenses in response to attack by aphids (Morkunas *et al.* 2011).

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Soybean (*Glycine max* (L.) Merr.) is considered one of the important food and industrial crops on the international level, owing to containing about 30% of cholesterol free oil, about 40% of protein which is similar in its nourishing value to the animal protein and beneficiary secondary metabolites such as isoflavones, phenolic compounds and saponins (Sakthivelu *et al.* 2008).

This work is aimed to study the effectiveness of two elicitors, methyl jasmonate and nitric oxide, for controlling cotton leaf worm infestation under field condition and also to test their effects on soybean growth, pigments content, osmolytes, soluble protein, total lipid, phospholipid, glycolipid, lipid peroxidation and H<sub>2</sub>O<sub>2</sub>.

### Materials and Methods

A field experiment was conducted in Agriculture Research Centre farm, Egypt during the 2014 season. Day temperature ranged from 28 to 45°C with an average of 36.7 ± 3.1°C while that at night was 22.3 ± 2.2°C. Daily relative humidity averaged 43.5 ± 4.6%, in a range between 31.1 and 57.3%. Soybean seeds (*Glycine max* L. Merr. cv. Giza 21, 22, 35, 82, 83 and 111) were obtained from Agriculture Research Centre, Ministry of Agriculture, Giza, Egypt. Seeds were selected for uniformity by choosing those of equal size and with the same colour. The selected seeds were washed with distilled water, sterilized with 1% sodium hypochlorite solution for about 2 min and thoroughly washed again with distilled water and left to dry at room temperature (25°C) for about 1 hr. Rhizobial inoculants were applied as peat slurry containing 10<sup>7</sup> *Rhizobium*/g. Uniform air dried seeds were sown in rows with 60 cm spacing and hills were spaced 10 - 15 cm apart within each plot in the field on the 12<sup>th</sup> June. Thinning was done before first irrigation to secure two plants/hill. The soil had a clay loam texture (sand 20%, silt 25%, and clay 55%). Experiment was laid out in randomized complete block design, which consisted of three replications, and plots area 21 m<sup>2</sup> (4.2 × 5.0 m). Each plots had of six rows and space between plants were 50 cm. After 30 days after sowing the first group was sprayed with MeJA (20 µM), the second group was sprayed with SNP (500 µM) and the third group was sprayed with distilled water and serve as control. The treatment was repeated every 4 days three times. At 45 DAS, ten plants were randomly chosen from each replication and the following parameters were studied: shoot length (cm), fresh and dry weight/plant and biochemical components.

According to the size of eaten part of leaf (the defoliation is measured as a percentage of the leaf area destroyed by the pests); the accumulative damage caused by the defoliator larvae was estimated by scoring the damage (0 to 5) of each of 10 randomly chosen leaves. Rate of infestation was calculated according to the formula given by (Kasopers 1965).

Total photosynthetic pigments of leaves were determined by spectrophotometric method recommended by Vernon and Seely (1966). The total soluble protein content in the supernatant was determined according to Lowry *et al.* (1951). Free amino acids content was estimated according to Moore and Stein (1954). Lipid peroxidation was determined by estimating the malondialdehyde content following the method of Heath and Packer (1968). The H<sub>2</sub>O<sub>2</sub> level was colorimetrically measured as described by Jana and Choudhuri (1981). Lipids were extracted three times for air dried shoots of soybean plants according to Navari-izzo *et al.* (1989). Glycolipids were estimated as their monosaccharides content by the anthrone sulfuric acid reagent (Hedge and Hofreiter 1962). Lipid phosphorus was estimated by the molybdate blue colour (Woods and Mellon 1941).

All data were subjected to statistical analysis and means were compared by Duncan's multiple range test using Mstat C commuter package.

### Results and Discussion

The percentage of soybean leaves area eaten by cotton leaf worm was recorded in Fig. 1. Giza 82 and Giza 22 are the most susceptible genotypes for the cotton leaf worm and the percentage of eaten area was (34 and 28%). In addition, Giza 83 and Giza 21 are the moderate resistant but Giza 35 and Giza 111 are shown to be more resistant and the eaten area percentage was (22, 20, 18 and 13%), respectively. Results obtained are in agreement with Zayed (2007) who showed that Giza 111 is considered to be resistant genotype for cotton leaf worm as consumed leaf area recorded was (38.10 cm<sup>2</sup>) comparing with Giza 82 and Giza 22 which are susceptible genotypes with consumed leaf area (59.51 and 54.15 cm<sup>2</sup>), respectively.

Also, it was found that treatment of soybean plants with MeJA and/or SNP enhanced the resistance in susceptible genotypes by decreasing leaves eaten area percentage comparing with untreated plants. In addition, MeJA treatment achieved better results than SNP treatment. These results are in conformity with findings of Thaler *et al.* (2001) who found that the application of jasmonic acid caused reduction in the population of *Frankliniell occidentalis* and aphids in field plots of tomato.

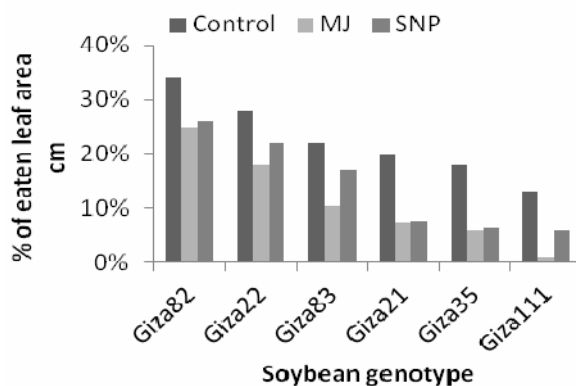


Fig. 1. Effect of foliar spray with MeJA and SNP on leaf area percentage eaten by cotton leaf worm of soybean genotypes.

As shown in Fig. 2, treatment with MeJA and SNP caused significant increases in shoot length, shoot fresh and dry weight in all soybean genotypes under natural field infection with cotton leaf worm. These results are in accordance with that of Abdelgawad *et al.* (2014) who found that pre-soaking maize grains in MeJA led to increases in plant height, fresh and dry weight of plant which supports our results that methyl jasmonate have enhancement effect on plant growth. Simaei *et al.* (2012) found that treatment with SNP clearly increased shoot fresh weight, on the other hand slightly enhanced shoot dry weight of soybean plants under normal conditions. Generally growth criteria in susceptible plants showed less value than resistant genotypes in both treated and untreated plants.

A significant increase in total photosynthetic pigments contents was recorded in soybean plants foliar sprayed with MeJA and SNP (Fig. 2). Also it is clear that susceptible plants possess less pigments content than resistant types under natural infection with leaf worm. Similar to our results obtained, Meyer and Whitlow (1992) found that spittlebug feeding decreased photosynthetic pigments content below control levels in goldenrod. Elicitors like methyl jasmonate can reverse the decreasing in photosynthetic rate caused by herbivorous insects

(Abdelgawad *et al.* 2014). Also, spraying of cotton plants with SNP enhanced total chlorophyll content under abiotic stress (Shallan *et al.* 2012).

The stronger overall stimulatory effect of MeJA on photosynthetic pigment accumulation could be due to its stronger effect on the chlorophyll synthesis pathway specially  $\delta$ -ALA (amino levulinic acid) (Beale 1978). MeJA treatment caused an increase of active cytokinin concentration which enhances chlorophyll accumulation in potato plant (Kovac and Ravnkar 1994). The decrease in the photosynthetic pigments may be due to the inhibition of pigment biosynthesis, Mg deficiency or due to damage of palisade tissue which contains the chloroplast or to the effect of ROS on these pigments (Khatab 2007).

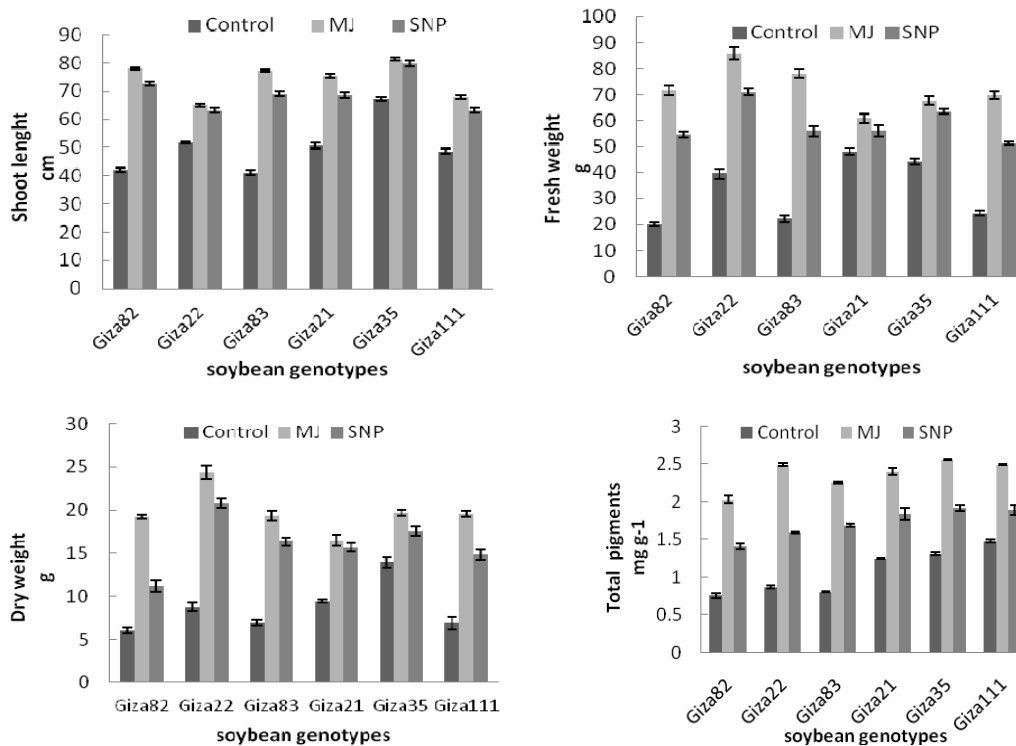


Fig. 2. Effect of foliar spray of MeJA and SNP on three morphological criteria and total photosynthetic pigments content of soybean genotypes under natural infection with cotton worm.

Plants may tolerate herbivore damage (i.e., reduce the impact of herbivory) through compensatory processes such as photosynthetic enhancement or increased growth rates (Strauss and Agrawal 1999).

Foliar spray of different soybean genotypes with MeJA and SNP caused significantly increased in total soluble protein and total free amino acids content as compared with control plants under natural field infection with cotton worm (Fig. 3). Alkylation of amino acids reduces the nutritional value of plant proteins for insects, which in turn negatively affects the insect growth and development (Bhonwong *et al.* 2009). These results are similar to War *et al.* (2012) who found that proteins content were increased in three groundnut genotypes damaged with insect as

compared to uninfested control plants. In general, the induction was greater in the insect-resistant genotypes than in the susceptible one. Increase in the protein concentration may be due to the generation of defense-related proteins after stored insect's infestation. Plants produce a number of defense-related enzymes and other protein-based defensive compounds (Chen *et al.* 2009; Helmi and Mohamed 2016).

Plants when damaged by herbivorous insects increased amounts of antinutritive, toxic proteins and secondary metabolites that interfere with oviposition, feeding, digestion, and absorption of essential nutrients by the insects (Smith and Clement 2012).

MeJA and SNP treatment suppressed the MDA in all soybean genotypes as compared with untreated plants (Fig. 3). These results showed that MeJA significantly reduced the formation of MDA under natural infection with cotton worm. Also, high amount of MDA contents were observed in susceptible plants than resistant plants under the same conditions. These results are similar to the results obtained by Farouk and Osman (2012) who found that infestation bean plants with spider mite induced the accumulation of thiobarbituric acid reactive substances in bean shoots followed by an increase in membrane permeability due to the hyper accumulation of hydrogen peroxide which indicating occurrence of lipid peroxidation.

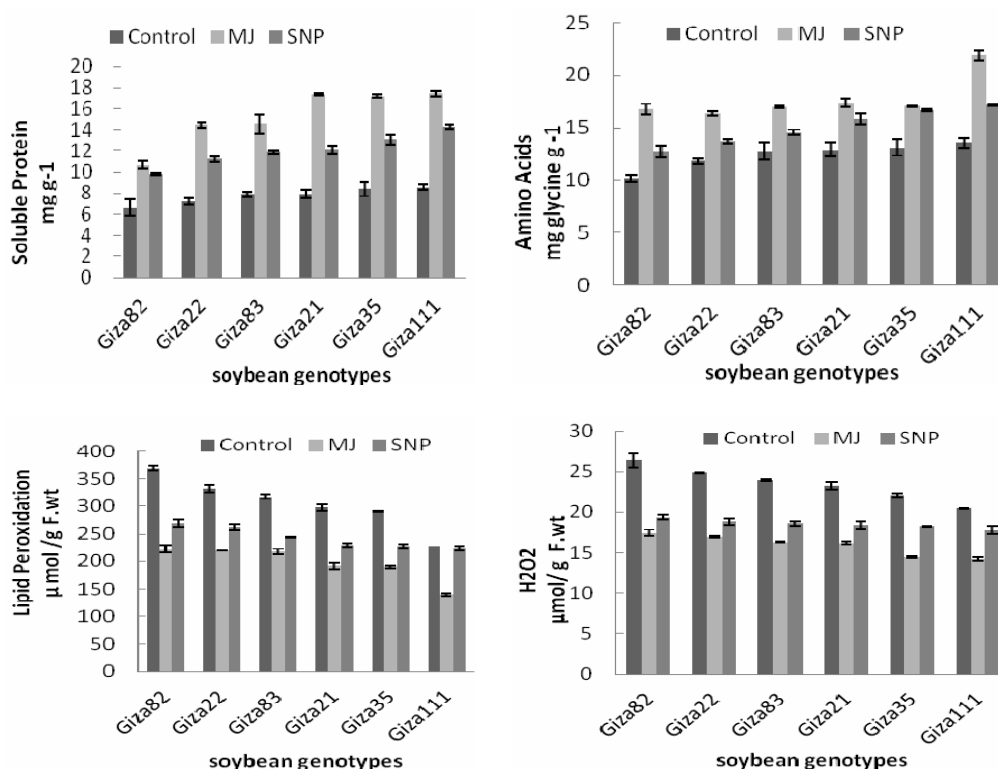


Fig. 3. Effect of foliar spray of MeJA and SNP on osmolytes contents and oxidative damage of soybean genotypes under natural infection with cotton worm.

H<sub>2</sub>O<sub>2</sub> content was significantly decreased in treated plants comparing with untreated plants which show higher contents of H<sub>2</sub>O<sub>2</sub>. MeJA treatment was more effective than SNP in decreasing

H<sub>2</sub>O<sub>2</sub> contents. At the same time soybean plants differ between each others in their H<sub>2</sub>O<sub>2</sub> content according to its susceptibility to cotton leaf worm stress. Resistant genotypes showed less H<sub>2</sub>O<sub>2</sub> content than susceptible genotypes. Insect feeding can also cause hydrogen peroxide, a type of reactive oxygen species, to be produced (Bi and Felton 1995). Too much hydrogen peroxide can be toxic to the plant and result in cellular damage.

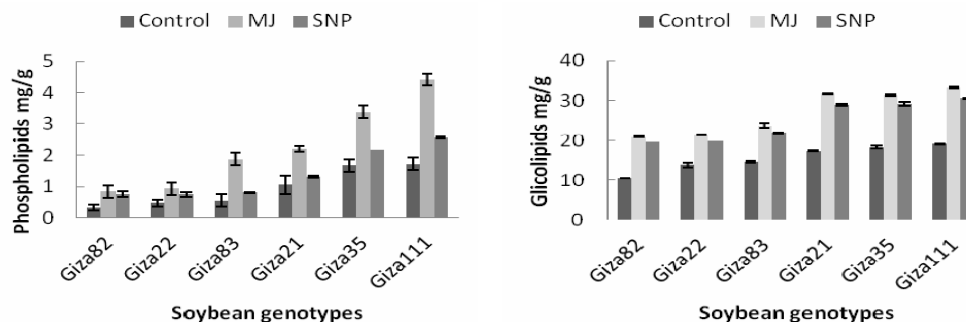


Fig. 4. Effect of foliar spray of MeJA and SNP on phospholipid and glycolipid contents of soybean genotypes under natural infection with cotton worm.

Generally, MeJA and SNP treatments caused significant increase in the glycolipids and phospholipids contents in all soybean genotypes as comparing with untreated plants. In addition, SNP treated plants caused significant increase in phospholipids content in all soybean genotypes except the susceptible genotypes (Giza 82 and 22) (Fig. 4). Phospholipid accumulation has been observed in *Arabidopsis thaliana* in response to wounding and thus may play a role in biotic stress signaling necessary for resistance to herbivore attack (Bargmann *et al.* 2009). Also, SNP as NO donor was found to enhance the accumulation of phospholipids in cucumber treated plants (Lanterimari' al *et al.* 2008).

In conclusion, treatment of soybean plants with MeJA and SNP would be effective enhancing the ability of these plants to resist cotton leaf worm infection. MeJA was more effective than SNP.

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