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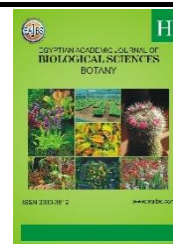
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Act of Malondialdehyde and Total Phenolic Content Under Bean Yellow Mosaic Virus Infection and Biostimulants Application

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ABSTRACT

Bean yellow mosaic virus represents a stress effect on fava bean plants. This study was conducted to assess the lipid peroxidation and total phenolic content (TPC) of fava bean plants under bean yellow mosaic virus infection and its management by the biostimulants; *Rhizobium* and *Bacillus* species spraying application. The obtained data revealed that virus-infected plants maximized stress factors, the content of malondialdehyde (MDA), while it decreased in other applications. TPC significantly increased in *Bacillus*, and *Rhizobium* isolates spraying treatments with 20.99 and 22.44 mg/100g extract, respectively, compared with control. The current work provides bio-resistance by *Bacillus* or *Rhizobium* isolates effectively minimizes the adverse effects of challenges such as stressing viral infection and representing an environmentally friendly commercial value for plant growers.

INTRODUCTION

Fava bean, *Vicia faba* L, is one of the oldest crops grown and used as a protein source for humans and animals (Bishnoi *et al.*, 2012). This crop fixes atmospheric nitrogen in a symbiotic relationship with rhizobium bacteria in the soil (Karla, 2009). It plays a significant dietary role in supplying proteins, carbohydrates, essential elements, and vitamins to rural and urban people. The fresh pods and green seeds are eaten boiled or are used to prepared curries; ripe seeds are used as pulse, often as soup “dhal” (Sultana, 2001).

Viral diseases have a significant status because they cause direct damage to the host and predispose the plant to secondary invaders (Mahgoub *et al.*, 1997). Bean yellow mosaic virus (BYMV) is the most common and prevalent virus among fava bean viruses (Sofy *et al.*, 2019). 89% of the Egyptian fava bean fields surveyed with high-level BYMV symptoms (80–100% infection) (Makouk *et al.*, 2003).

To control ROS and protect the cells, plants possess several low molecular weight antioxidants (ascorbate, phenolic compounds, tocopherols) and enzymes (SOD, CAT, APX), scavenging ROS and regenerating the active forms of antioxidants. Under normal conditions, ROS production and scavenging are well regulated. In this way, this enzyme system can

eliminate the damaging effects of toxic oxygen species (Chen and Asada, 1989 and Nunez *et al.*, 2003).

This work aimed to determine the malondialdehyde (MDA) content and total phenolic content of fava bean plants under bean yellow mosaic virus infection and its management by *Rhizobium* spp or *Bacillus* spp spraying application method.

MATERIALS AND METHODS

Virus Isolate and Treatments:

In this study, bean yellow mosaic virus isolate was used to inoculate the fava bean plants and biostimulants as *Bacillus* and *Rhizobium* bacterial isolates were sprayed to manage the virus infection, and all treatments illustrated in Table (1)

Determination of Lipid Peroxidation:

Lipid peroxidation was measured as malondialdehyde (MDA) content, a thiobarbituric acid reactive substance, according to Heath and Packer (1968). Leaf samples were homogenized in 0.1% TCA in a ratio of 1:5 (w/v) and centrifuged at 12,000×g for 30 min at 4°C. A 1-ml of the leaf extract was incubated with 4 ml of 20% TCA containing 0.5% thiobarbituric acid (TBA) for 30 min at 95°C. The mixture was incubated at 95°C for 30 minutes and then quickly cooled on ice. After centrifugation at 10,000×g for 15 min, the reaction product absorbance was measured at 450, 532, and 600 nm. The lipid peroxidation level is expressed as mmole/g FW of MDA-TBA complex formed using an extinction coefficient of 155/mM/cm.

Total Phenolic Content (TPC) Determination:

The Folin–Ciocalteu test was chosen to measure the TPC of fava bean extracts. The test was performed by referring to the method developed by (Velioglu *et al.*, 1998) with some modifications. The crude sample was prepared by liquefying 10 mg of the extract in 10mL of the solvent to yield a 1 mg/mL concentration. About 100 µL of the extract (1 mg/mL) was combined and mixed with 0.75 mL of the Folin–Ciocalteu reagent (diluted 10-fold with deionized water previously) in the test tube. The liquid mixture was allowed to stand for 5 minutes at room temperature. 0.75 mL of sodium carbonate (Na₂CO₃) was added to the mixture, and the test tube was shaken gently to mix them. After 90 minutes, the absorbance of the mixture was measured using the UV-Vis spectrophotometer at 725 nm. A calibration curve of standard reference was established using gallic acid (range of concentration from 0.01 to 0.05 mg/mL) as standard references plotted. TPC was revealed as gallic acid equivalents in milligrams per 100g of the extract.

RESULTS AND DISCUSSION

Malondialdehyde (MDA) Content:

The lipid peroxidation level in leaf tissues was measured as the malondialdehyde (MDA) concentration Table (1). Lipid peroxidation levels were significantly higher in pathogen-inoculated leaves when compared with un-inoculated controls. It was observed that MDA accumulation levels increased with time in the inoculated plants and were markedly higher with control. One of the biomarkers for oxidative stress is the lipid peroxidation measured by malondialdehyde (MDA) content. The free radical and toxic H₂O₂ collects electrons from lipid molecules present inside the cell membrane, which eventually causes lipid peroxidation (Flora *et al.*, 2012). MDA is the decomposition product of polyunsaturated fatty acids of biomembranes. Its increase shows that plants are under high-level oxidative stress, and MDA could be a great indicator of membrane disorder in plants exposed to pathogen colonization (Loreto and Velikova, 2001). This work data indicated

increased lipid peroxidation levels as indicated by accumulated MDA content in response to virus application. This finding is inconsistent with Sobhy *et al.*, 2020 in Pb-stressed wheat and Sewelam *et al.* (2017) in droughted wheat .

Table 1: Treatments, malondialdehyde (MDA) concentration, and total phenolic content

Treatments	Malondialdehyde (MDA) mmole/g	Total phenolic content (mg/100g extract)
Control	90.03	11.07
Bean yellow mosaic virus	133.04	12.4
<i>Rhizobium</i> spp.	59.41	22.44
<i>Rhizobium</i> spp. (spray) + Bean yellow mosaic virus	81.5	13.6
<i>Bacillus</i> spp.	56.31	20.99
<i>Bacillus</i> spp. (spray) + Bean yellow mosaic virus	77.63	19.28
LSD (MDA)		14.2
LSD (Total phenolic content)		4.2

Total Phenolic Content:

TPC activity is the process of figuring out the amount of phenolic content in the samples Table (1). TPC significantly increased in *Bacillus*, and *Rhizobium* isolates spraying treatments with 20.99 and 22.44 mg/100g extract, respectively, compared with control. Phenolic compounds in the plants have redox properties, and the properties allow them to act as antioxidants (Baba and Malik, 2015). One of the most efficient mechanisms that plants use to protect themselves from oxidative stress is various detoxifying ROS that is overproduced under environmental stresses. Such detoxification prevents cell injury and tissue dysfunction. This reduction in phenolic compounds might also be attributed to the role of their hydroxyl and carboxyl group in the scavenging of free radicals and chelating heavy metals (Yadav *et al.*, 2016) or due to the utilization of simple phenols in the formation of complex compounds like flavonoids, tannins, and lignins (Mondal *et al.*, 2015).

Bacillus produces antimicrobial metabolites that can be used as a substitute for synthetic chemicals or supplements to the use of biopesticides, and bio-fertilizers, for controlling plant diseases (Ongena *et al.*, 2005). *Bacillus subtilis* also enhances the synthesis of enzymes and PR proteins in host tissues in tobacco, resulting in increased resistance to mosaic virus, as evidenced by the reduced level of mosaic symptoms observed in plants treated with *B. subtilis* than in non-treated plants (Lian *et al.*, 2011).

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