

A polishing the harmful effects of *Broad Bean Mottle Virus* infecting broad bean plants by enhancing the immunity using different potassium concentrations

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Abstract

Broad bean mottle virus (BBMV) infects a wide range of hosts, resulting in significant production reductions. The lack of adequate and effective control methods involves implementing novel BBMV control strategies. Herein, we demonstrate the effect of different potassium concentrations (20, 40, and 60 mM) against BBMV in broad bean plants. Potassium could control BBMV infection in broad bean by inhibiting the virus. In addition, infection with BBMV caused a significant decrease in morphological criteria, SPDA, photosynthetic characteristics, phytohormones, and mineral content in broad bean leaves compared to control plants. The levels of reactive oxygen species (ROS) (hydrogen peroxide, hydroxyl radical, and oxygen anion) and ROS scavenging enzymes (catalase, superoxide dismutase, peroxidase, phenylalanine ammonia-lyase, chitinase, and 1,3 - glucanase) increased significantly in plants inoculated with BBMV alone or in the presence of K⁺. In addition, proline and phenolic compounds increased significantly after being infected with BBMV. In conclusion, treatment with a high potassium concentration (60 mM) alleviates the adverse effect of BBMV on broad bean plants by boosting secondary metabolites, phytohormones, and enzymatic antioxidants.

Keywords: disease severity; phenolic compounds; photosynthetic characteristic; phytohormone; proline; SPDA

Introduction

The faba bean (*Vicia faba* L.), also known as the broad bean, horse bean, field bean, or tick bean, is among Egypt's first domesticated crops. It is one of the world's most important winter-sown legume crops, with

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significant value as a low-cost food that is high in protein (20-25% in seeds) and carbohydrates (Robinson *et al.*, 2019; Mowafy *et al.*, 2022). In several Asian and African nations, it is the main source of protein for the impoverished (Sofy *et al.*, 2020b). *Vicia faba* is infected by the *Broad bean mottle virus* (BBMV), which belongs to the *Bromovirus* genus and Bromoviridae-positive-sense RNA viruses (Makkouk *et al.*, 2012). North Africa, West Asia, Morocco, Portugal, Tunisia, Sudan, Egypt, Algeria, Syria, and the United Kingdom are among the countries where the virus has spread (Makkouk *et al.*, 2012). Mottling, mosaic, or severe mosaic, leaf deformity, and plant stunting are all indications of viral infection in broad bean plants. Necrosis may appear in some broad bean genotypes. Seed quality can be harmed by the virus, which causes necrosis and shriveling in the seeds. Lentil, chickpea, pea, soybeans, *Trifolium* spp., *Phaseolus vulgaris*, and *Melilotus albus* are among the virus's natural hosts (Makkouk *et al.*, 2012). The virus is spread via beetle vectors and *Spodoptera exigua* larvae, but the viruliferous larvae are lost after the initial inoculation feeding (Ahmed and Eisa, 1991). Mechanical sap-inoculation and grafting are both ways to spread the virus. In broad bean, up to 1.4% of the virus is transferred by seed (Fortass and Bos, 1992; El-Dougdoug *et al.*, 2012).

Plant viral infections are one of the most common diseases that limit the growth, development, and production of several crops, posing a threat to agriculture's long-term viability and costing farmers a significant amount of money each year to combat (Sofy *et al.*, 2021b; Sofy *et al.*, 2021c). Therefore, it is critical in a sustainable agriculture system to focus on discovering safe plant disease control alternatives that do not harm the environment or public health and also cause an increment in the amount and quality of plant crops. For example, plant disease might initially be controlled by genetics or resisted by plant nutrients, which are among the most natural eco-friendly way to disease resistance (Sofy *et al.*, 2021a).

Chemical pesticides are utilized to manage plant virus diseases in a restricted number of cases. Pesticide residues in food crops can be minimized by reducing pesticide uses and/or doses through the application of nutrients, particularly those related to resistance or plant disease control, where these nutrients are not considered a substitute for pesticides but play a vital role in pest management programs (Mansour *et al.*, 2020; Sofy *et al.*, 2020a). Mineral nutrition can affect resistance mechanisms by 1) causing thicker cell walls to form as mechanical barriers. 2) synthesis of natural defense molecules, such as antioxidants, phytoalexins, and flavonoids, which protect against infections, where some organic and inorganic fertilizers may be effective for managing plant diseases via stimulants of resistance in a variety of plant species (Prakash and Verma, 2016; Maksoud *et al.*, 2022).

The presence of potassium (K^+) in fertilization programs has a wide range of effects on plant diseases. Furthermore, crop quality and disease resistance were linked to a number of crucial practical factors, with K^+ being one of the most essential. K^+ is a macronutrient that is considered necessary for plant growth and responsible for regulating metabolic processes within plants by boosting the effectiveness of multiple plant enzyme functions (Ashley *et al.*, 2006). K^+ can affect many processes within plants, including photosynthesis, sugar and starch formation, cell division, protein synthesis, improving the quantity and quality of plant crops, and maintaining water balance in plant tissues (Wang *et al.*, 2013; El-Nagy *et al.*, 2020). Also, a high K^+ supply was found to reduce the severity of virus diseases, such as the *Tobacco mosaic virus*, and leaf blight severity is caused by *Helminthosporium* (Sharma *et al.*, 2005).

Furthermore, encouraging epidermal cells to produce thicker outer barriers avoids disease attacks (Zörb *et al.*, 2014). That caused alterations in the major primary metabolite profiles of plants and distribution, as well as hormonal pathways (Amtmann *et al.*, 2008). More than 2,000 researches were examined, and a link between K^+ status and disease incidence were discovered. In 69%, 70%, and 41% of the investigations, having a high K^+ level reduced bacterial, fungal, and viral diseases, respectively (Amtmann *et al.*, 2008).

The stomatal guard cell closes the pores entirely against several bacterial infections that enter through the stomata when there is a high concentration of K^+ . Plants lacking K^+ , on the other hand, have problems with stomatal control (Melotto *et al.*, 2006). Diminished viral mobility about the host could possibly result from reduced transpiration. Viruses like TMV have been reported to travel large distances via the phloem (Séron and Haenni, 1996). Plant viruses are increasingly being discovered in the xylem and are capable of causing systemic

infection via xylem transport (Otulak and Garbaczewska, 2012; Wan *et al.*, 2015). Furthermore, the velocity of transpiration impacts the virus's migration through the apoplastic pathway and horizontal translocation from the phloem to the surrounding tissues (Sofy *et al.*, 2012; De Schepper *et al.*, 2013). A decrease in the stomatal index could also represent a host reaction to avoid additional infection. Many diseases, including bacteria, fungi, nematodes, and protists, employ stomata to access the plant host (Grimmer *et al.*, 2012).

The application of K^+ fertilizers can considerably reduce the incidence of Soybeans mosaic virus (SMV), as K^+ is the most abundant inorganic solute in plant cells and is implicated in plant responses to abiotic and biotic stressors. As a result, changing the potassium status can help to slow the progression of viral infections. As a result, K^+ transporters are possible targets for soybeans virus resistance (Zhou *et al.*, 2014).

Little information was observed about the role of K^+ in alleviating viral infection. So, this study was to evaluate the physiological and biochemical effects of different potassium concentrations (20, 40, and 60 mM) as foliar applications on growth, biochemical composition, and tolerance to *Broad bean mottle virus* (BBMV).

Materials and Methods

Plant materials

The seeds of broad bean (*Vicia faba*) cultivar 'Giza 461' were offered friendly from Agriculture Research Centre, Giza, Egypt. First, the seeds were sterilized in a 1% sodium hypochlorite solution for two minutes until washed thoroughly with distilled water several times. Then, the sterile seeds were soaked in distilled water for 8 hrs at room temp, followed by drying air for two hours.

Source of the virus isolate

The Egyptian isolate of *Broad bean mottle virus* (BBMV) was obtained from the Agriculture Botany Department, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt. The viral isolate was verified serologically using ELISA kits kindly presented from the Agriculture Research Centre, Giza, Egypt, and a double-antibody sandwich enzyme-linked immunoassay test (DAS-ELISA), according to Clark and Adams (1977).

Treatments and BBMV inoculation

The pot experimentation was made to assess the effect of formulated and safe antiviral agents (20, 40, 60 mM KH_2PO_4) against BBMV infection. A pot experiment was conducted in the Soil and Water Department Greenhouse, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt, during the successive winter season of 2019/2020. Plastic pots were filled with 10 kg sandy loam soil. The soil samples of the pot experiment were taken before cultivation, air dried, squashed, and sieved through a 2.0 mm sieve for determination. Some physical and chemical analyses of the soil samples and used compost were shown in Table 1 according to Arnold (1986).

The distribution of groups was as follows:

- T1: Sprayed plants only with distilled water as absolute control plants (AC).
- T2: Sprayed plants only with 20 mM KH_2PO_4 .
- T3: Sprayed plants only with 40 mM KH_2PO_4 .
- T4: Sprayed plants only with 60 mM KH_2PO_4 .
- T5: Infected plants only with BBMV (Challenge control; ChC).
- T6: Infected plants with BBMV followed by sprayed with 20 mM KH_2PO_4 .
- T7: Infected plants with BBMV followed by sprayed with 40 mM KH_2PO_4 .
- T8: Infected plants with BBMV followed by sprayed with 60 mM KH_2PO_4 .

Table 1. Physical and chemical analysis of the soil samples and used compost

Soil analysis								
Practical size distribution								
Coarse sand (%)		Fine sand (%)		Silt (%)		Clay (%)		Texture class
48.14		24.11		16.50		11.25		Sandy loam
Moisture content (%) at:			pH 1:2.5	ECe (dS m ⁻¹)	CEC (cmolc kg ⁻¹)	OC (%)	OM (%)	CaCO ₃ (%)
FC	PWP	AW						
12.75	4.75	7.81	7.85	1.80	3.10	0.25	0.40	2.13
Soluble ions (mmolc l ⁻¹)								
Cations				Anions				
Ca ⁺⁺	Mg ⁺⁺	Na ⁺	K ⁺	CO ₃ ⁼	HCO ₃ ⁻	Cl ⁻	SO ₄ ⁼	
2.30	2.90	12.30	0.45	0.00	2.90	11.75	3.30	
Available macronutrients (mg kg ⁻¹)								
N			P			K		
30			7			35		
Compost analysis								
pH (1:10)	EC dS/m	O.C %	OM %	C/N ratio	Total macro nutrients %			
6.73	3.03	20.10	34.57	13.22	N	P	K	
					1.52	0.54	1.00	

FC= Field capacity, PWP = Permanent wilting point, AW= Available water, pH= 1:2.5 w/v for soil water suspension, pH= 1:10 w/v for compost water suspension, ECe= electrical conductivity for soil paste extract, EC = electrical conductivity for compost water extract (1:10), CEC= Cation exchange capacity, OC= Organic carbon and OM= Organic matter.

To increase the spread of various KH₂PO₄ concentrations, two drops of Tween 80 were added, and the whole plant shoots were sprayed until the leaves were completely coated with the solution. Three days after spraying 20, 40, and 60 mM KH₂PO₄, BBMV inoculation was applied. The BBMV inoculation was made by extracting infectious sap from fresh infected broad bean plant leaves that showed severe symptoms by adding 100 mM phosphate buffer solution (pH 7.5) in a sterilized mortar and pestle. The virus-containing supernatant was centrifuged at 5000 ×g for 10 minutes just after crushed plant tissue through double layers of muslin. The virus-containing supernatant was employed as a viral inoculum. Healthy plant leaves were gently dusted with (600 mesh) carborundum then mechanically infected with virus-inoculum using a cotton swab that had been soaked in the viral inoculant (Sofy *et al.*, 2014). Control and treatment plant samples were collected for evaluation. In addition, 21 days after inoculation, the proportion of infected plants and the severity of the symptoms were investigated by the following rate: 0 = no symptoms; 2 = vein clearness; 4 = mild mosaic; 6 = yellowish with mild mosaic; 8 = severe mosaic; 10 = server mosaic with green vein band.

The following formula was used to calculate disease severity (DS) values according to Yang *et al.* (1996)

$$DS\% = \frac{\sum(\text{Disease grade} \times \text{Number of plants in each grade})}{(\text{Total number of plants} \times \text{Highest disease grade})} \times 100$$

Growth Indices and biochemical parameters

Ten plants were randomly selected from each treatment after three weeks of inoculation to measure plant height, stem diameter, and leaf biomass. In addition, three samples were harvested randomly from each treatment to determine different biochemical parameters.

Chlorophyll content and photosynthetic characteristics

A SPAD-502 chlorophyll meter (Konica Minolta, Inc., Tokyo, Japan) was used (Faizan *et al.*, 2018). The photosynthetic properties of the fully extended topmost plant leaves (stomatal conductance (gs), transpiration rate (E), net photosynthetic rate (PN), and intercellular CO₂ concentration (Ci)) were assessed using the Faizan *et al.* (2018) method.

ROS scavenging enzymes

Samples of broad bean leaf were frozen in liquid nitrogen and crushed in a 5 mL extraction buffer to prevent proteolytic activity (0.1 M phosphate buffer, pH 6.8, containing 0.5 mM EDTA). During centrifugation at 15,000 ×g for 20 minutes at 4 °C, enzyme extracts were collected, and their activities were determined in the supernatant. The Aebi (1984) technique evaluated catalase (CAT; EC 1.11.1.6) activity. The reaction mixture was combined with 9.96 mL of H₂O₂ phosphate buffer to make a final volume of 10 mL (0.1 M, pH 6.8). A UV spectrophotometer measured a decrease in absorbance at 240 nm.

The activity of superoxide dismutase (SOD; EC 1.15.1.1) was determined using the method published by Dhindsa *et al.* (1981). SOD mixtures included nitro blue tetrazolium chloride (NBT) (2.25 mM), EDTA (3.0 mM), methionine (200 mM), sodium carbonate (1.5 mM), phosphate buffer (100 mM), and riboflavin (60 mM), all of which were included in 3 mL reaction mixtures (100 mM, pH 7.8). Test tubes were kept in a light-filled area. The absorbance was measured at 560 nm using a spectrophotometer. One enzyme unit was defined as the volume of enzyme extract corresponding to a 50% reaction inhibition. Castillo *et al.* (1984) described a method for measuring peroxidase (POX; EC 1.11.1.11). The POX assay mixture included 1 mL phosphate buffer (pH 6.1), 0.5 mL guaiacol, 0.5 mL H₂O₂, 0.1 mL enzyme, and 0.9 mL water in a volume of 3 mL. Using a spectrophotometer, the absorbance of the solution was determined at 470 nm. Assis *et al.* (2001) method was used to determine phenylalanine ammonia-lyase activity (PAL; EC 4.3.1.5) level. Using the extraction buffer, the reaction mixture of 0.5 mL enzyme extract and 150 mM L-phenylalanine was increased to volume 3 mL. For 30 minutes, PAL was incubated at 40 °C. Following the production of E-cinnamic acid, PAL activity was measured at 290 nm using a UV- spectrophotometer. Chitinase (EC 3.2.1.14) was measured using colloidal chitin as the substrate and dinitro salicylic acid as the reagent, according to the method of Monreal and Reese (1969). The optical density was found at a wavelength of 540 nm using a spectrophotometer.

Abeles and Forrence (1970) method was used to determine 1, 3 -glucanase (EC 3.2.1.39). The substrate was laminarin, while the reagent was dinitro salicylic acid. At 500 nm, the absorbance was measured.

Determination of oxidative damage marker

Patterson *et al.* (1984) technique for examining H₂O₂ was used. Half a gram of leaf samples was mixed with 5% TCA in an ice bath. The mixture was centrifuged at 12,000 ×g for 10 minutes, and the supernatant was combined with TiCl₂. The residue was recovered and centrifuged after being extracted in H₂SO₄. The obtained supernatant was utilized to quantify hydrogen peroxide at 410 nm using a spectrophotometer.

To determine O₂^{•-} production, Jabs *et al.* (1996) used a technique. For 10 minutes, half a gram of broad bean leaves was ground up in phosphate buffer (pH 6.8) and centrifuged at 12,000 ×g. Hydroxylamine hydrochloride was then added to the supernatant. After incubating at 25 °C for one hour, the mixture was blended at the same temperature for 20 minutes with alpha naphthylamine and sulfanilamide. A spectrophotometer set to 530 nm was used to estimate the absorption wavelength.

The supernatant was combined with 100 M FeCl₃, 104 mM EDTA, 1 mM H₂O₂, and 100 M ascorbate to a final concentration of 1 mL and heated at 37 °C for one hour to determine ⁻•OH. The absorbance was measured with a spectrophotometer at 532 nm, according to Babbs *et al.* (1989).

The content of the proline of dry broad beans leaves was determined using the Bates *et al.* (1973) method. First, sulfosalicylic acid (3%) was mixed with a half gram of leaf, which was subsequently filtered through Whatman filter paper. Next, 100 mL of the plant extract supernatant was combined in equal parts with ninhydrin solution and glacial acetic acid. Before mixing with 5 mL of toluene, the sample was heated to 100 °C and then cooled. A spectrophotometer was used to measure the absorbance of the toluene layer at 528 nm.

According to Ziouti *et al.* (1992), the content of free phenols was evaluated. Half gram of broad beans leaf sample was ground in an ice bath with 2 mL of (80%) methanol. The homogenate was centrifuged three times at 7000 ×g for three minutes each time. Folin-Ciocalteu reagent and sodium carbonate were mixed with

100 μ L of the supernatant. The blue color formed after 30 minutes of incubation at 40 °C was measured at 760 nm using a spectrophotometer.

Estimation of hormone content

Endogenous hormone concentrations (indole-3-acetic acid (IAA), gibberellic acid (GA₃), abscisic acid (ABA), jasmonic acid (JA), and salicylic acid (SA)) were measured according to the method of Knecht and Bruinsma (1973). At 4 °C in the dark, a five-gram of broad fresh leaf beans sample was mixed in 80% cold methanol overnight. A rotary evaporator was used to evaporate the extract to an aqueous phase after filtering it through Whatman No.1 paper. The residue was dissolved in 0.1 M phosphate buffer (pH 8.0) and stored for 24 hours at 18 °C. The extract was centrifuged at 4 °C for 17,000 \times g. For phenol binding, two grams of sample PVP were added, filtered through Whatman No.1, rinsed with phosphate buffer (0.1 M, pH 8.0), and the final volume was finished to 30 mL. After that, partition extraction was performed twice using diethyl ether (1:2 volume), and the organic phase was discarded each time. The pH of the aqueous phase was then adjusted to 2.5 using 5 N HCl, and the aqueous phase was partitioned twice with diethyl ether before being discarded. The organic phase was evaporated to dryness and then dissolved in 5 mL H₂O, pH was adjusted to 2.5 using 1 N acetic acids, and then C18 sep-pack cartridge reversed-phase was used to separate the endogenous plant hormones (IAA, GA₃, ABA, JA, and SA) using high-performance liquid chromatography (HPLC) according to the method of Lee *et al.* (1989) using MeOH–acetic acid (2%) as mobile phase and maintaining flow rate at 1.0/min.

Estimation of mineral ion contents

Leaf samples were dried for 24 hours at 60 °C before pulverizing in an inox grinder. Using the microwave process on a MARSX press system, dried plant material was dissolved in a concentrated sulphuric and perchloric acids (3:1 v/v) mixture. The nitrogen (N), potassium (K⁺), phosphorus (P), and manganese (Mn) concentrations in the supernatant were determined. Following Bremner (1960), N was calculated using a micro-Kjeldahl apparatus (Ningbo Medical Instruments Co., Ningbo, China), P was calculated using Jackson (1967), and K⁺ was calculated using a flame photometer (Page *et al.*, 1982). The amount of Mn in the sample was determined using an atomic absorption spectrophotometer (PE-100 Perkin Elmer Boston USA). According to Houba *et al.* (1996) method.

Statistical analysis

At a 0.05 level of significance, the data were analysed using SPSS (Social Science version 26.00) statistical software in a completely randomized trial (Gomez and Gomez, 1984). The two-way ANOVA with Tukey's post hoc test variance analysis with Levene's study's parametric distribution was used to obtain the quantitative analysis. The error margin and confidence interval were set at 5% and 95%, respectively. GraphPad Prism 8 was used to create the graphs.

Results and Discussion

Confirmation of BMMV

Specific BBMV polyclonal antibodies have serologically confirmed the infectious sap of the *Broad beans mottle virus* (BBMV) by DAS-ELISA.

Systemic protection against BBMV in broad bean using potassium

Broad bean (*Vicia faba* cv. 'Giza 461') infected with BBMV showed distinct symptoms involving vein clearing; mild mosaic; yellowish with mild mosaic; severe mosaic; and severe mosaic with green vein banding with disease severity of 90% (Figures 1B and Table 2) in comparison to healthy leaves with no symptoms (Figure 1A). But 40 mM KH_2PO_4 treated plants (40 mM KH_2PO_4 + V) displayed a dramatic and significant decrease in disease severity percentage (DS: 34.66%) (Figure 1D, Table 2), when compared to 20 mM KH_2PO_4 treated plants (20 mM KH_2PO_4 + V) (DS: 60%) (Figure 1C, Table 2). On the other hand, plants treated with 60 mM KH_2PO_4 (60 mM KH_2PO_4 + V) revealed a highly significant reduction in disease severity percentage (DS: 16.6%) (Figure 1E, Table 2). Also, 20 mM KH_2PO_4 treated plants (20 mM KH_2PO_4 + V), 40 mM KH_2PO_4 treated plants (40 mM KH_2PO_4 + V), and 60 mM KH_2PO_4 treated plants (60 mM KH_2PO_4 + V) showed significantly lower virus concentrations in the symptomatic leaves of the challenged plants, with ELISA values of 0.399, 0.260, and 0.199, respectively, compared to the challenge control (0.611) (Table 2). All treatments, on average, lowered viral infection by 26.7%, 53.34%, and 66.67%, respectively.

Table 2. Influence of BBMV infection and 20, 40, 60 mM KH_2PO_4 treatments on virus concentration, percentage of infection (%), and disease severity (DS) of broad bean leaves

Treatment	Virus concentration	Percentage of infection (%)	D.S (%)
Challenge control (ChC)	0.611	100	90
20 mM KH_2PO_4 + V	0.399	73.3	60
40 mM KH_2PO_4 + V	0.260	46.66	34.66
60 mM KH_2PO_4 + V	0.199	33.33	16.6

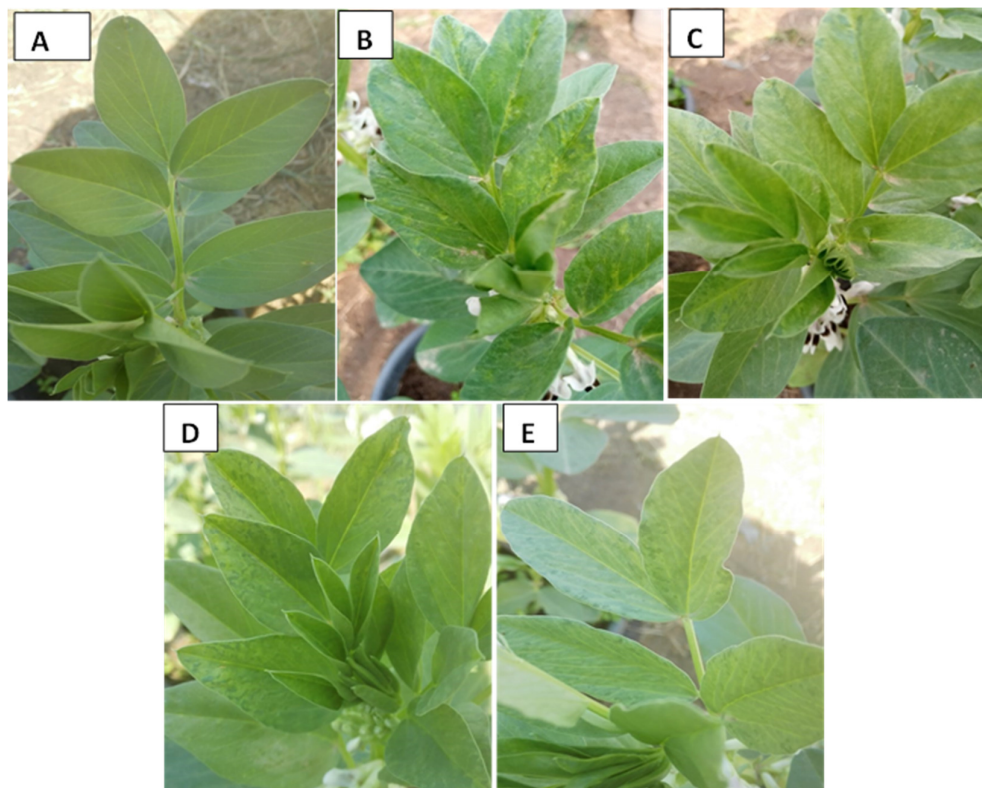


Figure 1. Broad bean of healthy leaves (A), BBMV infected leaves (B), and BBMV infected leaves treated with 20 mM KH_2PO_4 (C), 40 mM KH_2PO_4 (D), and 60 mM KH_2PO_4 (E)

Effect of potassium on morphological criteria under BBMV infection

Data in Figure 2 shows that foliar treatment with different concentrations of 20, 40, and 60 mM KH_2PO_4 caused a significant increase in fresh, dry weight, plant height, and stem diameter of broad beans plants over control plants. On the contrary, BBMV infection caused a significantly reduced in fresh weight (18.3%), dry weight (16.2%), plant height (4.9%), and stem diameter (18.2%) in broad beans plants as compared to non-infected plants. Furthermore, foliar applications of 20, 40, and 60 mM KH_2PO_4 reduce the negative effects of BBMV infection on morphological criteria of broad bean plants when compared to infected plants (Figure 3). The most pronounced increases in fresh weight (45.6%, 41%), dry weight (44.6%, 39.1%), plant height (37.1%, 18.8%), and stem diameter (96.6%, 86.2%) were detected in broad bean plants treated with the high concentration of 60 mM KH_2PO_4 in uninfected and infected plants, respectively. On the other hand, inoculation with BYMV induced a decline in shoot length, number of leaves, leaf area, number of branches, shoot fresh and dry weight of broad beans plants, according to Sofy *et al.* (2020b). Furthermore, the role of potassium as a cation (+) in several physiological processes and the movement of water and nutrients across the xylem might be related to the favorable effects of K^+ on broad bean morphological characteristics (Malvi, 2011). Potassium's effect on plant growth could be related to its relationship with the efficiency of the leaf as a CO_2 assimilator (Ibrahim *et al.*, 2015), triggering phytohormone, regulating cellular pH, improving N uptake, and functioning as an activator to enzymatic systems (El-Nagy *et al.*, 2020). In this regard, Ibrahim *et al.* (2015) stated that foliar spray of potassium citrate boosted potato plant length, number of branches, and total leaf area.

Similar outcomes have been recorded by Ibrahim *et al.* (2015) observed that treating potatoes with potassium citrate at 2000 ppm plus folic acid at 100 g L^{-1} resulted in only negative reactions with *Potato leafroll virus* (PLRV) and *Potato virus X* (PVX) using DAS-ELISA. The beneficial role of potassium citrate against viral diseases could be linked to an increase in phenolic compounds and the activity of related enzymes, such as PAL. That is thought to be the key enzyme for phenolic acid synthesis and the synthesis of most other secondary metabolites, and PPO, which protects plants from pests and diseases (Helmi and Mohamed, 2016; Ashry *et al.* 2018). This role could be explained by the antioxidant activity of phenolic compounds against reactive oxygen species. Sugimoto *et al.* (2010) also found that host defensive responses cause substantial K^+ accumulations inside of penetration-stopping locations of fungal hyphae. Although the physiological role of K^+ in disease resistance is unknown, nutritional factors that encourage host resistance have been linked to changes in protein or amino acid availability, lower cell permeability, and inhibit tissue susceptibility to maceration and penetration (Dordas, 2008).

Many studies have shown that applying K^+ fertilizer to plants can help protect them against various infections (Wang *et al.*, 2013). This is likely due to sufficient K^+ input allowing plants to dedicate more resources to developing more robust plants. That can help prevent phytopathogen invasion like the production of reactive oxygen species (ROS), phytohormones including auxin, ethylene, and jasmonic acid (Wang *et al.*, 2013), boosting nutritional resources and altering primary metabolism (Zörb *et al.*, 2014). Furthermore, Araujo *et al.* (2015) found that foliar spraying mango plants with potassium phosphite decreased internal necrosis and mango wilt. For example, KCl applied to the foliage helps reduce wheat powdery mildew (Dordas, 2008). The effect of K^+ on improving disease resistance in plants has been related to a lowering in the competition for nutritional resources between the pathogen and its host (Holzmueller *et al.*, 2007). Plants with this nutritional status can devote more energy to constructing stronger cell walls to ward off pathogens and insects and obtain more nutrients for plant defense and damage repair (Mengel and Kirkby, 2001). When there was enough K^+ , the stomata could operate correctly during airborne pathogen infections (particularly from bacteria and viruses), avoiding viral invasion by quick stomata closure (Pervez *et al.*, 2007).

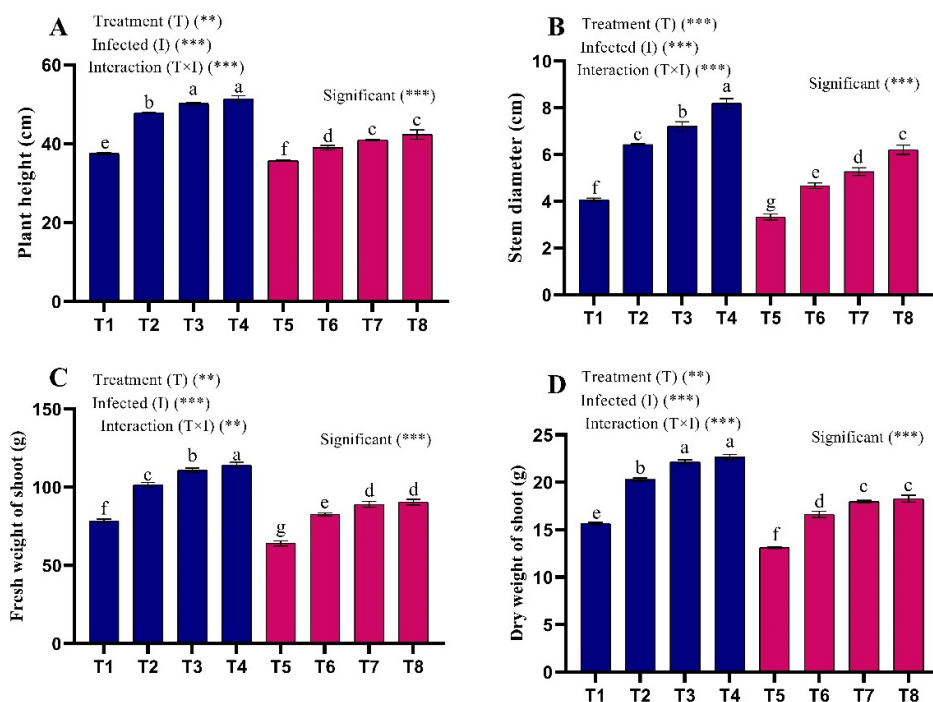


Figure 2. Effect of foliar spray with different concentrations of KH_2PO_4 on broad bean growth under BBMV infection (ChC) as compared with AC control

The values are the SE (n=10) means. A Tukey test showing at $p \leq 0.05$ indicates significant differences between mean values within each bar followed by a lower-case letter. In the two-way analysis of variance (ANOVA), there are significant and very significant differences indicated by ** and ***, respectively

Effect of potassium on chlorophyll content and photosynthetic characteristics under BBMV infection

Figure 3 shows the effect of potassium on SPAD chlorophyll values and the photosynthetic characteristics [net photosynthetic rate (PN), stomatal conductance (gs), intercellular CO_2 concentration (C_i), and transpiration rate (E)] in the leaves of broad bean plants under BBMV infection. SPAD chlorophyll (10.7%), PN (58.2%), gs (44.4%), C_i (40.5%), and E (58.7%) values were significantly decreased in broad bean infected with BBMV as compared to absolute control plants. Furthermore, foliar treatment with the different concentrations of 20, 40, and 60 mM KH_2PO_4 caused a significant increase in SPAD chlorophyll, PN, gs, C_i , and E values compared to control infected plants. The most effective concentration of KH_2PO_4 was the high concentration (60 mM) that caused a significant improvement in SPAD chlorophyll (55.9%, 37.3%), PN (47.2%, 93.9%), gs (88.9%, 140.0%), C_i (42.4%, 54.4%) and E (105.2%, 176.1%) values in uninfected and infected plants, respectively as compared to AC control and ChC control values, respectively.

Photosynthesis is among the most critical physiological processes for plant growth, so the plant-virus interaction suppresses photosystem II activity or influences pigment production in the chloroplast (Rahoutei *et al.*, 2000; Akladios and Mohamed, 2017). The findings of our study were in agreement with those of Sofy *et al.* (2021a), who discovered that SPAD chlorophyll values and photosynthetic characteristics were reduced in leaves of tomato plants due to infection with ToMV as compared to uninfected plants. In addition, Cacique *et al.* (2017) discovered that inoculating plants with *Ceratocystis fimbriata* and treating them with KCl improved photosynthetic performance and that gs improved in tandem with both A and C_i/Ca in the infected plants. K^+ affects photosynthesis in a variety of ways, according to Jin *et al.* (2011), including ATP generation, enzyme activation, the balance of electric charges required for stomatal opening/closure regulation, and photophosphorylation. Nutrition is highly connected to various yield components, with K^+ promoting photo-assimilates transit from the source to the sink (Zörb *et al.*, 2014). Because K^+ absorption into guard cells is a

vital stage in the stomatal opening, treatment of mango plants with KCl decreased the penetration of *C. fimbriata* due to closing the stomatal pores (Shabala and Pottosin, 2014). The superior photosynthetic efficiency of infected plants fed with a higher K⁺ rate could potentially be attributed to a direct action of K⁺ at the site of infection, which decreases disease severity (Jin *et al.*, 2011).

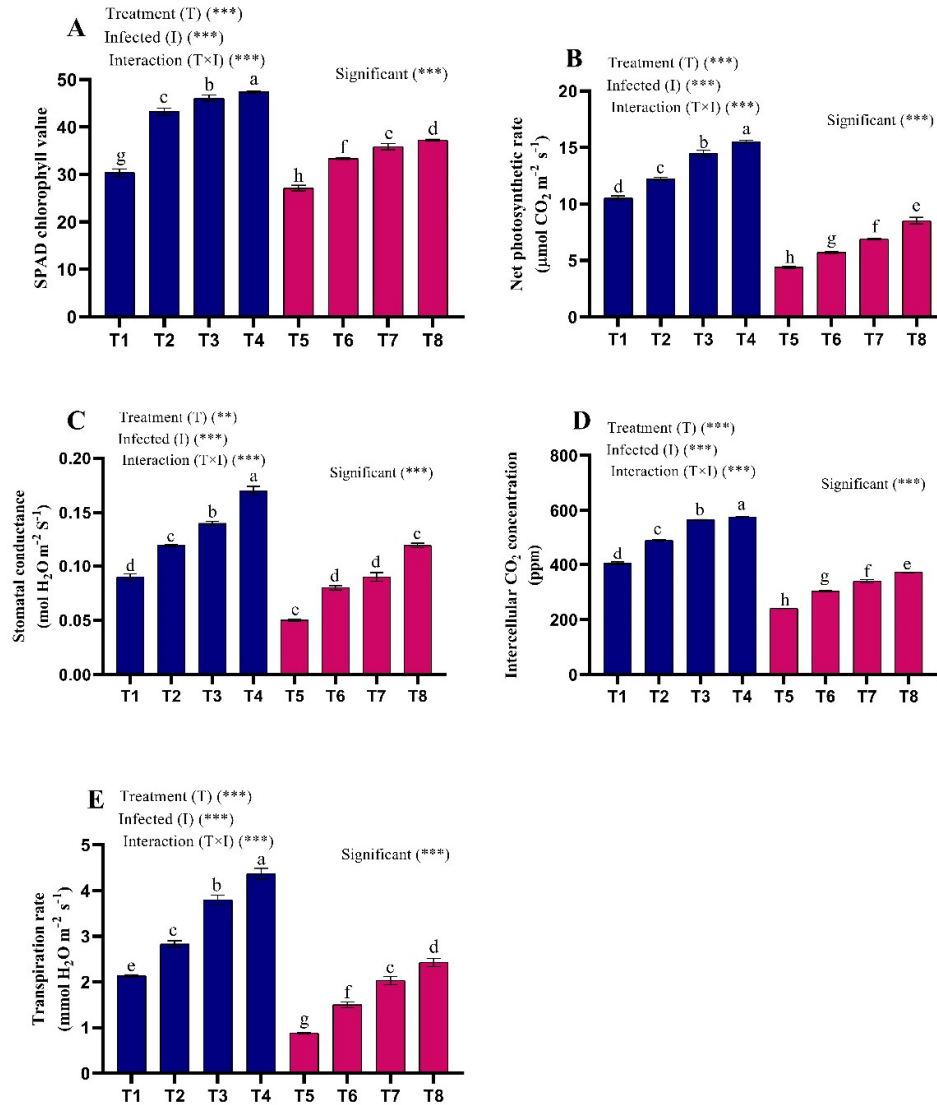


Figure 3. Effect of foliar spray with different concentrations of KH_2PO_4 on chlorophyll content and photosynthetic characteristics of broad bean under BBMV infection (ChC) as compared with AC control. The values are the SE (n=3) means. A Tukey test showing at $p \leq 0.05$ indicates significant differences between mean values within each bar followed by a lower-case letter. In the two-way analysis of variance (ANOVA), there are significant and very significant differences indicated by ** and ***, respectively

Effect of potassium on enzymatic antioxidants under BBMV infection

The influence of viral infection and treatment with different concentrations of 20, 40, and 60 mM KH_2PO_4 on the activity of antioxidant enzymes like catalase (CAT), peroxidase (POX), superoxide dismutase (SOD), phenyl ammonia-lyase (PAL), chitinase and β -1, 3 glucanases are shown in Figure 5. The broad bean leave challenged by BBMV (challenge control plants), the activity of CAT (21.2%), POX (19.5%), SOD (36.1%), PAL (42.9%), and β -1, 3 glucanases (227%) was significantly increased, but chitinase (63.3%) was significantly decreased as compared to absolute control plants. In addition, the activity of antioxidant enzymes (CAT, POX, SOD, PAL, and chitinase) increased significantly in response to the treatment with different concentrations of 20, 40, and 60 mM KH_2PO_4 compared to challenge control plants. The higher levels of CAT (32.9%), POX (15.9%), SOD (32.7%), PAL (64.5%), chitinase (75.7%), and β -1, 3 glucanases (13.7%) were detected in broad beans plants foliar sprayed with 60 mM KH_2PO_4 as compared to challenge control plants. Potassium promotes systemic resistance by activating defense-related enzymes such as CAT, POX, SOD, PAL, and chitinase. These pathogenesis-related enzymes are critical in plant resistance to viral diseases (Tamandegani *et al.*, 2021). ROS produced after viral infection reacts with proteins, lipids, and DNA, disrupting normal plant cell functions (Vitti *et al.*, 2015). As a result, plants produce both enzymatic and non-enzymatic compounds to reduce ROS accumulation and oxidative damage during infection (Lehmann *et al.*, 2015). The most crucial enzyme in the defense system, SOD that, dismutates superoxide into $\text{O}_2^{\bullet-}$ and H_2O_2 (Abu-Shahba *et al.*, 2022). ROS are poisonous and unstable, so CAT transforms them into less toxic and more stable components like $\text{O}_2^{\bullet-}$ and water (Gill and Tuteja, 2010). Glucanases are hypothesized to decrease viral spread by modulating the plasmodesmata's size exclusion limit (Dobnik *et al.*, 2013). The callose (β -1,3-glucan) formed in the plasmodesmata neck region acts as a physical barrier to virus cell-to-cell transfer, and glucanase deficiency causes virus movement to be delayed (Iglesias and Meins Jr., 2000). Glucanase was found to be critical for the dissemination of *Potato virus Y*, but not for its multiplication (Dobnik *et al.*, 2013). The current study found that adding potassium to broad bean plants boosted antioxidant activity, which helped to reduce the negative effects of virus infection. Potassium neutralizes numerous organic anions and other chemicals within the plant, helping to keep pH between 7 and 8, which is ideal for most enzyme processes. Potassium is also required for enzyme activation and protein synthesis (Cacique *et al.*, 2017). Higher phenolic compound concentrations may be associated with Mn availability, as seen in the current study associated with an enhancement in K^+ concentration. Mn is known to play a direct role in the activation of PAL, a crucial enzyme in the phenylpropanoid pathway that permits processes to produce different phenolic chemicals (Mohamed *et al.*, 2016; Moustafa-Farag *et al.*, 2020). Potassium phosphites activation of the phenylpropanoid pathway was linked to decrease internal stem necrosis and mango wilt, as described according to Araujo *et al.* (2015). Mn is also a cofactor of peroxidases, which is involved in the synthesis of lignin (Abu-Shahba *et al.*, 2021), as well as an essential component of the enzyme superoxide dismutase (SOD), which protects tissues from oxidative stress caused by pathogen and pests infection (Mohamed and Abd-El Hameed, 2014). After inoculation with *C. fimbriata*, Araujo *et al.* (2015) found that mango plants had increased enzyme activity and concentrations of metabolites associated with oxidative stress responses.

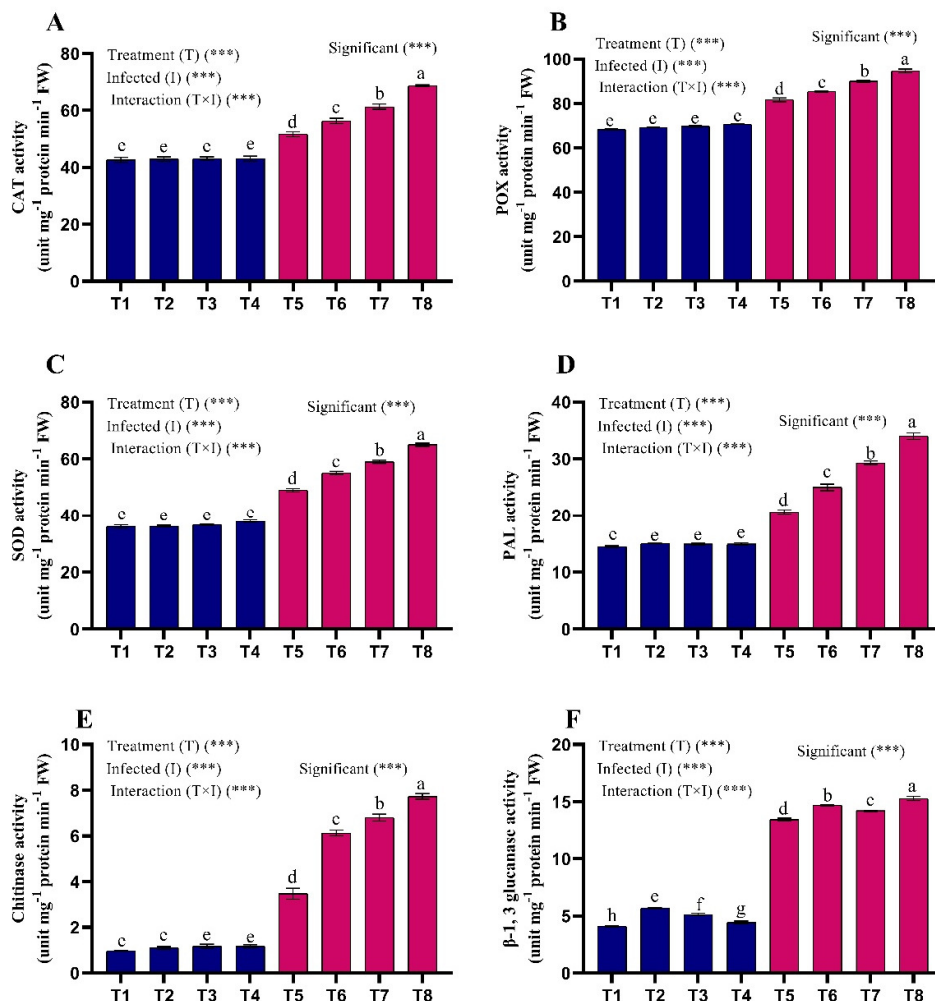


Figure 4. Effect of foliar spray with different concentrations of KH_2PO_4 on enzymatic antioxidants of broad bean under BBMV infection (ChC) as compared with AC control

The values are the SE (n=3) means. A Tukey test showing at $p \leq 0.05$ indicates significant differences between mean values within each bar followed by a lower-case letter. In the two-way analysis of variance (ANOVA), there are significant and very significant differences indicated by ** and ***, respectively

Effect of potassium on oxidative stress, proline and phenolic content under BBMV infection

BBMV infected broad bean leaves (ChC) showed a significant boost in the content of oxidative stress markers (hydrogen peroxide (H_2O_2), oxygen anion ($\text{O}_2^{\bullet-}$), hydroxide radical ($\text{}^{\bullet}\text{OH}$), proline content, and total phenol content by 87.3%, 58.7%, 91.9%, 99.1%, and 57.8%, respectively compared to AC values. In addition, broad bean plants foliar sprayed with different concentrations of 20, 40, and 60 mM KH_2PO_4 showed a significant reduction in H_2O_2 , $\text{O}_2^{\bullet-}$, and $\text{}^{\bullet}\text{OH}$ content but a significant boost in proline and total phenol content as compared to the AC and ChC controls (Figure 6). The highest decrease in H_2O_2 (28.3%), $\text{O}_2^{\bullet-}$ (29.6%), and $\text{}^{\bullet}\text{OH}$ (29.6%) content was detected in infected plants foliar sprayed with 60 mM KH_2PO_4 as compared to ChC control.

ROS formation is a common aspect of interactions between plant cells and viruses (Hernández *et al.*, 2016; Király *et al.*, 2021). The rapid generation of ROS causes oxidative damage during host-pathogen interactions. Singlet oxygen (1O_2), hydroperoxyl radical (HO_2), superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($^{\bullet}OH$) are the most significant ROS (Abu-Shahba *et al.*, 2022). The majority of ROS is produced by the Mehler reaction in chloroplasts, electron transport in mitochondria, and photorespiration in peroxisomes (Zhao *et al.*, 2016). The cellular scavenging capacity can be overwhelmed by a higher rate of ROS generation, resulting in fast accumulation of ROS, oxidative stress, and oxidative damage to cellular components (Gomaa and Dawood, 2021; Hernández *et al.*, 2016). Therefore, plants use a two-way ROS scavenging mechanism to mitigate oxidative harm. Superoxide dismutase, catalase, ascorbate peroxidase, and glutathione peroxidase are among the enzymes that make up the first way. Non-enzymatic antioxidants such as phenolic chemicals, proline, ascorbic acid, reduced glutathione, flavonoids, saccharides, and others make up the second way (Sofy *et al.*, 2020c). Antioxidant enzymes can help plants defend themselves against viruses (Sahhafi *et al.*, 2012; Hernández *et al.*, 2016). As a result, the activity of the ROS-scavenging system provides information on virus tolerance. Proline is a non-enzymatic antioxidant that can help to stabilize subcellular components, including cell membranes and proteins, as well as buffer redox potential and scavenge free radicals in stressful conditions (El-Beltagi *et al.*, 2019; El-Sheshtawy *et al.*, 2021). Furthermore, molecular chaperones have the tendency to preserve protein integrity and increase the activity of certain enzymes, such as nitrate reductase (NR) resistance under pathogen infection (Abd El-Rahman *et al.*, 2012; Abd El-Rahman and Mohamed, 2014). Among the several suitable solutes, proline is the only one that protects plants from single oxygen and radical damage induced by excess ROS (Szabados and Saviouré, 2010; Dawood *et al.*, 2021).

A sufficient K^+ supply frequently raises the phenol content in diseased plant tissues, aiding disease resistance (Prasad *et al.*, 2010). Mango resistance to *C. fimbriata* infection has been linked to phenolic build-up at infection sites (Araujo *et al.*, 2014). Also, treatment of mango plants with potassium phosphite caused inhibition of mango wilt and induced systemic resistance. This may be due to the enhancement of phenol content, the creation of an antifungal barrier, and the rapid deposition of tyloses (Araujo *et al.*, 2015; Mourad *et al.*, 2021).

Effect of potassium on phytohormones content under BBMV infection

Broad bean inoculated with BBMV showed a significant boost in the content of abscisic acid (ABA) by about 66.6% and a significant decrease in the contents of auxin (IAA), gibberellin (GA_3), jasmonic acid (JA), and salicylic acid (SA) by about 28.7%, 23.7%, 21.4%, and 18.8%, respectively, as compared to AC plants (Figure 7). In addition, treatment with different concentrations of 20, 40, and 60 mM KH_2PO_4 caused a significant boost in IAA, GA_3 , JA, and SA content and a significant reduction in ABA content in the BBMV-infected leaves of broad bean compared to ChC plants. The most effective concentration of KH_2PO_4 was 60 mM, which caused a significant increase in IAA (34.5%), GA_3 (23.5%), JA (97.3%), SA (64.0%), and a significant decrease in ABA (31.7%) content as compared to ChC plants.

Plant viruses employ a variety of mechanisms to facilitate viral replication and spread inside the cellular milieu of the plant (Islam *et al.*, 2018). Virus infections can cause direct or indirect disruption of phytohormone accumulation and signalling pathways.

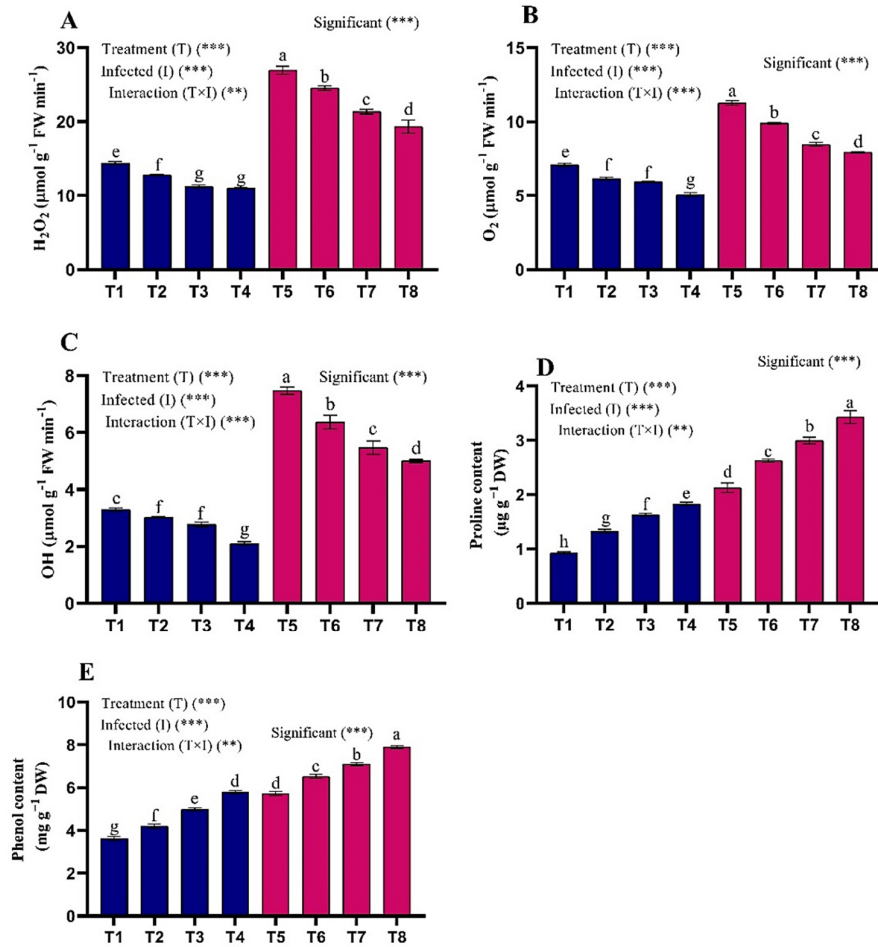


Figure 5. Effect of foliar spray with different concentrations of KH_2PO_4 on oxidative stress (A-C), proline (D) and phenol content (E) of broad bean under BBMV infection (ChC) as compared with AC control. The values are the SE ($n=3$) means. A Tukey test showing at $p \leq 0.05$ indicates significant differences between mean values within each bar followed by a lower-case letter. In the two-way analysis of variance (ANOVA), there are significant and very significant differences indicated by ** and ***, respectively.

In defensive mechanisms, ethylene (Et), jasmonic acid (JA), and salicylic acid (SA) play key roles (Tamaoki *et al.*, 2013). However, defense-related compounds such as abscisic acid (ABA), auxin (IAA), cytokinins (CK), gibberellins (GA_3) (Liu *et al.*, 2013), play important roles in plant physiology and development. Potassium elicitors have numerous advantages since they are not poisonous to pathogens; instead, they are detected by plant membrane receptors and promote the mobilization of a wide range of plant defences via innate immune stimulation (Jolanta and Darius, 2018). The synthesis of phytohormones such as jasmonic acid, auxin, cytokinin, and ABA also increases due to treatment with potassium ions (Sharaf *et al.*, 2009; Nam *et al.*, 2012). Numerous genes involved in jasmonic acid production are activated by K^+ availability, increasing jasmonic acid levels. In different studies, potassium is used as a factor that can stimulate the plant's defence mechanism, for example, by providing it through roots or leaf tissue (Sofy *et al.*, 2020d). Our findings are consistent with those of Gao *et al.* (2018), who discovered that 3 mM K^+ significantly increased the formation of phenolic compounds like cinnamic, ferulic, and salicylic acids and that each of the three acids can markedly restrict *Heterodera glycines* in vitro, implying that salicylic acid may play a part in the plant's disease resistance. Pathogen activity and viral infection may have been suppressed by salicylic acid as an allelochemical (Sofy *et al.*, 2021b).

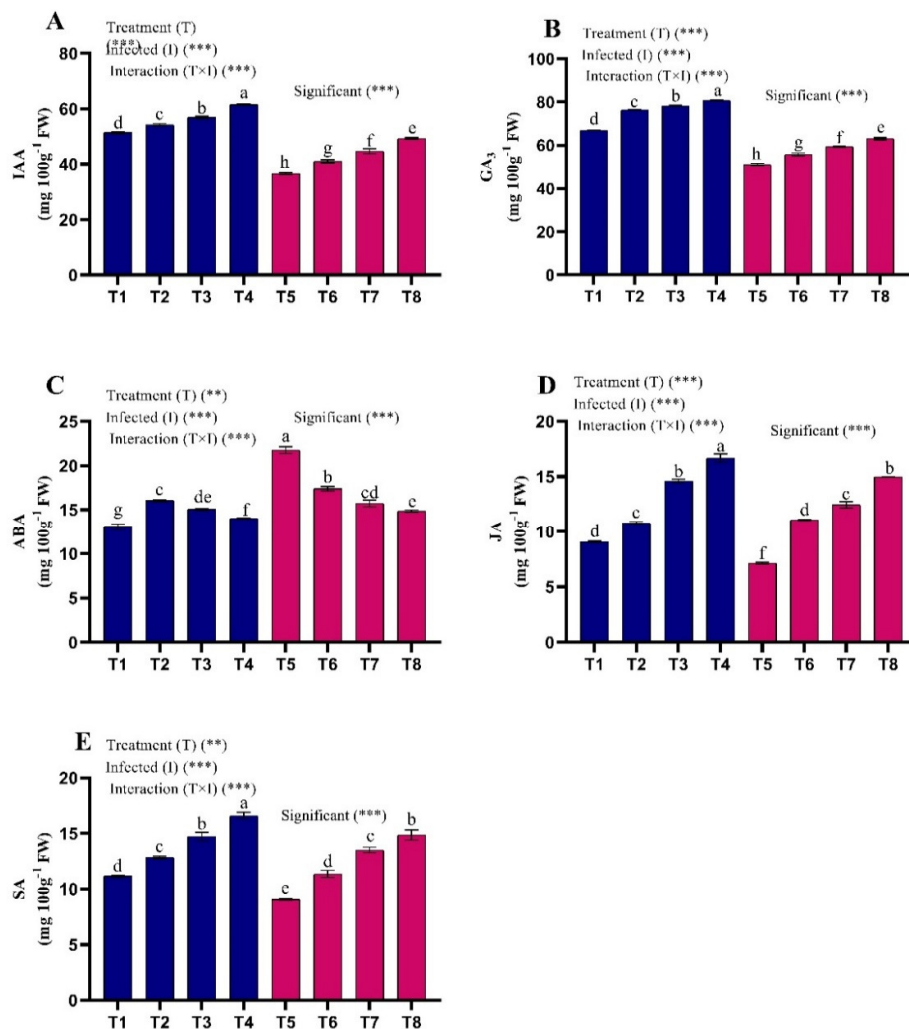


Figure 6. Effect of foliar spray with different concentrations of KH_2PO_4 on phytohormones content of broad bean under BBMV infection (ChC) as compared with AC control

The values are the SE (n=3) means. A Tukey test showing at $p \leq 0.05$ indicates significant differences between mean values within each bar followed by a lower-case letter. In the two-way analysis of variance (ANOVA), there are significant and very significant differences indicated by ** and ***, respectively

Effect of potassium on minerals content under BBMV infection

Data in Figure 7 shows that NPK and Mn contents in the leaves of broad bean plants were significantly decreased by about 32.7%, 25.0%, 28.8%, and 28.64%, respectively, after infection with BBMV as compared with AC plants. In addition, foliar spray with different concentrations of KH_2PO_4 (20, 40, and 60 mM) showed a significant increase in NPK and Mn content in broad bean leaves as compared with AC and ChC plants. The higher levels of N (117.8%, 93.1%), P (100.0%, 100.0%), K (196.6%, 146.4%) and Mn (12%, 28.2%) were detected in uninfected and infected plants, respectively, foliar sprayed with 60 mM KH_2PO_4 as compared to absolute control (AC) and challenge control (ChC), respectively.

The presence of a low concentration of K^+ in CMV-infected cucumber leaves increases the likelihood of virus establishment (Shakeel *et al.*, 2016). Potassium is a critical nutrient since it is mobile (Jones Jr *et al.*, 1991) and can get blocked in the phloem due to CMV infection (Shakeel *et al.*, 2016). Manganese and

potassium work together in a direct synergistic way. Manganese activates decarboxylase, dehydrogenase, and oxidase enzymes, which are critical for photosynthesis, nitrogen metabolism, and nitrogen absorption (Jolanta and Darius, 2018).

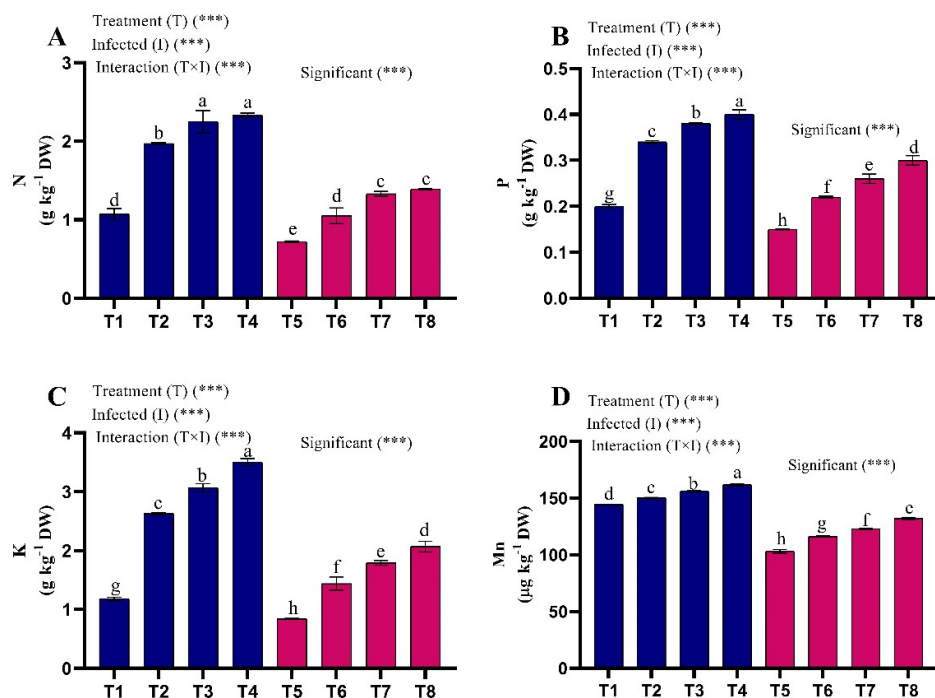


Figure 7. Effect of foliar spray with different concentrations of KH_2PO_4 on the minerals content of broad bean under BBMV infection (ChC) compared with AC control

The values are the SE (n=3) means. A Tukey test showing at $p \leq 0.05$ indicates significant differences between mean values within each bar followed by a lower-case letter. In the two-way analysis of variance (ANOVA), there are significant and very significant differences indicated by ** and ***, respectively

Conclusions

Because there is little information on the use of minerals such as potassium to reduce the negative effects of viruses, the current study focuses on potassium in suppressing BBMV in broad bean plants. The findings suggest that broad bean plants exposed to higher KH_2PO_4 (60 mM) rates became more resistant to the BBMV, most likely as a result of improved morphological and physiological performance. That favours the mounting of host defence responses by increasing secondary metabolites, defence phytohormones, and antioxidant enzyme activity. As a result, developing management methods that favour increasing KH_2PO_4 supply could be a beneficial strategy for reducing BBMV severity.

Authors' Contributions

Conceptualization: MRS, AGM, A.E.-A.M.A., and ARS; Data curation; Formal analysis; Investigation; Methodology; Resources; Software: MRS, AGM, A.E.-A.M.A., and ARS.; Validation; Visualization: MRS, AGM, A.E.-A.M.A., EER, HIM, MEE and ARS.; Writing - original draft: MRS, HIM, and ARS.; Writing - review and editing: MRS, AGM, A.E.-A.M.A., EER, HIM, MEE and ARS. All authors read and approved the final manuscript

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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