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Mineral Responses of *P. Vulgaris* L. To Telfairia Mosaic Virus Infection

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Abstract: *Mineral responses of P. vulgaris to Telfairia mosaic virus (TeMV) infection were investigated. Saponins, alkaloids, tannins and reducing sugars showed significant (P< 0.05) reductions in response to TeMV infection with percentage reductions of 89.3%, 54.5%, 46.3% and 18.3% respectively. Protein showed insignificant increase of 4.3% in inoculated samples. Percentage decreases recorded for fat, fiber and carbohydrate responses to TeMV infection was 28.6%, 20.8% and 14.2% respectively. Values for protein and moisture were not significant. Reductions in the contents of mineral elements were also significant with percentage reduction values for Na (95.3%), Mg (40.6%), K (32.2%), P (14.6%), Fe (13.6%) and Cu (11.9%). Values for Zn and Ca were not significant. The virus caused 56.9% reduction in phytate and increases of 22.8% (HCN) and 11.3% (total oxalate) in inoculated samples. The responses of vitamins to TeMV infection revealed reductions in all the vitamins investigated. Responses of amino acids to the virus depicted insignificant increases in inoculated compared to healthy. The results of this study reveal that minerals of P. vulgaris L. responded differently to TeMV infection. Some exhibited significant reductions in the nutritional quality of the legume while others showed marginal increases in inoculated samples in response to TeMV infection.*

Key Words: *Mineral responses, Phaseolus vulgaris, Telfairia mosaic virus infection.*

I. Introduction

Phaseolus vulgaris L. (Common bean) is a major grain legume that is consumed worldwide for it edible grains and pod. *Phaseolus vulgaris* is an herbaceous annual plant. It is a climbing vine that grows up to 2-5 m long [1]; [2]. It represents a great source of nutrition for millions of people and is second most important legume crop after soybean [3]. It has been documented that [4] common bean is cultivated as a major food crop in many tropical, subtropical and temperate areas of Americas, Europe, Africa and Asia. It grows well on soils with pH ranging from 4-9. It does better on well-drained, sandy loam, silt loam or clay loams, which are rich in inorganic content [5]. The leaf is occasionally used as a leaf vegetable and the straw used for folder [6]. Common bean is one crop that cannot withstand water logging though some cultivars do well in standing water [5].

Common bean is grown and consumed principally in developing or third world countries with high prevalent poverty rate. It is cultivated in Latin America (with Brazil and Mexico as the most important producers), eastern Africa (where per capita consumption is the highest in the world in countries such as Burundi, Rwanda and Uganda) [7], and in Asian countries such as China and Myanmar. *Phaseolus vulgaris* is an important dietary component in Nigeria. For countries such as Brazil and Mexico, it is a major source of protein. Daily intake of total protein in some of the least developed countries, such as Burundi, Rwanda and Uganda, is 40%, 31% and 15% respectively. For other developing countries, the total protein derived from common bean is Nicaragua (19%), Cuba (13%) and North Korea (11%). For a major producer like Brazil, *P. vulgaris* provide 9% of protein. Beans are also a major source of calories for residents of these countries. In addition, common bean plays an important role as a source of minerals especially iron and zinc [8]; [7], for which it also complement cereals.

The high level of poverty in the third world countries has led to untold challenges in the health of both the rural and urban poor. The high cost of purchasing plant minerals in supplementary form is alarming. The populace now depends on plant directly to obtain minerals in order to meet their nutritional requirement since plant sources of minerals are cheaper than minerals in food supplement. Apart from high cost, the danger associated with synthetic food products due to preservatives has also necessitated the dependence on natural food and life is gradually returning to plants (Nature). Minerals are inorganic nutrients occurring naturally in plants and are important in the maintenance of body functions whether in large or small l amounts. They are needed for optimal nutrition and in the prevention of diseases. Minerals are essential in supporting body cells and structure and also in the regulation of many body processes. Our physical well-being rely more on the minerals we take into our system [9] so increases or decreases in these mineral is important in determining whether or not they meet or exceed the recommended dietary allowance (RDA) taking into account the fact that low nutrient diet have an injurious impact on health [10].

Viruses affect plants and alter their physiological and biochemical content with significant increases and decreases in infected host plants. Viruses have been reported to limit both the growth and nutritional contents of many vegetables and legumes [1], [12], [13], [14], [15]. Virus diseases of legume crops are characterized by yellowing, stunting and poor pod-setting. Viruses which can produce the above symptoms in legumes include: Bean leaf roll virus (BLRV), Beet western yellow virus (BWYV), Soybean dwarf virus (SBDV), Legume yellows virus (LYV) (Luteoviruses). Over fifty viruses have been isolated from common beans: abutilon mosaic, alfalfa mosaic, alsike clover mosaic, Argentina sunflower, asparagus bean mosaic, 4 strains of bean chlorotic ring spot, 7 strains of bean local chlorosis etc. (Adams, 1980) (6). Bean common mosaic virus BCMV and Bean common mosaic necrosis virus BCMNV have also been reported in the field. [16].

Telfairia mosaic virus is another virus that has been reported in common bean. Common bean (*Phaseolus vulgaris*) is a susceptible host of Telfairia mosaic virus [17], [18]. Researchers have studied the production, genetic diversity and origin, minerals, isolates of common bean viruses etc. [7], [19]. However, no literature exists on the effect of Telfairia mosaic virus (TeMV) infection on the minerals *P. vulgaris*. The present study hereby examines the responses of minerals of *P. vulgaris* to infection caused by TeMV.

II. Materials And Methods

2.1. Collection of seeds and planting

Seeds of *P. vulgaris* used in this study were obtained from Goldie market in Calabar. Viable seeds were sown in steam-sterilized garden soil in 16 cm diameter polyethylene bags and maintained in a greenhouse at 25 ± 3^{o}C of the Botanical Garden, University of Calabar, Nigeria.

The inoculums used were prepared from virus infected leaves of *T. occidentalis*. The leaves were homogenized in a pre-sterilized cold pestle and mortar in disodium phosphate (Na_2HPO_4) buffer 0.03 M pH 8.0. Mechanical inoculation of seedlings at 3-4 leaf staged was carried out by application of inoculums on *Phaseolus vulgaris* dusted with carborundum (800 mesh) as an abrasive. The inoculated leaves were then rinsed with water and left for symptom expression. The control plants were also dusted with carborundum and treated only with buffer. A total of thirty plants each (control and inoculated) were used. Three replicates each for inoculated and controlled were used. The set up was monitored for symptom expression such as mosaic, severe leaf malformation and distortion characteristic of TeMV infection.

2.2 Preparation of samples for analysis

Four months post-inoculation and control plants were harvested, and seeds sun-dried (for five days), grounded into powder in an electric mill (National Food Grinder, Model MK 308, Japan). One hundred grams were taken from the grounded samples for the determination of crude phytochemicals, antioxidant properties, proximate, vitamins, amino acids and elemental composition analysis.

2.3. Quantitative determination of minerals of *P. vulgaris* in response to TeMV infection

Phytochemicals were determined using standard procedures. For instance alkaloids and free fat was determined using [20], tannins [21], saponins, flavonoids [22], [23], proximate analysis by Association of Official Analytical Chemists [24]. Vitamin (Vit. A, C and Nicotinamide (Spectrophotometric method), thiamine B and Riboflavin (B_2), Minerals like Ca, Fe, Mg Zn and Cu were determined using Absorption Spectrophotometer ((Pye Unicam SP8-190, Spec. UK) as outlined in [24]. Sodium and potassium were estimated by Flame Photometry Jahway Analyzer (P. F7, UK). Phosphorus [24]. Hydrocyanate, phytate, oxalate content were determined by the methods of [23], [25] and [26] as described by [25]. Amino acids by the method of [27].

2.4. DATA ANALYSIS

Data obtained were analyzed using the student t-test at 95% confidence limit. Values presented were means of three replicates.

III. Results

TABLE 1: The responses of crude phytochemicals of *Phaseolus vulgaris* to Telfairia mosaic virus (TeMV) infection

Phytochemicals	Inoculated(mg/100g)[a]	Healthy (mg/100g)[a]	PercentageDifference (%)[b]
Alkaloids	7.02 ± 0.01	15. 40 ± 0.1	54.4
Saponins	0.30 ± 0.1	2.80 ± 0.02	89.3
Tannins	0.43 ± 0.01	0.58 ± 0.1	25.9
Flavonoids	ND	ND	
Reducing compounds	39.12 ± 0.11	47.91 ± 0.10	18.3

a = values are means ± SD of three replicates; b = values were obtained by expressing the differences between the values of the healthy and inoculated plant as a percentage of the healthy, ND = Not detected

Results in TABLE 1 revealed marked decreases (P<0.05) in saponins (89.3%), alkaloids (54.4%), tannins (25.9%) and reducing compounds (18.3%) of *P. vulgaris* in response to TeMV infection in inoculated samples compared to the healthy.

TABLE 2: Proximate responses of *P. vulgaris* to Telfairia mosaic virus (TeMV) infection.

Proximate content	Inoculated(mg/100g)[a]	Healthy (mg/100g)[a]	PercentageDifference (%)[b]
Protein	21.80 ± 0.2	20.90 ± 0.02	4.3
Fat	1.00 ± 0.1	1.50 ± 0.02	28.6
Fiber	3.80 ± 0.02	5.01 ± 0.02	20.8
Carbohydrate	69.80 ± 0.03	61.14 ± 0.01	14.2
Moisture	89.70 ± 0.01	92.30 ± 0.01	2.8

a = values presented are means ± SD of three replicates; b = value were o btained by expressing the differences between the values of the healthy and the inoculated plants as a percentage of the healthy.

The results as presented in TABLE 2 showed that infection of *P. vulgaris* by TeMV engendered significantly reductions (P<0.05) in fat (28.6%), fiber (20.8%) and carbohydrates (14.2%) contents. However, protein and moisture contents showed marginal reductions.

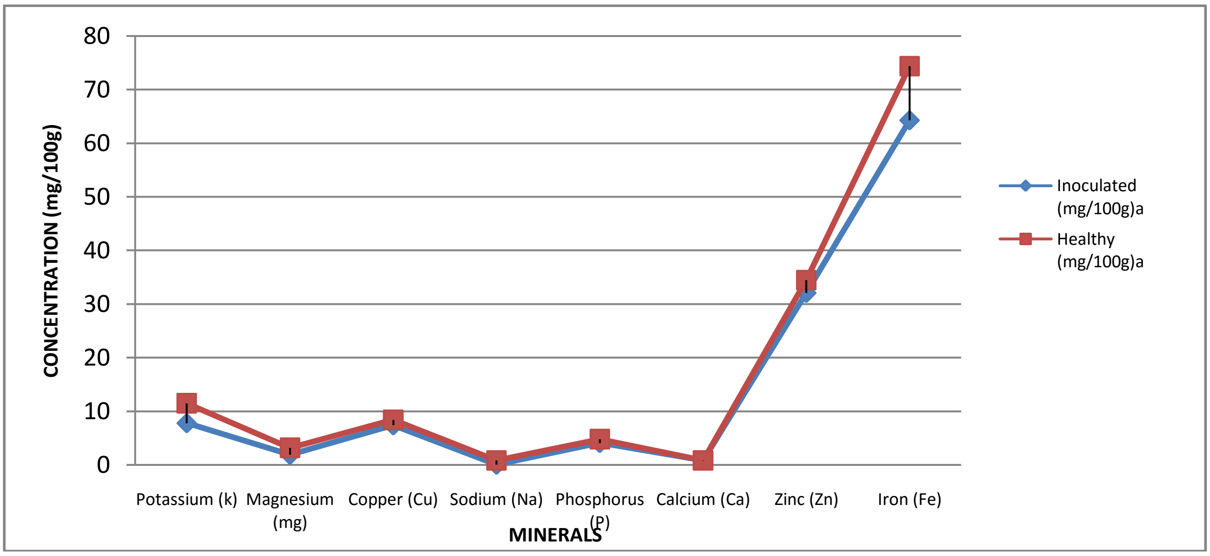

FIGURE 1: Mineral responses of *P. vulgaris* to Telfairia mosaic virus (TeMV) infection.

Mineral responses of P. vulgaris to infection of TeMV are presented in Fig.1 Except for Ca and Zn whose values were comparable to those of the healthy samples, those for Na, Mg, K, P, Fe and Cu were significantly(P< 0.05) reduced in response to TeMV infection. Percentage reduction values for Na, Mg, K, P, Fe and Cu were 95.3%, 40.6%, 32.2%, 14.6%, 13.6% and 11.9% respectively.

TABLE 3: Antioxidant responses of *P. vulgaris* to Telfairia mosaic virus (TeMV) infection.

Antioxidants	Inoculated(mg/100g)[a]	Healthy (mg/100g)[a]	PercentageDifference (%)[b]
Total oxalate	41. 27 ± 0.02	37.09 ± 0.02	11.3
Soluble oxalate	30.56 ± 0.02	27.94 ± 0.02	9.4
Hydrocyanate (HCN)	2.32 ± 0.02	1.89 ± 0.2	22.8
Phytate	60.40 ± 0.02	140.20 ± 0.2	56.9

a = values are means ± SD of three replicates; b = values were obtained by expressing difference between the value for the healthy and inoculated plant as a percentage of healthy.

The result presented in TABLE 3 indicated that the virus caused significant (P<0.05) decrease in phytate (56.9%). There were however increase in infected samples obtained for hydrocyanate (22.8%), total oxalate (11.3%) and soluble oxalate (9.4%).

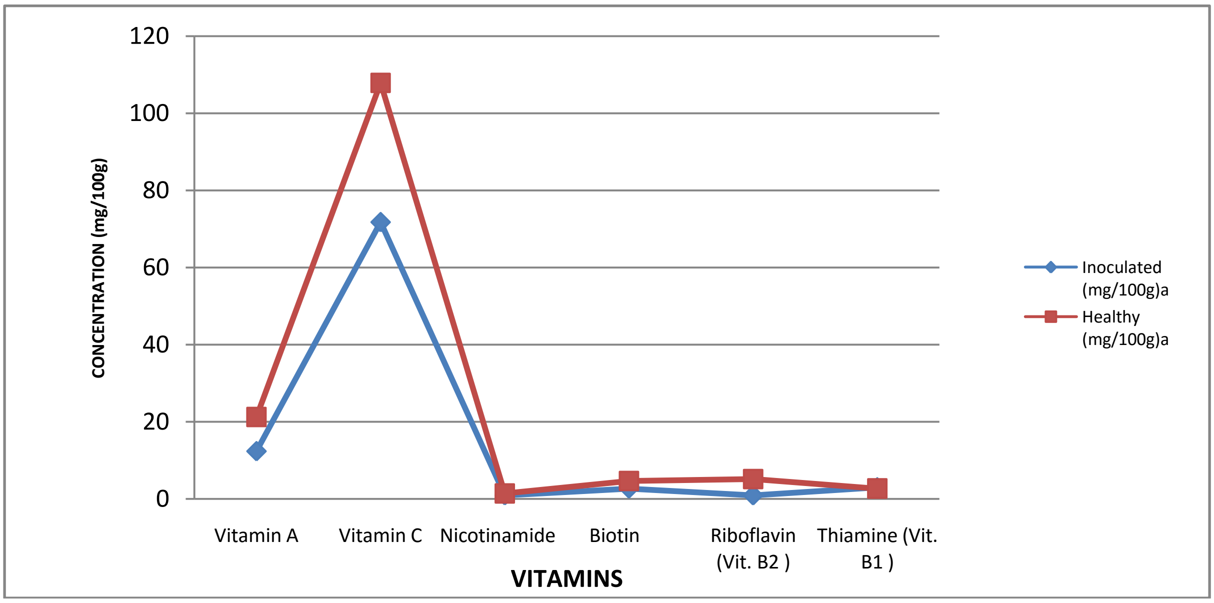

FIGURE 2: Vitamin responses of *P. vulgaris* to Telfairia mosaic virus (TeMV) infection.

Vitamins of *Phaseolus vulgaris* showed reductions in response to *Telfairia mosaic virus* infection. A range in percentage reductions values from 32.7% (for nicotianmide) to 47.3% (for riboflavin) was recorded (Fig. 2).

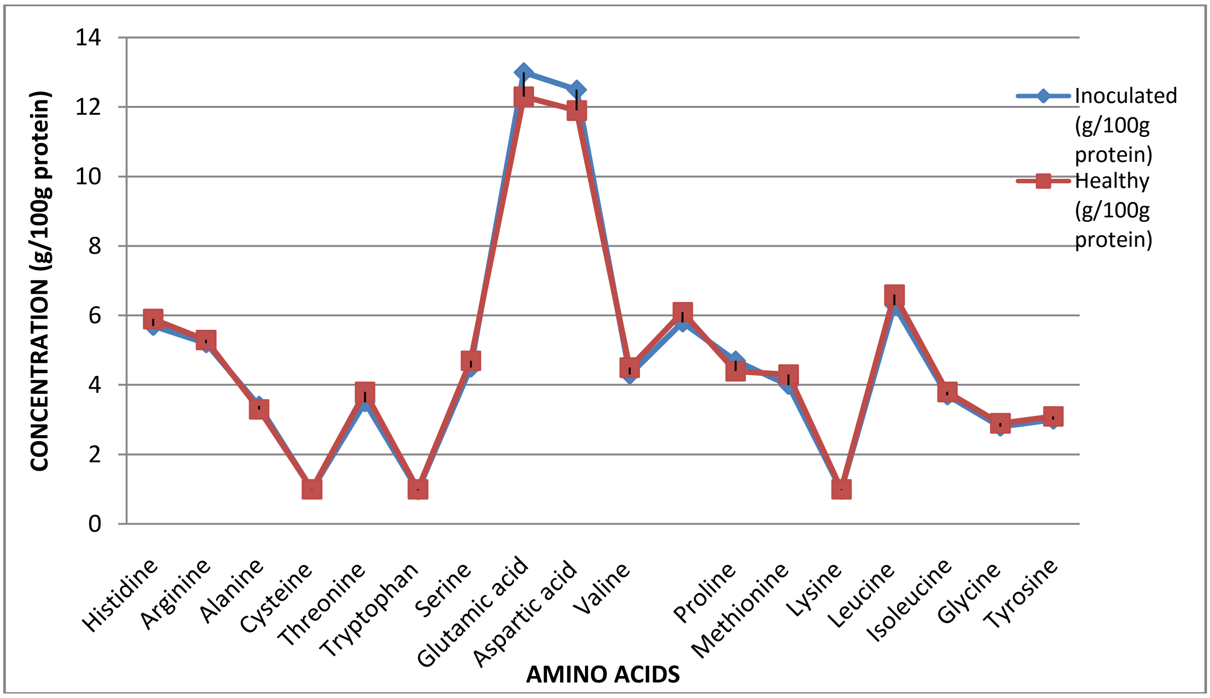

FIGURE 3. Amino acids responses of *P. vulgaris* to Telfairia mosaic virus (TeMV) infection.

Protein showed insignificant increases in inoculated samples TABLE 2. Amino acids which are constituents of proteins also showed insignificant increases in inoculated samples. Lowest and highest percentage increases in inoculated samples were 1.7% for histidine and 7.0% for methionine. More increases were recorded for aspartic acid (5.0%), threonine (5.2%), glutamic acid (5.7%), proline (6.8%) and methionine (7%) as compared to 4.3% increase of protein in inoculated samples of *Phaseolus valgaris* (Fig.3).

IV. Discussion

The response of minerals of *Phaseolus vulgaris* L. to Telfairia mosaic virus infection was investigated. From this study, the virus induced significant reductions in saponins, alkaloids, tannins and reducing compounds. Various plant metabolites have been reported to be altered due to viral infection [28], and

[29], Saponins are used in the manufacturing of shampoos, insecticides and various drugs preparation and synthesis of steroid hormones [30]. A reduction in medically useful alkaloids is a common feature and an important economic aspect of virus disease. Alkaloids are known to play some metabolic role and control development in living systems and have a protective role in animals [31]; [32]; [33]. Tannins have been found to form irreversible complexes with proline-rich proteins [34] resulting in the inhibition of the cell protein synthesis. Medically, this is important for the treatment of inflamed or ulcerated tissues [34]. Indeed plants that have tannin as their main component are astringent in nature and are used for treating intestinal disorders such as diarrhea and dysentery [35]. The reduction of these phytochemicals components in *P. vulgaris* by TeMV infection reduces their effectiveness in disease prevention.

Results of this study also revealed significant reductions in fat, fiber and carbohydrate. These findings are in consonance with reports by [33] in *Amaranthus hybridus* infected by TeMV. Reduction in starch has been reported by [36] in *Cucumis melo L.* infected by Cucumber mosaic virus. [33] in *Cucurbita moschata* inoculated with Moroccan watermelon virus (MWMV). Adequate intake of dietary fiber can lower the serum cholesterol level, risk of coronary heart disease, hypertension, constipation, diabetes, colon and breast cancer [37]. However, values obtained for protein and amino acids were slightly higher in inoculated samples than in the healthy. This result is in agreement with reports b [38]; [39], [40] who recorded increases in protein content in inoculated samples in Potato-PVY, *V*icia *faba*-BYMV and Alfalfa-AMV combinations.

The response of minerals of *Phaseolus vulgaris* to TeMV infection depicted significant reductions in Na, Mg, k, P, Fe, and Cu respectively in inoculated samples compared to the healthy. Minerals whether major (100 mg/day) or trace (mg/day) are inorganic compounds that exist in the body either as charged atoms or as part of complex molecules and are required for normal metabolic activities. These minerals play important roles in the body; Na (nerve transmission & body water balance), Mg (protein formation and enzyme cofactor), K(muscle contraction, nerve transmission, electrolyte balance), P (Nucleic acids, bone and tooth formation, cell membranes, and ATP formation), Fe (hemoglobin) and Cu (enzyme component and red blood cell formation). The reductions in these mineral with attendant consequences in *P. vulgaris* is worrisome. Zinc and Ca were marginally reduced. Reduction in contents of the mineral elements observed in this study is consistent with those of [7]; [40]; [12]; [33]. Total oxalate, soluble oxalate and hydrocyanate (antioxidant) contents in this study were significantly higher in inoculated plants sample compared to healthy ones. This finding is in agreement with previous work by [12]. In contrast, the antioxidant phytate was significantly reduced in inoculated samples compared to the healthy.

This study revealed marked decreases in the all vitamins contents in inoculated plants compared to the healthy. Previous reports of losses in vitamins in other plants-virus combinations abound [12]; [33]. Vitamins are essential in proper nutrition. Vitamin A helps to regulate cell development and boost the immune system. It is needed particularly for good vision and healthy skin. It also improves the body's healing ability. Deficiency results in blindness and xerophthalmia. Vitamin C is required for healthy skin, bones and muscles. It plays an important role in the manufacture of collagen which is the connective tissue that holds bones together. The deficiency causes scurvy [41]; [42]; [43]. Thiamine (Vit.B_1) is essential in the breakdown of carbohydrates prior to the Krebs cycle, deficiency causes beriberi. Nicotinamide act as coenzymes for oxidation-reduction reactions in many energy yielding metabolic pathways. Pellagra develops in thiamine deficiency [44]. Protein in this study showed marginal increases in inoculated plants, amino acids being constituent of protein also showed insufficient increases in inoculated samples. This increase could be due to the virus multiplication which entails the synthesis of virus specific abnormal protein that accumulates and ultimately raises the percentage over healthy [45].

Plant viruses can cause serious losses to most if not all major crops upon which depend for [46]. *Phaseolus vulgaris* is the second most important legumes crop in the world after soybean. Common bean presents a great source of nutrition for millions of people. It is a food security crop in Nigeria. It is a short-duration crop (2.5 – 4 months), it helps to shorten the hunger periods and for providing quick cash. Its early maturity and capacity to provide a range of food products (leaves as well as, fresh pods and dry grain) also helps provide a more balanced diet to vulnerable communities members (the under five, pregnant mothers and chronically ill people [3]. Common bean is the world's healthiest food and is widely consumed throughout the world. It is a rich source of protein, dietary fiber, phytochemicals, lectins, trypsin inhibitors among others [47]. *Phaseolus* vulgaris has been reported as an excellent functional food because of its high levels of diverse chemical content that has shown to protect against oxidative stress, diabetes, cardiovascular disease, different types of cancer and metabolic conditions [48].

V. Conclusion

From the nutritional point of view, the significant reductions in these health promoting phytonutrients (alkaloids, saponins, tannins, Na, Mg, K, P, Fe, Cu, fat, fibre, carbohydrate, phytate and vitamins caused by TeMV reduced the nutritional value of this legume. The human body is said to contain about 4.0% mineral elements, of which 0.05%-2.0% is contributed by the macro elements, and the remaining by micro-elements. The main source of mineral elements to man is plant materials [49]. Results of this study revealed that the responses of minerals *P. vulgaris* toTelfairia mosaic virus infection should be given attention. Efforts should be geared towards the prevention of TeMV infection on *P. vulgaris* in order to guarantee nutritive value. The following recommendations are made; the use of virus-free seeds, use of resistant variety and biotechnological research should be intensified on the production of virus-resistant *P. vulgaris*.

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