Changes in protein, amino acids composition and leaf cells of beet plants (*Beta vulgaris* L.) due to *Beet mosaic virus* (BtMV) infection

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Abstract: *Beet mosaic virus* (BtMV) is one of the most devastating diseases of cultivated beet plants. On studying the effect of BtMV on the chemical constituents of beet plants, BtMV-infected beet plants show high significant increase compared with healthy beet plants in the content of total protein. There was a progressive increase in protein contents of healthy and infected plants with increase in plant age. BtMV inoculated plants recorded 7.93, 8.53, and 19.27 percent increase in protein content over healthy plants at first, second and third week's respectively after inoculation. Protein band of molecular weight of 31 kDa was appeared. No similar protein band was observed in samples of healthy plants. BtMV-infected beet plants contain significant lower content of total free amino acids than that of the healthy ones. There was a progressive decrease in free amino acids contents of healthy and infected plants with increase in plant age. BtMV inoculated plants recorded 35.27, 58.83, and 24.00 percent decrease in free amino acids content over healthy plants at first, second and third week's respectively after inoculation. The concentrations of amino acids like alanine, aspartic acid, glutamic acid, phenylalanine, serine, threonine and tryptophan were more in infected plants up to 21 days after inoculation. Trace amounts of arginine, glycine, leucine, lysine, and valine were detected in healthy plants and their contents were more in infected plants. Cystine, proline and tyrosine were absent in healthy leaves whereas they were present in traces in infected plants at 14 days after inoculation and could not be detected in the succeeding stages of analysis**.** BtMV reduced more of leaf measurements such as medvein thickness, blade thickness, palisade tissue thickness, spongy tissue thickness, vascular bundles length, vascular bundles width, number of xylem vessels and xylem vessel diameter.
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1. Introduction

Beet, *Beta vulagaris* L. is an important crop cultivated in large area in all over Egypt. The importance of *Beet mosaic virus* (BtMV) has been recognized for many years wherever beet are grown(**Polak, 1981; Karl *et al*., 1983; Merkuri and Russo, 1983; Wenzl *et al.,* 1986; Rogov *et al.,* 1991; Heidel and Rush, 1994; Rush and Heidel, 1995; Wisler *et al*., 1997; Juretic, 1998; Dusi, 1999; Mali, 2000; Piszczek, 2000; Choueiri *et al*., 2001; and Okhovvat *et al*., 2001).** The virus belongs to the genus potyvirus and infects a little number of plants belongs to vegetable plants. BtMV causes losses with different percents **(Bojnansky *et al*., 1983; Koch, 1986; Briest and Kegler, 1987; Dusi *et al*., 2000; and Wintermantel, 2005). Nemchinov *et al*. (2004)** concluded that, the viral genome of BtMV comprises 9591 nucleotides, excluding the 3' terminal poly (A) sequence, and contains a single open reading frame (ORF) that begins at nt 166 and terminates at nt 9423, encoding a single polyprotein of 3086 amino acid residues. A 3' untranslated region of 168 nucleotides follows the ORF. The deduced genome organization is typical for a member of the family Potyviridae and includes 10 proteins: P1, HC-Pro, P3, 6K1, CI, 6K2, NIa-VPg, NIa-Pro, NIb and coat protein (CP).

Here, we report the changes in the chemical constituents, especially protein and amino acids composition, of beet plants due to BtMV infection, as well as, what cells showed abnormalities caused by BtMV.

2. Material and Methods

2.1. Virus isolate

Virus inoculum was the crude sap obtained by trituration of frozen leaves of beet plants (*Beta vulgaris* L. cv. Pleno) seedlings showing mosaic symptoms. These symptoms developed 14 days after inoculation with a single local lesion obtained from *Chenopodium quinoa* leaves that were inoculated with sap extracted from naturally infected beet plants (*Beta vulgaris* L. cv. Pleno). Inoculation of leaves was carried out by rubbing with finger after their being dusted with carborandum as described by **Rawlins and Tompkins, (1936)**.

2.2. Protein and amino acids determination

All experiments were repeated twice. Four replicates were used for each treatment. Healthy and

infected beet plants were collected in the morning, each sample was analyzed twice. Protein content was determined according to microKjeldahles as outlined by **A.O.A.C. (1995).** Free amino acids content mg/g dry weight were determined colorimetrically according to the method described by **Jayarman (1981)**. The individual amino acids present in the healthy and infected leaves were identified by Chromatography according to **Moore *et al.*, (1958)**.

2.3. Protein gel-electrophoresis

0.5g of leaves of healthy and infected plants was hand ground in a volume of 0.1 ml (SDS) sample buffer cracking solution. Extracts were added in 1.5 cm eppendorf centrifuge tube according to **Laemmli (1970).** Homogenates were heated at 95°C for 5 min then briefly centrifuged at 12,000 rpm to pellet cellular debris. The resulting supernatants (total protein extracts) were stored at -70°C until analysis by PAGE. The extract was separated by electrophoresis on 1mm thick 12.5% acrylamide slab gels. Gels were stained with Coomassie blue.

2.4. Histological changes

Virus infected and healthy plant tissues were killed and fixed in formalin acetic acid (FAA) solution for 2 days(10 ml Formalin + 5 ml glacial acetic acid + 35 ml distilled water + 50 ml ethyl alcohol 95%). Samples were dehydrated and cleared in n-butyl alcohol series **(Willey, 1971)** and embedded in paraffin wax of 56-58C. Cross and longitudinal sections 15 µ thick were cut using a rotary microtome, adhesive with Haupt's adhesive and stained with crystal violet erythrosine combination **(Sass, 1961)**, cleared in carbol xylene and mounted in canada balsam, and then examined under light microscopy.

3. Results and Discussion

Beet mosaic virus (BtMV) was obtained from a single local lesion produced on *Chenopodium quinoa* test plant. To insure the purity of the isolated virus, two cycles of consecutive serial transfer of single local lesion developed on *Chenopodium quinoa* were carried out. Virus was maintained on beet seedlings which were used as a source of virus during subsequent studies. Virus was easily transmitted by sap **(Glasa *et al.* 2000 and Mali, 2000).** *Chenopodium quinoa* was used as a local lesion diagnostic host because it reacts by BtMV with local lesions.

3.1. Protein content

Data in **Table (1) and Fig.(1)** show that, BtMV-infected beet plants show high significant increase compared with healthy beet plants in the content of total protein. There was a progressive increase in protein contents of healthy and infected plants with increase in plant age. BtMV inoculated plants recorded 7.93, 8.53, and 19.27 percent increase in protein content over healthy plants at first, second and third week's respectively after inoculation. This result was agreed with that obtained by **Bokhoven *et al.* (1990); Gao *et al.* (1994); Murthy *et al.* (1994); Manickam *et al.* (2000); Sutha *et al.* (2000); Milavec *et al.* (2001); Helmy and Maklad, (2002); Satish *et al.* (2002); and Patel *et al.* (2004). Sarma *et al.* (1995)** reported that, bhendi yellow vein mosaic bigeminivirus infection increased the chemical constituents of bhendi [okra] (*Abelmoschus esculentus*) leaves, such as total phenol, total sugar, non-reducing sugar, nitrogen and protein. **Cheema *et al.*, (2003)** showed that, protein content in two soybean varieties increased with infection with soybean yellow mosaic virus. **Haque *et al.*, (2005)** reported that, Changes in the chemical composition and physiological behaviour such as photosynthesis and respiration of Zucchini yellow mosaic virus (ZYMV)-infected pumpkin leaves (*Cucurbita moschata*) compared to healthy ones were determined in a net house. Observations revealed that ZYMV infection increased the protein content of pumpkin leaves compared to healthy ones. ZYMV infection in pumpkin leaves showed a decreased rate of photosynthesis and an increased rate of respiration. **Muqit *et al.*, (2007)** showed that, an experiment was conducted to determine the changes in ash gourd (*Benincasa hispida*) due to infection of *Papaya ring spot virus* (PRSV). Total protein was increased in the infected leaves due to PRSV. **Singh *et al.*, (2007)** reported that, primary and secondary metabolites like proteins have received considerable attention in relation to resistance in plants against diseases. Protein content was higher in plant parts infected with *Pea mosaic virus* (leaf, stem and root) than their healthy counterparts, but maximum protein content was found in diseased leaves followed by root and stem. **Poonam and Gupta (2008)** reported that**,** the infected samples of bean plant infected with bean common mosaic virus contained more protein than healthy ones. The higher protein content in virus infected plants is possibly due to the synthesis of virus coat protein and other virus associated non-structural proteins. **Rao *et al.*, (1989)** concluded that, the increased protein content in virus infected plants was **due to** increased activity of RNA synthetase or RNA polymerase.

3.2. Protein electrophoresis

Gel electrophoresis is a rapid method for detecting viral proteins. In the present study, protein band of molecular weight of 31 kDa was appeared **(Fig. 2).** No similar protein band was observed in samples of healthy plants. The same results were

obtained by **Fleming *et al.*, (1991), Abdel-Ghaffar *et al.*, (2003), Kimmo *et al.*, (2009) and Lin *et al.*, (2009).** Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) is a widely used technique for the separation and molecular weight estimation of individual proteins **(Melcher and Fletcher, 1999). Seifers *et al.*, (2000)** characterized a novel potyvirus isolated from maize. About 36 kDa protein was detected when virus was analysed by electrophoresis in polyacrylamide gels (SDS-PAGE). **Rozhnova *et al.*, (2001)** found that, the electrophoretic analysis of leaf proteins of potato plants revealed a 33-kDa polypeptide induced by the potato virus y. **Lin *et al.*, (2004)** characterized a new potyvirus isolated from jasmine (*Jasminum sambac*) in Taiwan. A 32-kDa protein was detected when virus was denatured with sodium dodecyl sulfate (SDS) and analysed by electrophoresis in polyacrylamide gels (SDS-PAGE).

3.3. Free amino acids content

Data in **Table (2) and Fig. (3)** show that, BtMV-infected beet plants contain significant lower content of total free amino acids than that of the healthy ones. BtMV inoculated plants recorded 35.27, 58.83, and 24.00 percent decrease in free amino acids content over healthy plants at first, second and third week's respectively after inoculation. This result was agreed with that obtained by **Jeyarajan and Ramakrishnan (1972); Chowdhury *et al.*, (1985) and Suteri (1985). Fiebig *et al.* (2004)** found a significant reduction in the concentration of the total amount of amino acids of barley plants infected with Barley yellow dwarf virus. **The decrease in total free amino acids may be due to the reduction in photosynthesis and the increase in respiration rate. Hemida (2005)** showed that, virus causing mosaic, mottling, malformation and distortion in faba bean (*V. faba*) was found in various fields in Assiut Governorate, Egypt. The virus isolate was detected and identified as *Bean yellow mosaic virus* (BYMV). Total free amino acids were estimated in leaves of two host plants (*Vicia faba* and *Phaseolus vulgaris*) inoculated with BYMV. In *Vicia faba* and *Phaseolus vulgaris* plants, the virus isolate induced a lower concentration in free amino acids.

3.4. Amino acids composition

Data in **Table (3)** show that, the concentrations of amino acids like alanine, aspartic acid, glutamic acid, phenylalanine, serine, threonine and tryptophan were more in infected plants up to 21 days after inoculation. Trace amounts of arginine, glycine, leucine, lysine, and valine were detected in healthy plants and their contents were more in infected plants. Cystine, proline and tyrosine were absent in healthy leaves whereas they were present in traces in infected plants at 14 days after inoculation and could not be detected in the succeeding stages of analysis. These results were agreement with that obtained by **Kaniewski and Micinski (1978); Randles *et al.*, (1980); Short and Davies (1987); Atreya *et al.*, (1991); Kakani *et al.*, (2001); Liu *et al.*, (2001); Kisaka and Kida (2003). Roberts and Ramasarma (1952)** observed the accumulation of serine, threonine, and proline in plants infected by *Turnip yellow mosaic virus*. **Bozarth and Diener (1963)** found increased concentration of glutamic acid, glutamine, serine, asparagine, aminobutyric acid and proline in PVY-infected tobacco plants. **Turka (1985)** concluded that, the concentration of some amino acids is considered a critical factor in determining the attractiveness of the crop to sucking insects and the resistance of the crop to attack. **Anan *et al.*, (1996)** reported that, contents of amino acids were measured in the fruits of transgenic tomato carrying the TMV [*Tobacco mosaic tobamovirus*] coat protein gene, non-transgenic tomato, cultivated species of tomato and their wild relatives. The results showed no significant differences in these chemical components between the fruits of transgenic and non-transgenic tomatoes. *Lycopersicon peruvianum* and *Lycopersicon hirsutum* had much higher concentrations of aspartic acid contents in fruits of transgenic tomato, non-transgenic tomato. *Lycopersicon peruvianum* were considerably higher than in the other species. *Lycopersicon cheesmanii* had the highest glutamic acid, asparagine and glutamine contents. **Zeh *et al.*, (2001)** indicated that, Methionine (Met) and threonine (Thr) are members of the aspartate family of amino acids. The enzymes cystathionine gamma-synthase and Thr synthase (TS) compete for the common substrate, O-phosphohomo-Ser with the notable feature that plant TS is activated through S-adenosyl-Met, a metabolite derived from Met. Increased levels of homo-Ser and homo-cysteine indicate increased carbon allocation into the aspartate pathway. In contrast to findings in Arabidopsis thaliana, increased Met content has no detectable effect on mRNA or protein levels or on the enzymatic activity of cystathionine gamma-synthase in potato.

Any particular amino acid required for virus synthesis is present in limited amounts in the normal amino acid pool of the host and may thus act as a limiting factor to virus infection. Asparagine participates in the biosynthesis of purine, pyrimidines, nucleotides and nucleosides. Because of increased amino acid content, it may also be possible that these amino acids may form part and parcel of the virus protein constituents **(Tu and Ford, 1970).**

Table 1. Effect of BtMV infection on protein content of beet plants at different weeks after inoculation.

Weeks after inoculation	Protein content(mg/g)		
	Healthy	Infected	Percent increase over healthy
First week	0.63	0.68	7.93
Second week	0.82	0.89	8.53
Third week	0.83	0.99	19.27
L.S.D at 5%	0.02		

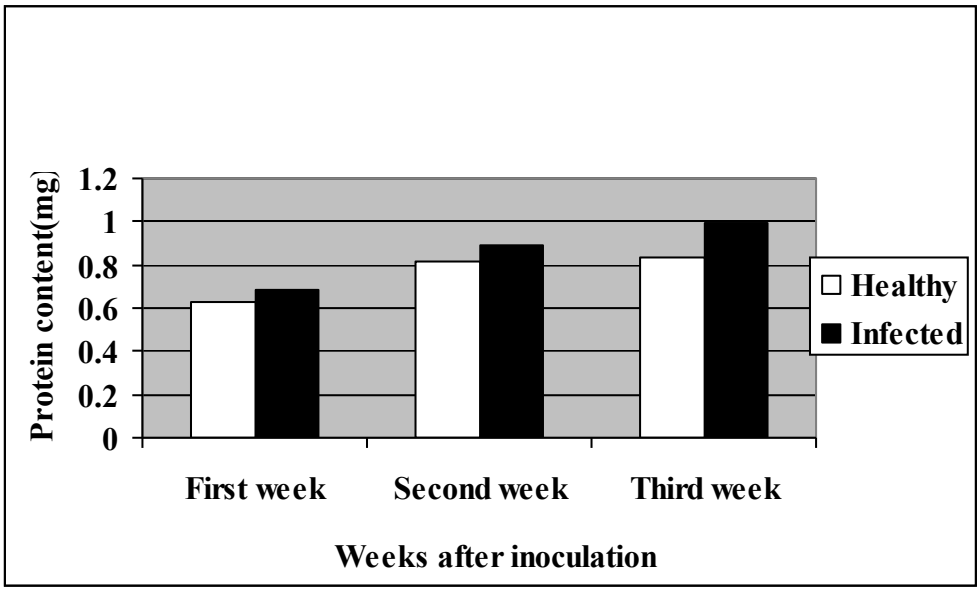

Figure 1. Protein content of BtMV-infected beet plants at different weeks after inoculation.

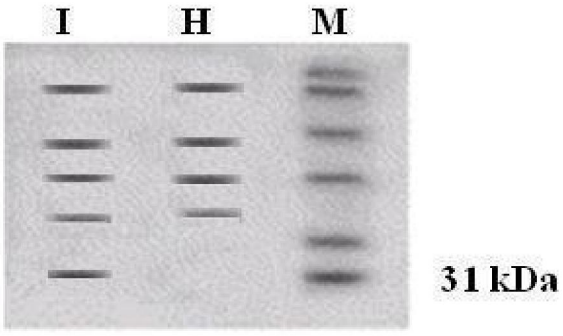

Fig (2): Electrophoresis of the protein of beet plants infected with BtMV in 12.5% SDS-PAGE. M: Marker proteins with molecular masses in kDa. I: Proteins from beet plants infected with BtMV. H: Healthy sample. BtMV protein was stained with Coommasie brilliant blue

Table 2. Effect of BtMV infection on amino acids content of beet plants at different weeks after inoculation.

Weeks after inoculation	Amino acids content(mg/g)		
	Healthy	Infected	Percent decrease over healthy
First week	3.72	2.75	35.27
Second week	9.53	6.00	58.83
Third week	8.37	6.75	24.00
L.S.D at 5%	0.09		

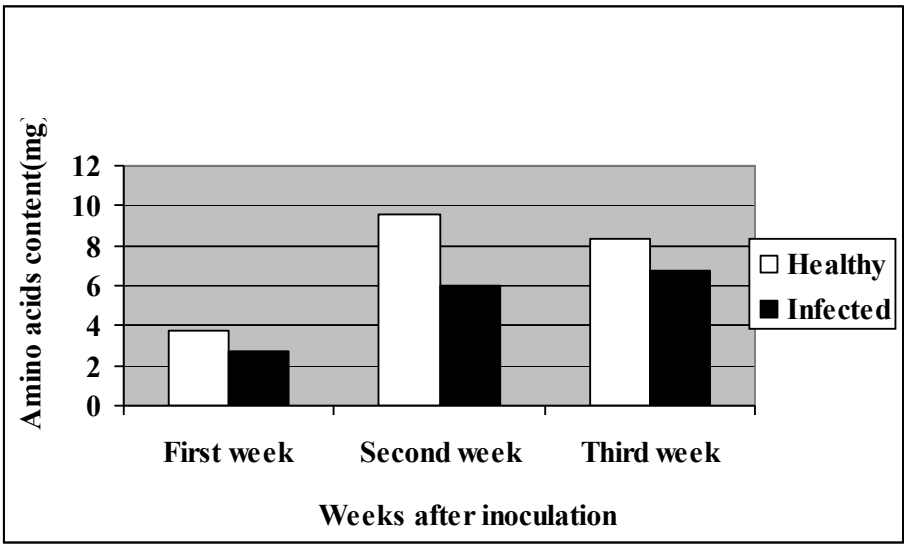

Figure 3. Amino acids content of BtMV-infected beet plants at different weeks after inoculation.

Table3. Effect of BtMV infection on amino acids composition of beet plants at different weeks after inoculation.

Amino acid	Weeks after inoculation					
	Healthy			Infected		
	First week	Second week	Third week	First week	Second week	Third week
Aspartic acid	++	++	++	++	+++	+++
Threonine	+	+	+	++	++	+
Serine	+	+	+	++	++	++
Glutamic acid	++	++	++	+++	+++	+++
Glycine	+	+	+	+	++	+
Proline	-	-	-	+	+	+
Alanine	++	++	++	+++	+++	+++
Cystine	-	-	-	-	+	-
Valine	+	+	+	++	+	+
Leucine	+	+	+	++	+	+
Tyrosine	-	-	+	+	+	+
Phenylalanine	-	+	+	+	+	+
Lysine	+	+	+	+	++	+
Histidine	-	-	-	+	-	-
Arginine	+	+	+	+	++	+
Tryptophan	+	+	+	++	++	++

(-): Nil; **(+):** Slight **; (++):** Mild **; (+++):** High

3.5. Histological changes

From **Table (4) and Fig. (4)**, it could be noticed that, BtMV had a lower effect on Av. Medvein thickness, Av. Blade thickness, Av. Palisade tissue thickness, Av. Spongy tissue thickness, Av. Vascular bundles length, Av. Vascular bundles width, Av. Number of xylem vessels and Av. Xylem vessel diameter. Similar results were obtained by **Dubey and Bhardwaj, (1982); El-Hammady *et al.* (1983); Eskarous *et al.* (1984); Buchter *et al.* (1987); Roberts, (1989); Tzeng *et al.* (1993); Singh and Rathi (1996); Ashraf *et al.* (1999); Reddy *et al.* (2006); Prestes *et al.* (2009). Gevorkyan *et al.* (1976) and Burdonov (1978)** showed that, infection with virus caused a reduction in the width of cells in the palisade parenchyma. The leaf blade is reduced in thickness. **Kaminska and Zawadzka (1977)** reported that, in trees infected by apple rubbery wood virus, the xylem was unevenly and poorly lignified. Cells were much smaller, vessels fewer and xylem rays larger. **Buzhoryanu (1984)** reported that, in virus-infected tobacco leaves there was a reduction in lamina thickness due to a contraction of cells, particularly the palisade layer and the parenchyma, and a reduction in the intercellular spaces. **El-Dougdoug *et al.* (1993)** evaluated the effect of *Citrus exocortis viroid* (CEVd) infection on the histology of young orange (*Citrus sinensis*) leaves. Light microscope investigation of the leaf petiole and cross sections of the leaf blade showed several histological changes. In general, CEVd-infection affected the conductive tissues. Infected phloem tissues showed less active sieve elements, and phloem radial thickness and secondary phloem fibers were reduced. The thickness of xylem tissue and vessel diameter was also reduced, as was the number and diameter of glands. Infection reduced the palisade layers. Also, **Sofy *et al.* (2007)** reported that, *Citrus psorosis virus* Egyptian isolate (CPsV-EG)-infection affects the upper epidermis of the leaf which is composed of non-tabular parenchyma cells covered by a thin layer of cuticle. Crystal idioblast (CI) containing cells are lacking in the palisade layer and protrude into the epidermis. The oil glands are lacking compared with healthy leaf. Secondary growth occurs in midvein and major lateral veins in smaller veinlets. The vein endings consist of a single trachoid strand of elongated parenchyma cells enclosed by the bundle sheath compared with healthy ones. **Vigliocco *et al.*, (1993)** studied histology of leaves of maize infected by *Maize rough dwarf fijivirus* (MRDV). It appeared that vascular bundles of the 2nd and 3rd order were first affected with accumulation of dense granular contents in some phloem cells, initiation of hyperplasia extending towards the abaxial epidermis and subsequent differentiation of xylem, phloem, and parenchymatous elements in the proliferating cellular mass. This cellular mass extending beyond the leaf epidermis constitutes an enation, a characteristic symptom of infection by MRDV. **IsHak and El-Deeb (2004)** reported that, the most important changes due to *Sweet potato chlorotic stunt virus* (SPCSV) infection were confined to the vein region. In general, almost all the anatomical characters of the midrib investigated by light microscopy were increased. However, a reduction was observed in the diameter of xylem vessels and phloem area as well as the thickness of the leaf blades. **Kunkalikar *et al.*, (2007)** showed that, *Ppapaya ring spot virus* brings about histological and histochemical changes in papaya upon infection. In diseased leaves, palisade cells were markedly distorted. The spongy cells lost their normal round shape with complete disintegration.

Table 4. Anatomical observation on beet leaf tissues infected with BtMV.

Measurements(µ)	Healthy	Infected
Average of Medvein thickness	1317	732
Average of Blade thickness	189	161
Average of Palisade tissue thickness	87	59
Average of Spongy tissue thickness	71	54
Average of Vascular bundles length	233	270
Average of Vascular bundles width	197	157
Average of Number of xylem vessels/bundle	75	65
Average of Xylem vessel diameter	35	20

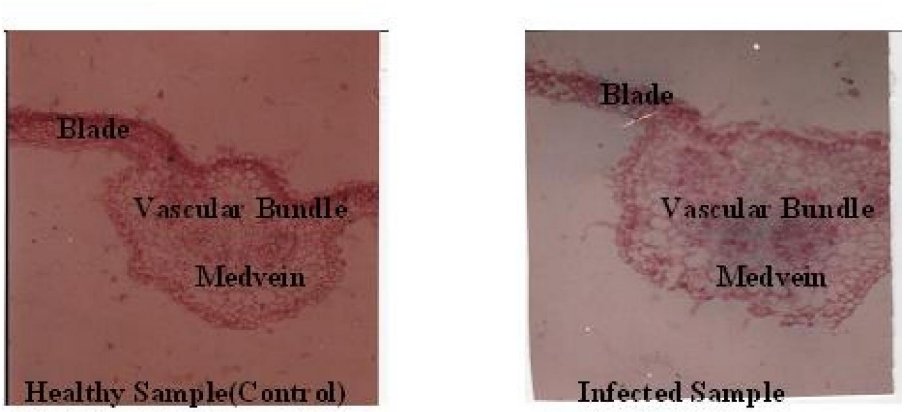

Fig.(4): Transections of beet leaf infected with BtMV in comparison to control one.

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