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Mango Malformation Disease in Egypt: 2- Physiological and Histopathological Changes in Malformed Inflorescences and Leaves and Therapy

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Mango (*Mangifera indica*) is affected with malformation disease (MMD). It was found that the malformed tissues contained viroid-like RNA. Due to infection, light microscope examination of leaf blade cross sections showed several histological changes. Phloem radial thickness and secondary phloem fibers were reduced. Xylem tissue thickness as well as vessel diameter was also reduced. The glands were also reduced in both number and diameter. The ultra-structure as shown by transmission electron microscope of infected leaves showed important variations. Infected cells developed the paramural bodies known as plasmalemmasomes. These bodies were different in size, shape and internal structure. Occasionally, other type of membrane structures, which resembled coiled watch spring like multilayered membrane body, were noticed. There were some reductions in the number of chloroplast, with aberration, in thylakoid membrane system, while grana were not well developed. Vesicles appeared in infected cell. Furthermore, the infected leaf cells showed degenerative effect as proved in the presence of mylein like bodies and in the large central vacuoles.

The MMD exhibited a significant decrease in gibberellin and auxin activities. In addition, natural inhibitors for gibberellin and auxins revealed significant activity in malformed inflorescences, compared with healthy ones. Cytokinin activities were significantly increased in malformed inflorescences. Also, it was noticed that cytokinins were act as natural active inhibitors.

The malformed inflorescences of all tested mango varieties had highly increase in Ca and Mn content compared with healthy ones. Also, the malformation disease caused a slight reduction in P and a high reduction in N, K, Fe and Zn contents.

The therapy of malformed mango trees with solution (X) containing micro and macronutrients and growth regulators resulted in an elongation of malformed inflorescences and leaves as well as a clear reduction in appearance of malformation phenomena.

Key words: Growth regulators, histological changes, mango malformation and paramural bodies.

Mango malformation is well known in India and has also been confirmed in most mango-growing countries, *i.e.* Pakistan, Bangladesh, Egypt, South Africa, Israel, Mexico, Brazil, Central America, Sudan, Cuba, Australia, U.S.A, and the United Arab Emirates (Kumar *et al.*, (1993). Losses due to malformation have not been accurately assessed because yield loss is not a linear function of disease severity. Nevertheless, malformed inflorescences in a tree do not bear fruit, thus causing losses in yield. In India over 50% of the trees are affected, with consequent heavy losses in yield (Kumar *et al.*, 1993). In South Africa, the disease is present on 73% of mango farms, with severity ranging from 1-70% (Rijkenberg and Crookes, 1984).

There are physiological and anatomical variation related to the disease. All viroid and viroid-like RNA induced the extreme stunting growth as primary symptoms. Other symptoms include epinasty, rugosity, twist and scission of leaves (Diener, 1988 and El-Dougdoug *et al.*, 1993 & 1998). They reported that the primary internal cytopathic changes, histological and ultrastructural due to infection. Light microscope investigations of leaf petioles and blade cross section figured out several histological changes. In general viroid infection clearly affected the conductive tissues. The glands were reduced in both number and diameter. The infection lessen palisade layers, whose cells showed almost cuboidal shapes with fewer chloroplast. The infected cells developed the paramural bodies known a plasmalemmasomes. There was some reduction in the number of chloroplast with aberrations in thylakoid membrane system while grana were not well developed. Vesicles were noticed in infected cell. Furthermore the infected leaf cells showed degenerative effect summarized in the presence of mylein like bodies and large central vacuoles (Hari, 1980, Diener, 1988, and El-Dougdoug *et al.*, 1993)

The involvement of hormonal imbalance was indicated by Majumder *et al.* (1970) who succeeded in obtaining considerable reduction in floral malformation by spraying the affected branches of varieties *Dashehi, Chausa* and *Bombay* Green with naphthalene acetic acid NAA (100 or 200ppm). Pandey *et al.*, (1973) suggested that the peculiar behavior of malformed shoots may be due to imbalance between growth promoters and growth inhibitors. Knight and Campbell (1995) mentioned that there were nutritional deficiencies (especially Zn, Fe, and Mn) associated with the strongly alkaline Egyptian soils (pH 7.5-8.0) and they also found the same nutritional deficiencies in malformed mango trees. Studies in India were not able to attribute a primary role to nutrients in inciting the disease (Singh *et al.*, 1991). Other reports indicated that hormonal imbalance in malformed tissues might be attributed to metabolic changes due to host-parasite interactions (Kumar and Beniwal 1992).

Finally, one hundred years since it was first recognized, the problem of mango malformation is still unresolved. Thus, this work aimed to continue investigations on the problem trying to cure it using simple practices.

Materials and Methods

Samples of healthy and naturally malformed inflorescences and leaves of mango of some varieties, *i.e.* Dabsha, Hindi, Taimour and Zebda, were collected from mango orchards at Qualubia Governorate. Samples were prepared for anatomical structure, ultrastructure, determination of endogenous hormones, macro and microelements.

Tissue processing:

Samples of both healthy and infected leaves were prepared to be examined by both light and electron microscopes. Transverse free hand sections of blade leaves were cut by hand into distilled water with a single edge razor blade. The sections were examined by light microscope. On the other hand, small leaf blade segments (about 1 mm) were cut from four different of healthy and malformed leaves. The selected tissue samples were fixed, processed for electron microscopy as described by Abdel-Ghaffar (1994) and ultrathin sections viewed with a Zeiss 10C Transmission Electron Microscope at National Research Centre, Dokki, Egypt.

Determination of macro and micro elements:

Macro and microelements in healthy and malformed inflorescences were determined according to the methods described in A.O.A.C, (1990).

Determination of endogenous hormones:

Gibberellin, auxin, cytokinin-like substances and inhibitors like-substances were extracted from healthy and malformed leaves as well as those sprayed with solution X according to the technique described by El-Antably (1976). Lettuce hypocotyl bioassay was used for testing gibberellin activities (Frankland and Wareing, 1960). Wheat coleoptile section was used in straight growth assay technique described by Bently and Hously (1954) for auxin activity. The determination of cytokinin activities was based on induction of chlorophyll formation in cucumber cotyledons (Fletcher and McCullagh, 1971). The experimental data were statistically analyzed by the short cut procedure described by Tukey (1953).

Therapy of mango malformation phenomena:

Mango trees cultivated in nursery or in the open field, which showed malformation of leaves and inflorescences, were sprayed with solution (X). The solution (X) was formulated to consist of macro and microelements and growth regulators. The solution was applied by 3 systems i.e. one time, twice with 60 days intervals and three times with 30 days intervals during growth period in mango orchards at different locations i. e., Shamal El-Tahrier, El-Khatatba, and Ismaielia.

Results

Samples of healthy and infected leaves were collected and carefully examined in order to direct a spotlight on the viroid-like RNA effects on plants. The histological and ultra-structural, physiological hormones, micro and macro-elements alteration were taken into consideration.

Anatomical features:

Microscopic examination revealed several changes in both blade and petiole due to viroid-like RNA infection (Table 1). In general, the obvious effect was confined to the conductive tissues. The viroid like RNA reduced the thickness of both phloem and xylem tissues. The infected phloem tissue contained much less active sieve

Table (1): Effect of mango malformation on some characters

Characters of leaf blade*	Healthy	Malformed
Phloem thickness (μm)	120.78**	76.10
Xylem thickness (μm)	220.60***	132.33
Blade thickness (μm)	737.00**	606.60
Xylem vessel diameter (μm)	50.21**	35.59
Midrib diameter (μm)	340.00***	178.00
Gland diameter (μm)	117.20	112.82
Xylem row (number)	25.50	30.50
Xylem vessels/row	3.75	5.00

* Average of three readings.
** Significance at 5%.
*** Significance at 1%.

elements. Moreover, the secondary phloem fibers were clearly reduced as a result of infection. The infected xylem vessels attained fewer diameters as compared with those of healthy ones. Consequently, the vascular cylinder dimension of leaf midrib was significantly lessened (Fig. 1&2). It was also found that infection has reduced both number and diameter of glands of blade and leaf midrib.

Ultrastructural studies:

Examination of mesophyll cells in ultra-thin sections prepared from malformed leaves revealed cytopathic effects in comparison with the healthy ones. Concerning malformed tissues, the following effects were noticed; 1- Granulation of the cytoplasm. (Fig. 3, photo C & E). 2- large number of different size of cytoplasmic vacuoles formed by invagination of plasmalemma (Fig. 3, photos, A, B&D) and tonoplast (Fig. 3, photo B). 3- Appearance of two types of vesicles in the vicinity of plasmodesmata. One type of vesicle contained fiberillar materials usually forming net works, the other was filled with a dense electron opaque materials (Fig. 3, photos E&F). Both types of vesicles were bound by single membrane and sometimes had ribosomes attached to them. 4- Malformation of mitochondria (Fig. 3, photos E&F). 5- The chloroplasts showed conspicuous alterations in shape (round, concave and clumping) and in internal structure (destroying of grana and thylakoid membrane (Fig. 3, Fig. 3, photos, A, B, C, D, E & F). 6- The infected cells showed some abnormalities in the cell walls, i.e. cell wall protrusion, depositions of electron dense materials near the walls (Fig. 3, photos, C, E & F). 7- There were paramural bodies with different sizes containing multiple membrane enclosed chambers and multivesicular bodies or plasmalemmasomes (PLS) can be correlated with symptoms and were not found in non-infected cells. Occasionally, other type of membrane structures which resembled coiled watch spring were noticed (Fig. 3, photos, C, D & E).

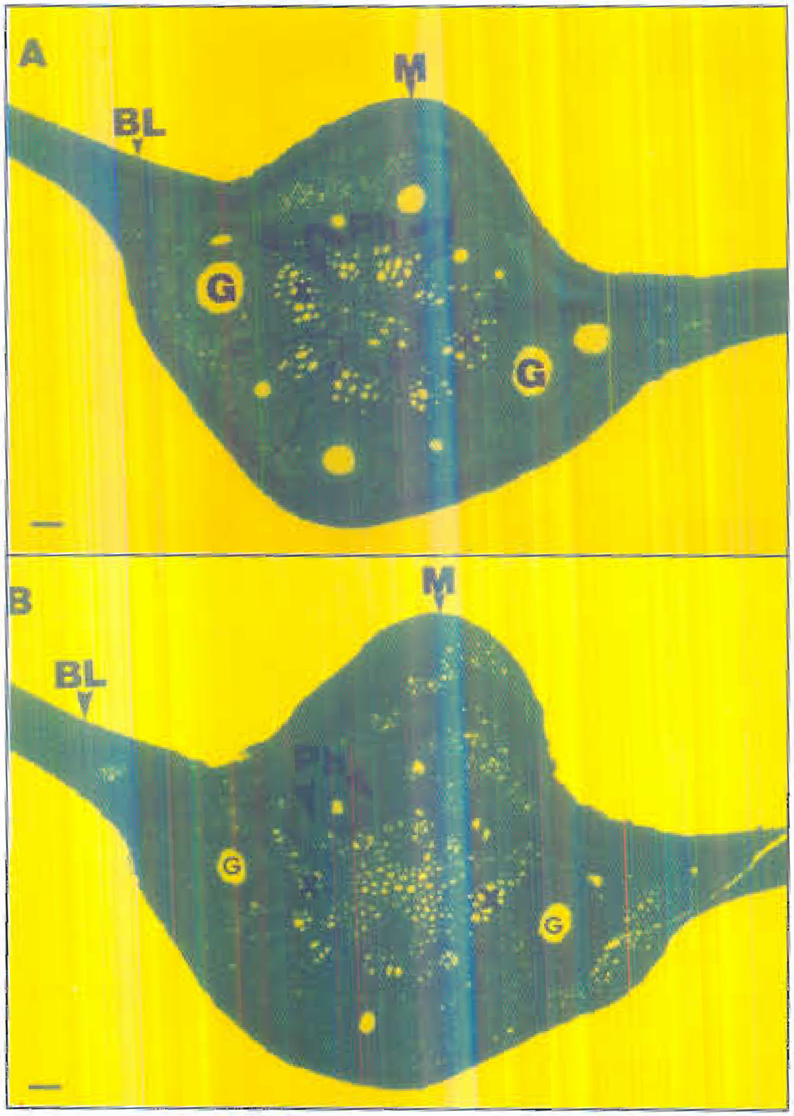

Fig. 1. Light micrographs of transverse sections of mango Zebda leaves, hand cross section of blade leaves. A= Healthy and B= Infected (40Xc). Whereas: Bl= blade, M= midrib, G= gland, Ph= phloem and X= xylem.

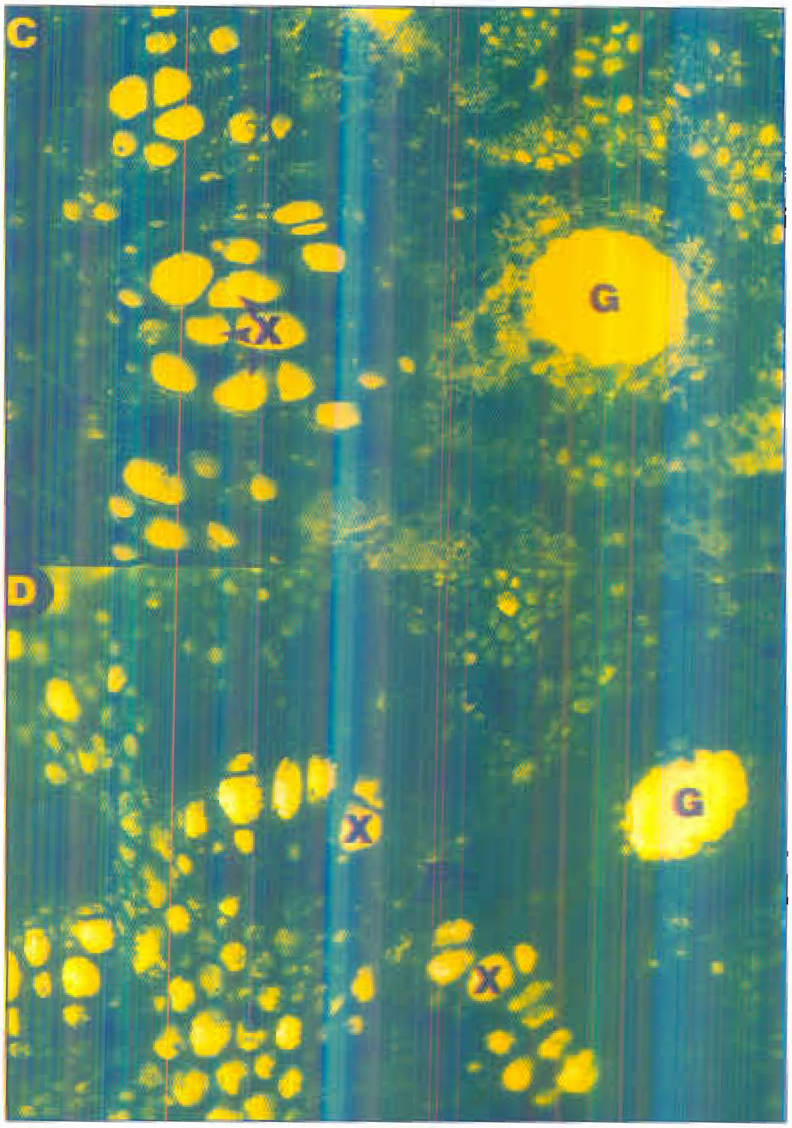

Fig. 2. Light micrographs of transverse section of mango Zebda leaves, hand cross section of petiole of C = healthy and D = infected (88Xc). Whereas: G= gland, Ph= phloem and X= xylem.

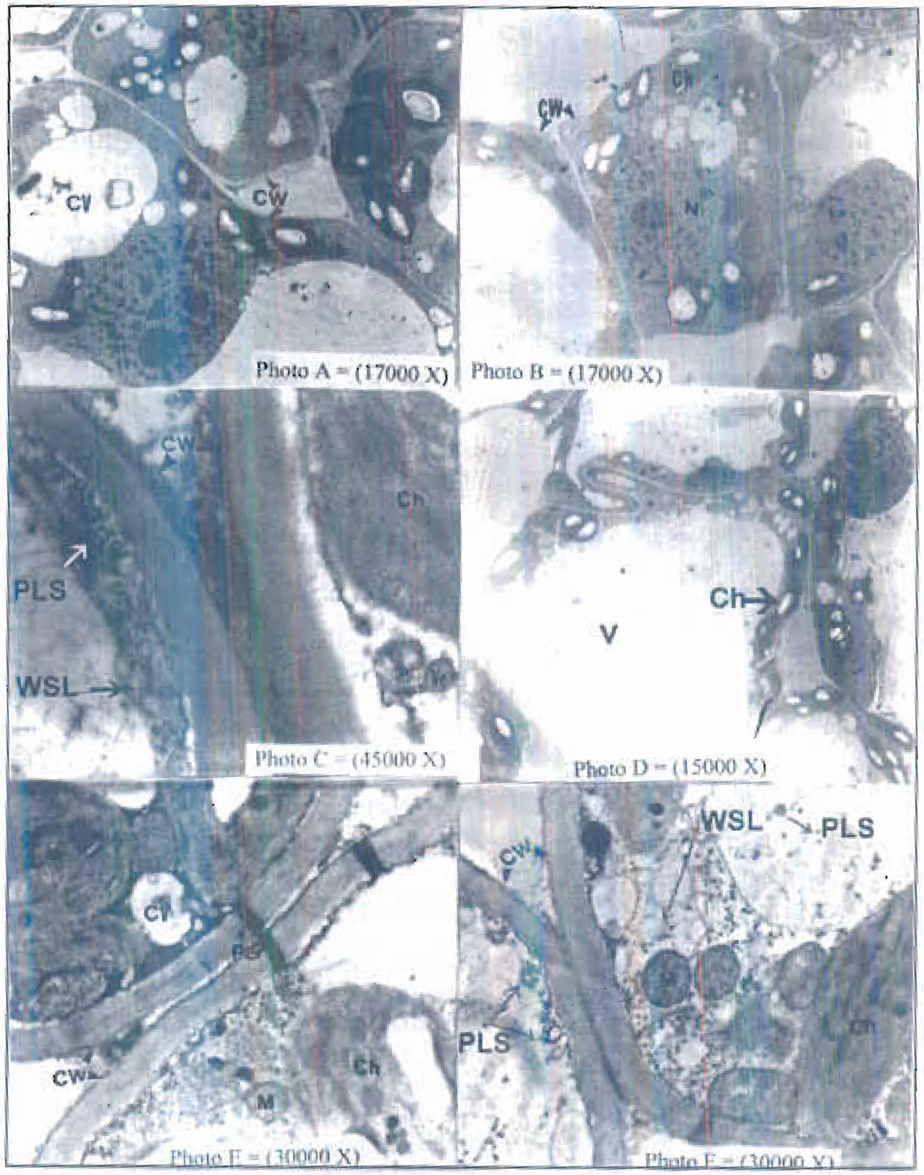

Fig. 3. Chloroplast of infected mango leaves and cytoplasm vacuoles (A&B). Watch spring like multilayered membrane body and multiple vesculated paramural bodies or plasmalemmasomes (C&F). Cell wall aberration occurring corrugated profile (A&C). The irregular internal structures are electron dense and differ in size (E&F).
Whereas: Ch= chloroplast, Cv= cytoplasm vacuole, N= nucleus, Cw= cell wall, V= vesicular, PLS= plasmalemmasomes, WSL= watch spring like, M= mitochondria and G = granules.

Effect of mango malformation on some macro and microelements in mangó inflorescences:

Data presented in Table (2) show that malformed mango varieties had a high increase in Ca and Mn contents compared with the healthy ones. On the other hand, the malformed mango showed a slight reduction of Phosphorus and high reduction of N, K, Fe, Mg and Zn contents.

Table 2. Effect of mango malformation on macro and microelements in healthy and malformed mango inflorescences

Elements	Mango inflorescences***							
	Dabsha		Hindi		Taimour		Zebda	
	H	M	H	M	H	M	H	M
Macro Elements (%)*								
N	1.49	1.16	1.86	1.29	1.36	1.10	1.28	0.96
P	0.56	0.39	0.76	0.70	0.41	0.38	0.38	0.34
K	0.79	0.56	0.76	0.43	0.86	0.49	0.71	0.54
Ca	1.41	1.68	1.42	1.86	1.22	1.54	1.35	1.88
Mg	0.66	0.68	0.83	0.64	0.73	0.71	0.76	0.61
Micro Elements (ppm)**								
Fe	311	286	322	290	281	257	298	266
Zn	38	26	54	41	24	19	46	31
Mn	29	44	46	78	41	63	30	46

* Calculated as percentage.
** Calculated as ppm.
*** H= Healthy inflorescences and M= Malformed inflorescences.

Effect of mango malformation infection on activities of endogenous phytohormons:
A- Gibberellins:

The gibberellins like substances, as well as, their inhibitors are determined in both healthy and malformed inflorescences and their nearest leaves of four mango varieties, i.e. Taimour, Zebda, Hindi and Dabsha. Data in Fig. (4) illustrated that, the gibberellin activities in healthy inflorescences and leaves and malformed inflorescences sprayed with solution X of four mango varieties were significantly higher than in the malformed ones., where, their values reached minimum in those malformed and non sprayed ones. In addition, natural inhibitors of gibberellins exhibited significant activity in malformed inflorescences meanwhile, did not appear in healthy and those sprayed with solution X. (Fig. 4). That was true for the four mango varieties. Moreover, Taimour variety showed the highest activity of natural gibberellin inhibitors followed by Zebda comparing with Hindi and Dabsha varieties. Meanwhile, Hindi and Dabsha varieties were the least significant. On the other side, in leaves those lies directly below healthy and malformed inflorescences did not exhibit clear differences regarding both gibberellin and inhibitors activities between them.

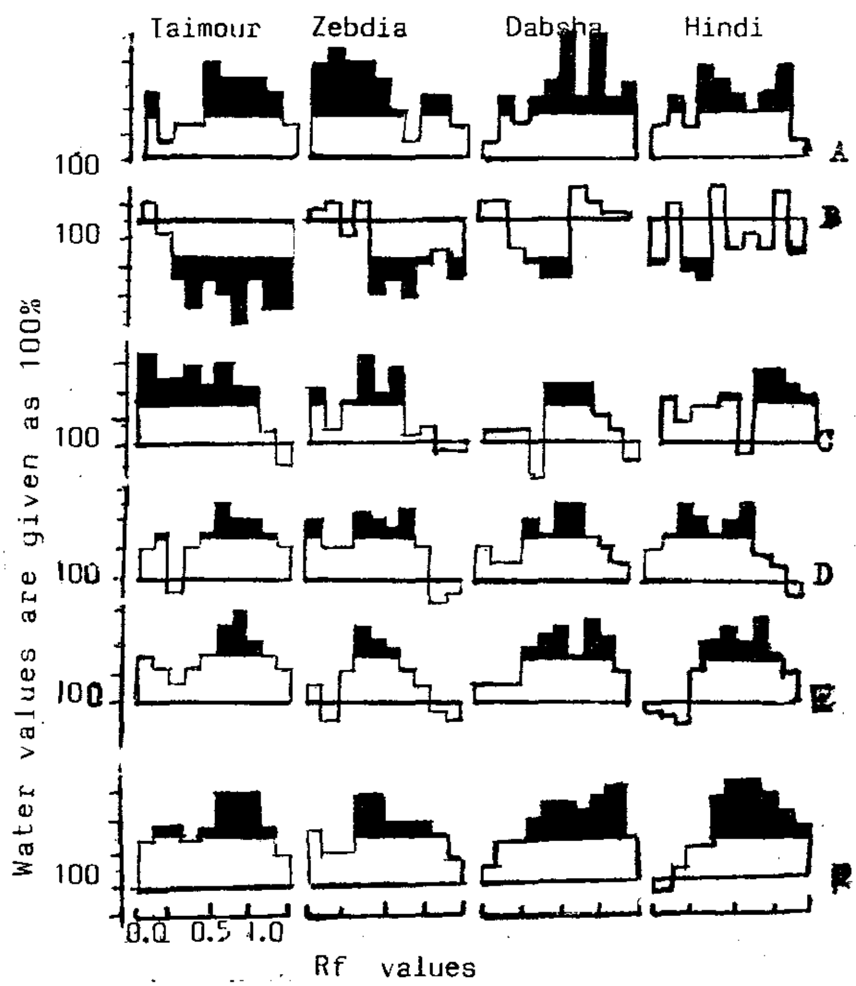

Fig. 4. Changes in gibberellin activity in inflorescences and their nearest leaves of four mango varieties.
Gibberellin activities were determined by lettuce seedlings bioassay test.
A- Healthy inflorescences, B- Malformed inflorescences, C- Malformed inflorescences post-sprayed with solution X, D- Healthy leaves, E- Malformed leaves and F- Malformed leaves post-sprayed with solution X

B- Cytokinins:

The endogenous activities of cytokinins were significantly increased in malformed inflorescences of the four mango varieties before spraying with solution X. This significant increase showed its maximum in Zebda variety followed by Taimour, meanwhile, the other two varieties, i.e. Hindi and Dabsha were nearly the same (Fig. 5). Also, it was noticed that cytokinins natural inhibitors activity did not appear in all mango varieties. On the other hand, in case of healthy inflorescences, not only did not exhibit significant differences in their cytokinins activity but also insignificant or small persistence of cytokinin inhibitors were also found in case of Zebda and Dabsha varieties. As for the activities of cytokinins and their natural inhibitors in leaves below healthy and malformed inflorescences, there were no clear differences existing between them (Fig.5). The spraying of the malformed inflorescences or leaves with solution X, showed no differences in the trend of activity of cytokinins and their inhibitors as compared with non sprayed ones (Fig. 5).

C-Auxins:

It was clearly noticed that auxins were higher in malformed sprayed compared with healthy inflorescences than malformed non-sprayed ones. Since, healthy inflorescences showed significant increase of this activity in mostly with different mango varieties. Meanwhile, malformed ones not only exhibited more less of auxins activity but also, to some extent inhibitors activity was present (Fig. 6). As for auxin activity in the nearest leaves those lies directly below inflorescences either healthy or malformed ones, it is obvious that no significant differences existed among them. Since, levels of activity were nearly the same in different varieties. Here, again, a change of hormonal balance appears to be in an intimate relationship of the malformation phenomena in the studied mango varieties.

Effect of spraying with solution X on malformed mango tissues:

The sprayed malformed inflorescences or leaves with solution (X) on seedlings and trees of mango were visually investigated. The visual investigation indicated that spraying of malformed tissue for one time, twice with 60 days intervals and three times with 30 days intervals during growth period in mango orchards at different locations i. e., Shamal El-Tahrier, El-Khatatba, and Ismaielia improves the growth of mango trees and led to disappearance of malformed growth after about 20-30 days post spraying Fig. (7), as well as, increased fruit setting on cured inflorescences of treated mango trees in Shamal El-Tahrier, and Ismaielia. The levels of endogenous phytohormons affected by this treatment. The cytokinin levels were increased in their biological activities as a result for this treatment. Furthermore, level of gibberellin and auxin contents in inflorescences and leaves were increased, indicating that the agent of malformed inflorescences and leaves had reduced in biological activities of endogenous phytohormons (Fig. 4, 5 & 6).

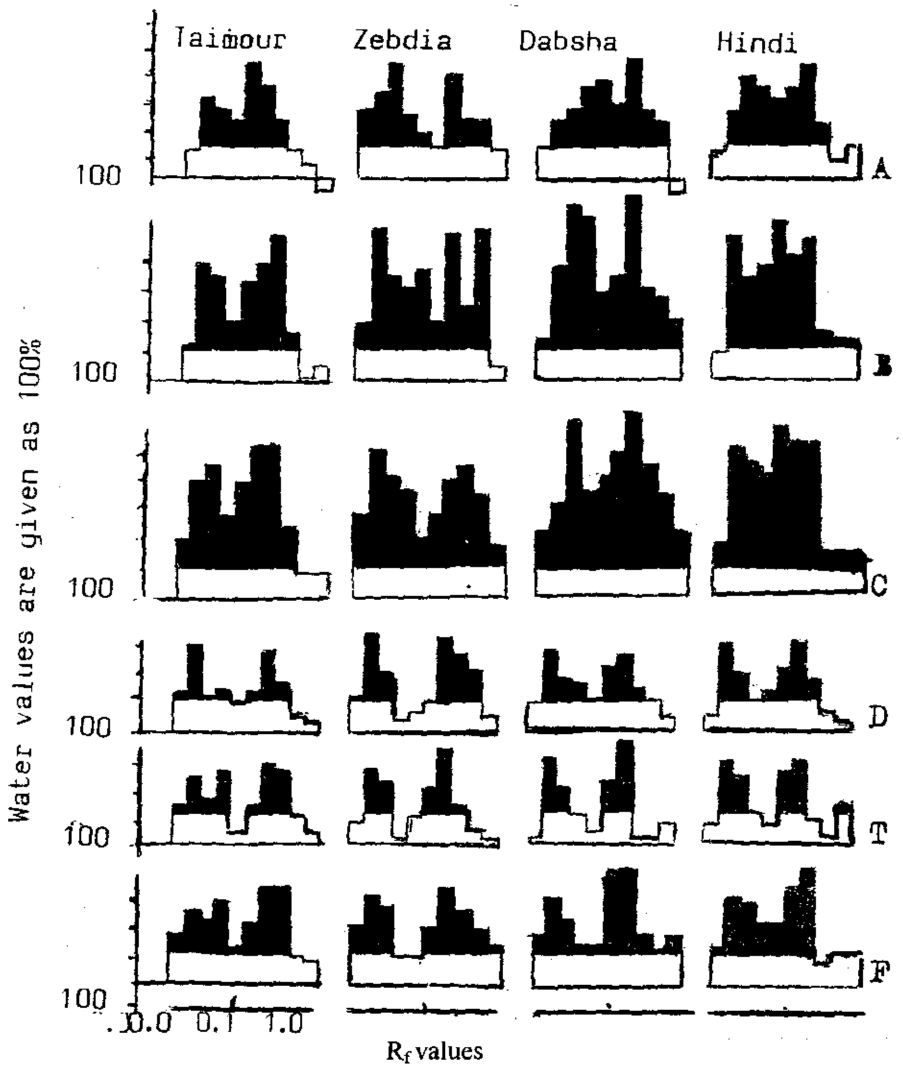

Fig. 5. Changes in the cytokinins activity in the inflorescences and their nearest leaves of four mango varieties.

Cytokinins activity was determined by the Amaranthus beta-cyanin bioassay test (water values are given as 100 percent)

A= Healthy inflorescences, B= Malformed inflorescences, C= Malformed inflorescences post-sprayed with solution X, D= Healthy leaves, E= Leaves nearest malformed inflorescences and F= Leaves nearest malformed inflorescences post-sprayed with solution X.

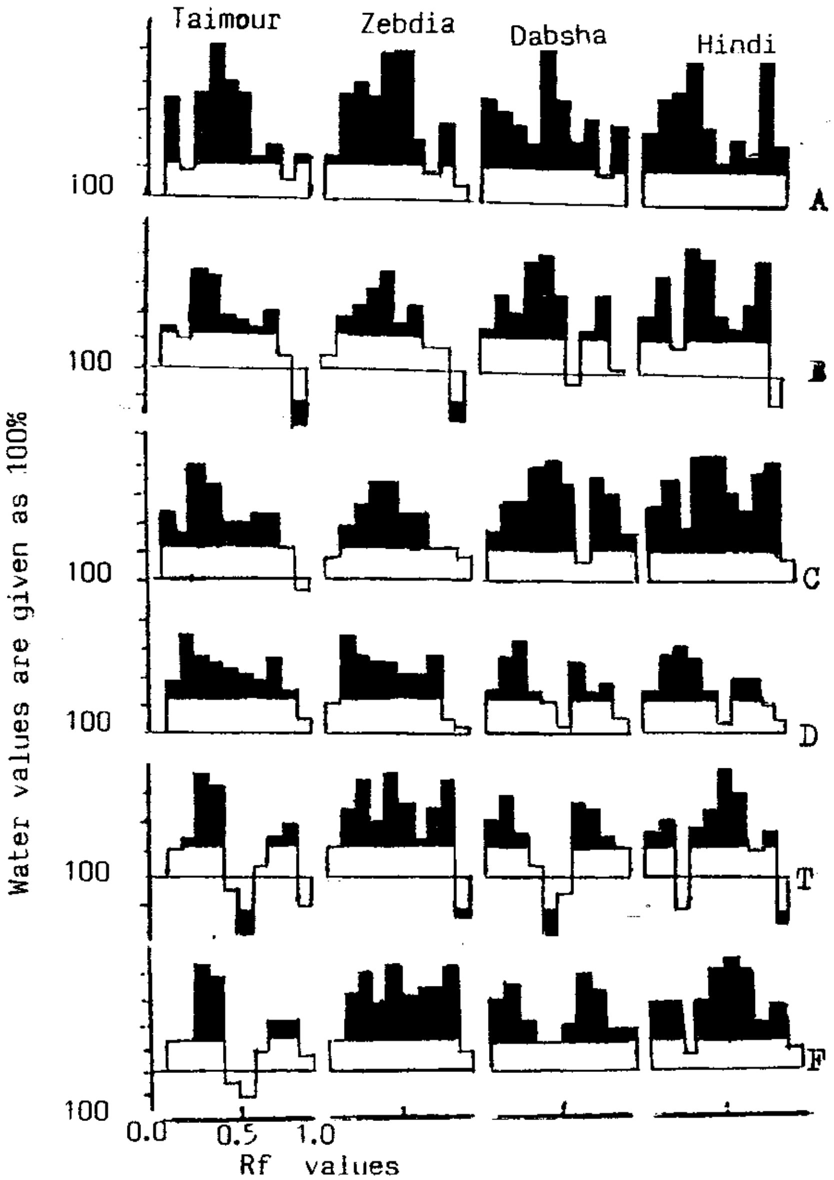

Fig. 6. Changes in the auxins content in the inflorescences and their nearest leaves of four mango varieties.
Auxins activity was determined by the wheat coleoptile straight growth bioassay test.
A= Healthy inflorescences, B= Malformed inflorescences, C= Malformed inflorescences post-sprayed with solution X, D= Healthy leaves, E= Leaves nearest malformed inflorescences and F= Leaves nearest malformed inflorescences post-sprayed with solution X.

B

Fig. 7. Mango inflorescences and leaves.
1- Malformed inflorescences and leaves pre sprayed with solution X.
2- Inflorescences and leaves post sprayed with solution X.

Discussion

Malformation of inflorescences and leaves of early natural infection on mango trees and seedlings (showing erect growth of in particular the production of small leaves with various forms of epinasty and an abnormal unevenness of surface) were found infected with viroid-like RNA [El- Dougdoug *et al.*, 2003, (in press)]. These samples were used for determination the physiological and histopathological changes compared with the healthy ones.

In the present work, it was found that the influence of viroid like RNA was similar to viroid and virus infection on physiological and biological effects (Diener, 1988, El-Dougdoug *et al.*, 1993 and El-Dougdoug *et al.*, 1998).

The viroid like RNA infection clearly affected the conductive tissues. It decreased phloem tissue thickness, fibers, xylem tissue thickness and vessel diameter. The glands in both number and diameter were reduced. As for leaf mesophyll cells infection, diminished palisade layers such tissue elements showed almost cuboidal shape with fewer chloroplast content. All mentioned anatomical changes could be attributed to indirect effects occurring in the host plant may be due to the lower auxin level resulting from viroid infection (Rodriquez *et al.*, 1978, El-Dougdoug, 1988 and El-Dougdoug *et al.*, 1993).

In infected mango cells, there were abnormal chloroplasts characterized by poor stacking of grana and the presence of loosely arranged thylakoid membranes. The thylakoid membranes were found to be pushed to the periphery because of the presence of electron dense granules. The only cellular abnormality described so far is the presence of hypertrophied nuclei in infected cells (Diener, 1988). The present investigation showed that the main cytopathic changes found that in viroid infected leaf cells were the presence of large numbers of paramural bodies and the abnormal development of the chloroplast organization. Paramural bodies of the type reported here also had been detected in infected cells, but not in healthy (Hari, 1980, El-Dougdoug *et al.*, 1993 and El-Dougdoug *et al.*, 1998).

The mango malformation led to reduction in macro elements (N, P, K and Mg) and microelements (Fe and Zn). Lee and Singh (1971) and El-Dougdoug (1988) obtained similar results.

The infected mango trees showed a significant decrease of gibberellins, auxins and like substances but increased levels of cytokinins in the malformed inflorescences and leaves comparing with healthy ones. These results agree with those obtained by Rodriguez, *et al.* (1978) for citrus exocortis viroid.(CEVd) and El-Dougdoug, (1988) for potato spindle tuber viroid (PSTVd).

Also, in this connection, Jindal and Hembera (1976) reported that, gibberellins could increase auxins levels, either by enhancement of auxin biosynthesis or by retardation of auxin destruction. Also, they indicated that gibberellins might act as suppressors for cytokinin production. The possibility of gibberellins mediated feed back inhibition of cytokinin must be recognized (Teitschevova, 1970). The increase in cytokinin activities may be the reason for occurrence of malformation in infected inflorescences. Spraying of malformed mango with solution X (consisting of macro, microelements and growth regulators) led to elongation of the malformed inflorescences and leaves and reduced appearance of symptoms and infectivity, as well as, increased fruit setting on cured inflorescences of treated mango trees in Shamal El-Tahrier, and Ismaielia. Lee and Singh, (1971) and Shawky, *et al.* (1978) found that irrigation with B, Mn, Co, Zn reduced virus and viroid infection symptoms on tomato and potato plants, as well as, increased the yield. On the other hand, it was proved that gibberellins could be also stimulating elongation independently of auxin. Therefore, the high content of gibberellin may be the cause of elongation in malformed inflorescences. Kazana and Kalsumi (1974) found that the high content of gibberellin might be the cause of spindling in infected potato tuber with PSTVd.

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مرض تشوه المانجو فى مصر:
٢- التغيرات الفسيولوجية والهستوباثولوجية فى نورات وأوراق المانجو المصابة والعلاج

خالد عبد الفتاح الدجدج*، جهاد محمد الهباء**، ممدوح حسين عبد الغفار*
* قسم الميكروبيولوجي – كلية الزراعة – جامعة عين شمس – مصر.
** قسم النبات الزراعي – كلية الزراعة بمشتهر – جامعة الزقازيق/فرع بنها – مصر.

وجد أن أنسجة أوراق وأزهار المانجو المشوهة تحتوى على تركيبات خيطية ودائرية للحمض النووى الريبوزى ذات وزن جزيئى منخفض مشابه للفيرويد. وقد أظهر الفحص بالميكروسكوب الضوئى للقطاعات العرضية لنصل الأوراق المصابة تغيرات هستولوجية. حيث أثرت الإصابة بوضوح فى الأنسجة الوعائية مسببة نقصا فى العناصر الغربالية لأنسجة اللحاء المصابة مع إختزال فى سمك الأشعة اللحائية وألياف اللحاء الثانوى. علاوة على ذلك نقص فى سمك نسيج الخشب بالإضافة إلى نقص فى متوسط قطر الوعاء الناقل فى ذلك النسيج كما أدت الإصابة إلى إختزال فى عدد ومساحة الغدد.

وقد أظهرت دراسة التركيب الدقيق لخلايا الأوراق المصابة تغيرات هامة بالمقارنة بالأوراق السليمة يذكر منها تطورات فى الجسيمات متعددة الطبقات والتى تعرف بالبلازماليماسومز Plasmalemmasomes حيث تباينت فى الشكل والحجم والتركيب الداخلى وتلاحظ أحيانا تركيبات أخرى تشبه Coiled watch spring . كما أدت الإصابة إلى خلل فى نظام أغشية ربط الجرانا Thylakoid membrane فى الكلوروبلاست. ونقص فى الجرانا وإختزال فى أعداد الكلوروبلاست. كما لوحظ وجود حويصلات فى الخلايا المصابة. وعلاوة على ذلك أدت الإصابة إلى تكسير فى الجسيمات المشابهة للمايلين Mylein like bodies مع تواجد فجوات عصارية مركزية كبيرة.

كما أدت الإصابة بالمرض إلى نقص معنوى فى نشاط الجبرلينات والأوكسينات بالإضافة إلى مثبطات الجبرلينات والأوكسينات الطبيعية فى الأزهار والأوراق المشوهة بالمقارنة بالسليمة. ولكن أدت إلى زيادة معنوية فى نشاط السيتوكينينات فى الأزهار المشوهة ونقص فى مثبطاتها الطبيعية.

وقد وجد فى الأزهار المشوهة فى كل أصناف المانجو المختبرة زيادة مرتفعة فى محتواها من الكالسيوم والمنجنيز بالمقارنة بالأزهار السليمة. ومن ناحية أخرى نقص قليل فى محتواها من الفوسفور ونقص مرتفع من النتروجين والحديد والبوتاسيوم والزنك.

وأظهر رش الأوراق والأزهار المشوهة بمحلول يحتوى على عناصر صغرى وكبرى ومنظمات النمو إلى إستطالة التكتل فى الشماريخ الزهرية وإستطالة الأوراق المشوهة وإختفاء ظاهرة التكتل.