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Cytogenetic and Ultra Structural Effects of *Narcissus tazetta* Extract on Root Meristem Cells of *Vicia faba* L.

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ABSTRACT

Water extract of *Narcissus tazetta* bulb were applied on both cytogenetical and ultra structural alteration in *Vicia faba* roots meristem cells. The experiment was carried out in two variants: (1) continuous treated with the extract for 3, 6, 12, 24 h and (2) treated at 3,6,12 h and followed by recovery time for 72 h. During the treatment, the mitotic activity was inhibited (24 h) depending on the long time of exposure of the extract. All treated resulted in gradual reduction of the mitotic activity. The mitotic activity reached its lowest value after 12 and 24 h. After time of treatment of the extracts, the mitotic activity was inhibited within 24 h and did not resume even after recovery. Treatment caused changes in the phase index, mainly as an increase in the number of prophases. After 24 h of treatment, in all phases, condensation and contraction of chromosomes were observed. After recovery, divisions did not resumed in all treatment, reaching even lower values than the control. C-mitosis, anaphase bridges, chromosome stickiness and micro- nuclei were observed in Narcissus extracts treated faba root-tip cells. In the current work combined both cytogenetical and ultra-structural effects of Narcissus extract on the root meristem cell of *Vicia faba* plants. Finally, the nucleolus material was extruded from the nucleus into the cytoplasm. Also, disruption of nuclear membranes, chromatin material, mitochondrial cristae disappear, formation huge autophagic vacuoles, and disintegration of organelles were observed.

Key words: *Narcissus tazetta*, *Vicia faba*, mitoses, biopesticides, nucleolus material

INTRODUCTION

According to the risks and damage caused by the synthetic pesticides, in recent years there has been a great increase in the number of the studies carried out to examine the effects of bio-pesticides in the agricultural context. Plant extracts, especially, the compounds of terpenoids, alkaloids and phenolics have been examined recently with respect to their effects on the growth and development of harmful insects (Erturk *et al*., 2004).

Many of natural plant substances have some of the characteristics of commercial synthetic pesticides, most natural plant products do not have the problem of creating harmful residues or break down materials that would damage plants or harm human beings and animals. However, much of the biological activity of natural products is still not well-documented. Some of the questions that remain regarding some compounds of the alkaloids, phenolics and terpenoids include: how long it takes for these natural products to disintegrate or disappear from nature (i.e.,underground water, soil)? (Mert and Betul, 2008).

Amaryllidaceae is one of large families in terms of bulbous plants such as *Pancratium, Narcissus, Galanthus* and *Leucojum*. The bioactive compounds of Amaryllidaceae species are known to possess a wide variety of biological activities including antitumor, antiviral, cytotoxic, anti-inflammatory, antinociceptive, anticholinergic and DNA-binding activities (Sener *et al*., 2003; Monarca *et al*., 2003). *Narcissus tazetta* is considered the oldest cultivated narcissus.

Narcissus plants have many medicinal uses due to containing many active constituents as secondary metabolites as alkaloids, many researchers proved that, narcissus could be used as anti-viral, anti-fungal, anti-tumor and many other uses. Insecticidal activity of the extracts of *Galanthus elwesii, Leucojum aestivum* and *Narcissus tazetta* ssp. *tazetta* were recorded as 90% or greater mortality within six days against Milkweed bug (Bowers *et al*., 1995). Liu *et al*. (2007) found that the extraction of *Narcissus tazetta* var. *chinensis* are effective against two insects *Sitophilus zeamais* (Motschlsky) and *Tribolium castaneum* (Herbst) and also, have strongly decreased the survival rate of tumor cell line, HL-60, K562, KT1/A3 and A3R (Liu *et al*., 2006). Ronsted *et al*. (2008) reported that alkaloid extracted from narcissus is effective against Alzheimer.

Piozzi *et al*. (1968) extracted from fresh bulbs of many varieties of daffodils two compounds have been extracted. Although their structures are related to many Amaryllidaceae alkaloids, the compounds show no basic properties since the nitrogen is amidic in character. The first substance, named narciclasine, shows a strong antimitotic activity and has been assigned structure VIII or its mirror image. The second compound, named narciprimine, has no antimitotic activity and has been given structure XII.

Bi *et al*. (1998) isolated an inhibitor form the secreted mucilage of *Narcissus tazetta* L. Bulbs. Based on X-ray analysis, the inhibitor was identified as Narciclasine (NCS), an Amaryllidaceae alkaloid.

Narciclasine (NCS) is a potent plant growth inhibitor. It shows a broad range of inhibitory effects on seed germination and seedling growth in wheat (*Triticum aestivum*). The chlorophyll content of light grown wheat seedlings was markedly reduced in the presence of NCS. The block of chlorophyll accumulation in the presence of NCS was most probably due to the block of the formation of 5-aminolevulinic acid, an essential chlorophyll precursor (Bi *et al*., 2003).

Narcissus tazetta lectin (NTL) with potent antiviral activity was isolated and purified from the bulbs of the Chinese daffodil *Narcissus tazetta* var. *chinensis*. This group of lectins shares a number of biological properties, such as rabbit erythrocyte hemagglutination, antiviral activity (Balzarini, 2006, 2007; Keyaerts *et al*., 2007; Ooi *et al*., 2010) and insecticidal effect (Gatehouse *et al*., 1995; Hogervorst *et al*., 2006; Ohizumi *et al*., 2009). Also, the biological activity of glucomannan isolated from Narcissus poetics plant which gave high growth, catalase and hormonal potential. The presence of high viscosity and biological potential of narpoetan isolated from bulb makes it possible to use the preparation with fungicides when germinating seed of spiked cereals and cotton (Umarov *et al*., 2009).

Many investigators used the electron microscope to observe the ultrastructural changes that occur in different cell organelles in response to chemical or physical agents. Soran *et al*. (1981) studied the micronuclei ultrastructure within *Vicia faba* root meristems after gamma-ray irradiation.

The aim of this study was to investigate the cytotoxic effects and ultra structural changes of *Narcissus tazetta* Bulb extracts which have properties to allow for potential use as a biopesticide by using the *Vicia* test.

MATERIALS AND METHODS

The experiment was carried out at the genetic and cytology Department, National Research Center, Cairo, Egypt, 2007-2011.

Plant material: The broad bean or *Vicia faba* (2n = 14) seeds var. Giza 3 were used for all the experiments and were obtained from the Crop Research Institute, Agricultural Research Centre, Giza, Egypt. Also, bulbs of *Narcissus* sp. (2n = 20), var. *Narcissus tazetta*.

Treatment materials: Preparation of aqueous narcissus extract: About 500 g of narcissus bulbs were homogenized in distilled water using blender at high speed for several minutes complete to 1 L and let for 48 h in room temperature then filtered and keep the filtrate in refrigerator till it used.

Reagents for electronic microscopy investigation:

- **Fixative solution** : 5% glutaraldehydes in 0.1 M sodium cacodylate Buffer, pH 7.3
- **Post fixative solution** : 1% osmium tetroxide in 0.1 mL sodium cacodylate buffer
- **Immersion mixture** : Mixture of resin and propylene oxide
- **Stain** : Toluidine blue for light microscope examination prior to final trimming
- **Stain for ultrathin sections** : Uranyl acetate 30 min followed by lead citrate for 15 min

Cytological studies procedures

Seed-germination: Seeds of *Vicia faba* (v. Giza 3) were used. Three replicates (15 seeds/replicate) were selected for each treatment and the control. The seeds were soaked in tap water for 12 h and then treated with aqueous extracts for different periods 3, 6, 12 and 24 h. The seeds were washed with water and then germinated in rolls of filter paper in large beakers with tap water at the bottom. The percentage of seed- germination was estimated when the seedling were three days old.

Mitotic study: Seeds of *Vicia faba* were soaked in tap water for 12 h and then germinated on filter paper rolls in large beakers with 3 cm height tap water at the bottom. After germination, (two groups, first group after treated and second group recovery for 72 h)1.5-3 cm long roots were immersed in the experimental agents at the top of 100 mL vials filled with aqueous narcissus extract, distilled water was used for the control treatment.

The immersed roots of *Vicia faba* were exposed to aqueous narcissus extract for variable times according to test (3, 6, 12 and 24 h, besides to control). Subsequently some root-tips of *V. faba* excised and fixed in ethanol: acetic acid (3:1 v/v). The fixed root- tips were kept in refrigerator for about 24 h before staining.

Another group of seedlings were taken off the test solutions, washed in water and transferred into tap water for 72 h. The recovered roots were cut, fixed.

Good preparations of chromosomes in *Vicia* are obtained by the Feulgen squash technique (Sharma and Sharma, 1980). Scour was taken from roots of 3 replicates (3 roots/replicate).

Electron microscopy: This investigation has been carried out at the Central Services Laboratory, at National Research Centre, Dokki, Cairo, Egypt. The ultra structural changes in the treated *V. faba* root-tip meristems were studied using the Transmission Electron Microscopy (TEM).

The technique used in this respect is summarized as follows:

- *Vicia faba* L. seeds were germinated as mentioned before in mitosis technique till their roots reached 1-3 cm long
- After immersion in the test extract for 3,6,12 h the roots tips were cut (not more than 1 mm) immediately and then fixed by immersion in fixative agent
- Dehydration was carried out by a series of increasing concentrations of ethanolic solutions starting with 40% and ending with absolute ethanol
- Clearing with propylene oxide for 30 min
- Infiltration with propylene oxide-Araldite Mixture (1:1) for 30 min
- Embedding in freshly prepared Araldite embedding mixture in labeled containers for 24 h. at 60°C. Then embedding mixture then polymerizes (hardens) forming plastic blocks suitable for cutting
- Cutting of semi thin: the sections were cut on an LKB ultra microtome [ULTRACUT-UREICH-JUNG]. At first 1 μm semi thin sections were cut, picked up on glass slides and stained with toluidine blue for light microscopic examination prior to the final trimming around a portion including the endometrial epithelial lining and the stromal tissue
- Mounting of the ultra thin sections on metal mesh copper grids. The ultrathin sections were stained with uranyl acetate for 30 min. followed by lead citrate for 15 min

The ultra thin sections were examined under EM10 (West Germany) Transmission Electron Microscope, at 80 Ku accelerating Voltage.

Statistical analysis: All cytological data were statically analyzed using t-test. The significant to control at 0.01 level.

Score was taken from 9 roots (3 roots/replicate). Percentage of shoot and root lengths, Mitotic Index (MI), total number of chromosomal aberrations and types of aberrations was estimated:

$$MI = \frac{\text{No. of dividing cells}}{\text{No. of total counted cells}} \times 100$$

$$\text{Chromosomal aberrations (\%)} = \frac{\text{No. of total aberrations cells}}{\text{No. of tatal counted cells}} \times 100$$

$$\text{Chromosome stickiness (\%)} = \frac{\text{No. of chromosome stickiness}}{\text{No. of total aberrations cells}} \times 100$$

RESULTS AND DISCUSSION

Seed-germination: The effects of *N. tazetta* extract on percentage of seed germination (relative to control), mitotic index, number and percentage of abnormal mitoses were recorded and summarized in Table 1. The percentage of seed germination decreased gradually from (85%) in control and reached to 50% after exposure for 3 h treatment. Then seed germination decreased and reached to the lowest value (5%) after 24 h treatment.

The results clear that narcissus extracts inhibited the seeds germination. The effect of tested extract on plant growth could be related to several phytochemical constituents in the extract. The inhibition of seed germination may be attributed to the percentage of alkaloids which may contains

Table 1: Seed-germination, mitotic index (MI), abnormal mitoses and interphase cells of *Vicia faba* after treatment with crude extraction of *Narcissus tazetta*

		Mitosis				Interphase	
Treatment	Seed germination (%)	No. of counted cells	No.	No. abnormal (%)	Abnormal cell (%)	MI (%)	No. of abnormal cells	Abnormal cell
Control	98	9000	990	19	1.92±0.30	11.00±0.360	30	0.37±0.08
3 h	50	9000	744	216	27.90±1.95**	8.27±0.51**	98	1.19±0.23**
6 h	25	9000	717	279	37.35±3.82**	7.97±0.37**	145	1.76±0.76**
12 h	16	9000	568	397	61.94±4.09**	6.31±0.95**	330	3.95±0.50**
24 h	4	9000	409	300	73.35±4.87**	4.54±0.13**	357	4.15±0.18**
Control 72	0	6000	876	96	3.83±0.96	14.00±0.07	8	0.16±0.60
R 3 h	0	6000	478	335	70.80±0.96**	7.97±1.90**	128	2.32±0.36**
R 6 h	0	6000	496	357	72.04± 1.00**	8.26±0.30**	146	2.66±0.36

** Highly significant to control at 0.01 level (t-test), Mean values are Mean±SE

Narciclasine (Piozzi *et al*., 1968; Bi *et al*., 1998; Lefrance *et al*., 2011). The results of this study are similar to those observed in several previous reported which noted that Narciclasine was isolated from the *Narcissus tazetta* L. bulbs which inhibited seeds germination and seedling growth of rice and Chinese cabbage. Narciclasine interacted with plant hormones in some physiological responses. Narciclasine suppressed the gibberellin- and induced α-amylase production in barley seeds and cytokinin- induced expansion and greening of excised radish cotyledons (Sasse *et al*., 1982, 1984; Bi *et al*., 1998).

Also, Umarov *et al*. (2009) found that the presence of high viscosity and biological potential of narpoetan which isolated from bulb Narcissus makes it possible to use the preparation with fungicides when germinating seed of spiked cereals and cotton. Na *et al*. (2011) found that NCS inhibitory effects on root growth of *Arabidopsis thaliana*.

Mitotic study: The results indicated that the *N. tazetta* extract exhibited a strong depressive effect on the mitosis of *V. faba* roots. The *N. tazetta* extract used; there was a negative correlation between the treatment extracts and the mitotic indices obtained from their action. As for the treatment, at 3, 6, 12 and 24 h the mitotic index was found to be 8.27, 7.97, 6.21 and 4.54%, respectively compared with the control 11% (Table 1). Inhibition of the mitotic index increased significantly with increase in the long time exposure of *N. tazetta*. The untreated roots (controls) had high mitotic indices in all the mitotic phases.

After time of recovery for 72 h, the percentage of mitotic index at 3 and 6 h treatment was still inhibited (Table 1). The percentage of mitotic index reached to 7.97 and 8.26% at 3 and 6 h treatment after recovery for 72 h compared to control 14%.

Where MI was the lowest this result proved that the cells undergoing mitosis are toxically (cytotoxic and genotoxic) affected by these treatment at the end of time treatment, the levels of toxicity due to appearance various chromosome-related to anomalies increase toxically affected, in the cells exposed to narcissus extract. Piozzi *et al*. (1968) reached that extracted from fresh bulbs of many varieties of daffodils two compounds have been extracted. Although their structures are related to many Amaryllidaceae alkaloids, the compounds show no basic properties since the nitrogen is amidic in character. The first substance, named narciclasine, shows a strong antimitotic activity and has been assigned structure VIII or its mirror image.

This suggests the suppression of mitotic activities in *V. faba* seeds treated with extracts, since mitotic index is a quantitative estimation of the mitotic activities in an organisms or a particular

organ of an organism. This observation corroborates the findings of Bakare *et al.* (2000), who recorded lower mitotic index values in the treated root cells of *A. cepa* when compared with the control root cells. Kuras *et al.* (2009) found alkaloids from *Uncaria tomentosa* bark were retard or inhibit mitosis and change mitotic phases in *Allium cepa* meristematic root tip cells according to treatment time by hours.

The results clear that, the highest rate of abnormal cell formation was observed after 24 h treatment. Mitotic toxicity causes irregular distribution or existence of spindle apparatus, so that C-mitosis is observed. It is proposed that C-mitosis is brought about by the impact of chemical poisons on spindle fibers and turbogenic events. Many studies proved that Narcissus bulbs have secondary metabolites highly effective against fungi, viruses and even herbs. Liu *et al.* (2006) found that the extraction of *Narcissus tazetta* var. *chinensis* are effective against two insects *Sitophilus zeamais* (Motschulsky) and *Tribolium castaneum* (Herbst) and also, the extract had strongly decreased the survival rate of the following tumor cell lines: HL-60, K562, KT1/A3 and A3R. Yuzbaoiolu, (2003) Eventuated a weak C-mitotic effect can prevent spindle fibers to reach a chromosome, and as a result of this, appearance of retarded chromosomes (Dane and Dalgic, 2003).

The extracts caused to the appearance of abnormal root meristem cells with increase in time of treatment when compared to the control. Yet it was also observed that these treatments caused statistically significant decreases in MI (at $p< 0.01$). All treatments caused high significant increase percentage of abnormal mitosis. The minimum percentage of abnormal cells was observed after treatment with 3 h (27.9%) and a highly statistically significant percentage was (73.35%) at 24 h treated with *N. tazetta* extract compared to control (1.92%). Mitotic chromosomal aberrations after recovery for 72 h for 3 and 6 h treatment showed that, the abnormality still higher were recorded 70.80 and 72.04%, respectively compare to control 3.83% (Table 1).

In this study, many various abnormalities were recorded along the experimental periods. Table 2 clears the number and percentage of different types of aberration in interphase and mitotic stages. In interphase, higher percentage of micro-nuclei and binuclear reached to 89.65 and 19.38 after treated with 6 h and 3 h respectively. Percentage of chromosome disturbed reached to 51.82 after treated with 24 h. Chromosomal stickiness was more frequent type of abnormalities recorded in all the mitotic stages. The percentage of stickiness reached to 76.26% after treated for 24 h (Fig. 1a, h). Chromosome stickiness may result from chromatin fibers' sticking to each other or breaking due to erroneous or inadequate condensation of these fibers, as a consequence of this, movement of mitotic spindle fibers together with inner-chromosome stickiness when the chromosome is drawn to the pole causes secondary anomalies (bridge and fragment occurrence). Fiskesjo (1985) proved that stickiness in chromosomes is an indication of the high toxicity of the chemical substance and usually this may kill the cells with the irreversible damages. These abnormalities have also been reported for several extracts and chemicals already investigated

Table 2: Different types of abnormalities occurring in the mitosis and interphase cells in *Vicia faba* after treatment with different times with *Narcissus tazetta* extract

	Abnormalities in interphase stage				Different types of abnormalities in the mitoses						
Treatment	No. of abn. cells	Mic (%)	Binuc (%)	Ch dis (%)	No. of Abn. cells	Disturbed (%)	Sticky (%)	Micronuclei (%)	Laggard (%)	Metaphase (%)	Bridges (%)
3 h	98	80.61	19.38	0.00	216	10.65	5.55	32.87	15.28	11.11	24.54
6 h	145	89.65	10.34	0.00	279	11.47	35.84	3.58	2.15	41.94	5.02
12 h	330	67.27	4.24	28.48	397	3.27	71.79	20.91	1.26	1.51	1.26
24 h	357	45.94	2.24	51.82	300	13.33	76.00	9.33	0.00	0.00	0.00

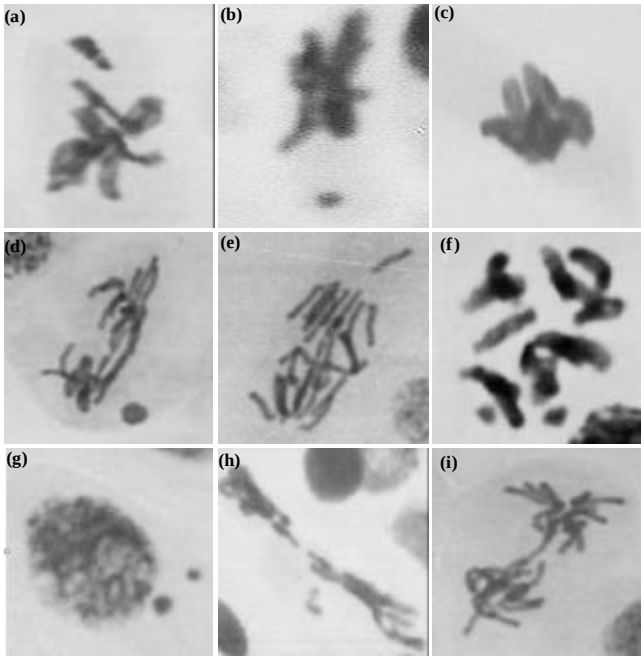

Fig. 1(a-i): Abnormal mitotic activity observed after *Narcissus tazetta* extracts treatment in root-tip cells of Vicia faba (a) Metaphase with lagging chromosome, after 12 h, (b) Metaphase with macro nucleus after 6 h, (c) A sticky metaphase after 24 h, (d) Anaphase with bridge and macro nuclei after 12 h, (e) Anaphase with lagging chromosome after 6 h, (f) Disturbed metaphase after 6 h, (g) Interphase with two micro nuclei after 12 h (h) Sticky anaphase and lagging chromosome after 12 h and (i) Anaphase with bridge after 6 h

(Badr and Elkington, 1982; Nwakanma *et al*., 2009). Stickiness usually leads to the formation of anaphase and telophase bridges and this end up inhibiting metaphase and cytokinesis, respectively and thus hampering cell division. According to Patil and Bhat (1992) suggested that, stickiness is a type adhesion involving mainly the proteinaceous matrix of chromatin. Disturbance of the chromosome was observed at metaphase and anaphase and recorded in a considerable percentage which reached to 13.33 % after treated for 24 hrs (Fig. 1f).

The induction of bridge was observed in anaphase and telophase stages. The highly percentage of bridges reached to 24.54% after treated for 3 hrs (Fig. 1d, i). Bridges might be attributed to the stickiness at anaphase (Lui *et al*., 1994), or due to the formation of dicentric chromosome as a result of breakage and reunion (Shehata *et al*., 2000).

Lagging chromosome was observed at metaphase and anaphase after all treatment. The highly percentage reached to 15.28% (Fig. 1a, e, h). Considerable micro- nucleuses were recorded. The highly percentage of micro-nucleus were 20.91% (Fig. 1b, d, g). For all treatment the occurrence of bridge, lagging and micro-nuclei may lead to loss of genetic materials. The formation of micro-nuclei is regarded as an indication of the mutagenicity of their inductions (Gustavino *et al*., 1987; Monarca *et al*., 2003; Rosa *et al*., 2003; Liu *et al*., 2004). Micronuclei are formed as a result of lagging chromosomes or a centric breakage. Mitosis anomalies such as bridge and micronuclei result from clastogenic effects on nucleus chromosomes (Grant, 1978; Yuzbaoiolu, 2003).

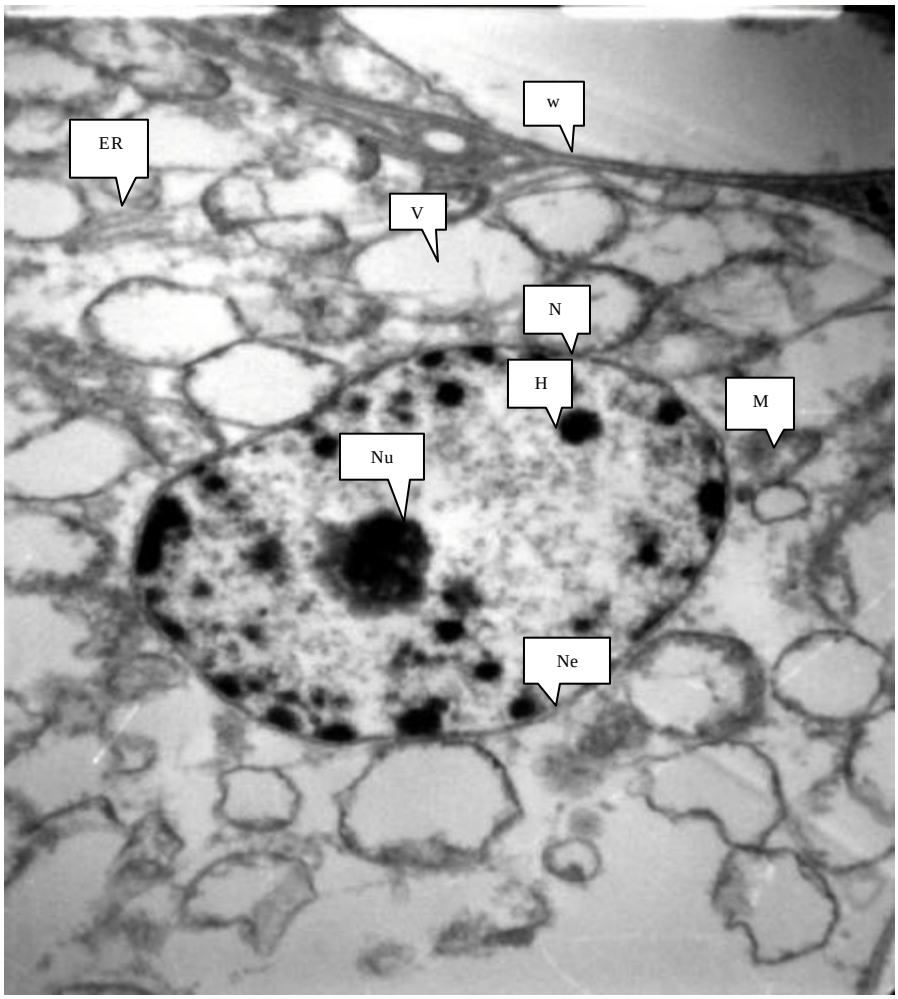

Fig. 2: TEM micrographs of ultra thin sections of untreated (control) root tips of *Vicia faba* showing normal cell enclosed by an electron-dense wall (W), V: Vacuoles, N: Nucleus, Nu: Nucleolus, Ne: Double membranes nuclear envelope, ER: Endoplasmic reticulum, M: Mitochondria, H: Patches of heterochromatin

Observation of ultra-thin sections by transmission electron microscopy: Untreated specimens (control), showed a normal cell enclosed by an electron-dense cell wall. The cell is nearly filled by vacuoles and a normal nucleus. The nucleus was surrounded by an organized nuclear envelope and contained one nucleolus; patches of electron-dense heterochromatin, most of them were seen beside the nuclear envelope. The euchromatin occupied most of the nucleoplasm. The newly formed wall after the cell division is an electron-lucent in its appearance. Each cell was characterized by many vacuoles and a cytoplasm with numerous organelles (Fig. 2).

The cells from treated specimens with narcissus extract after 3 h, show big nucleolus inside the nucleus. Small sparse patches of heterochromatin are seen inside the nucleus. Many vacuoles with electron-dense bodies at their periphery are also detected groups of mitochondria appeared empty of cristae and deformed (Fig. 3a, b). In addition unidentified electron-lucent bodies (may be lipid)

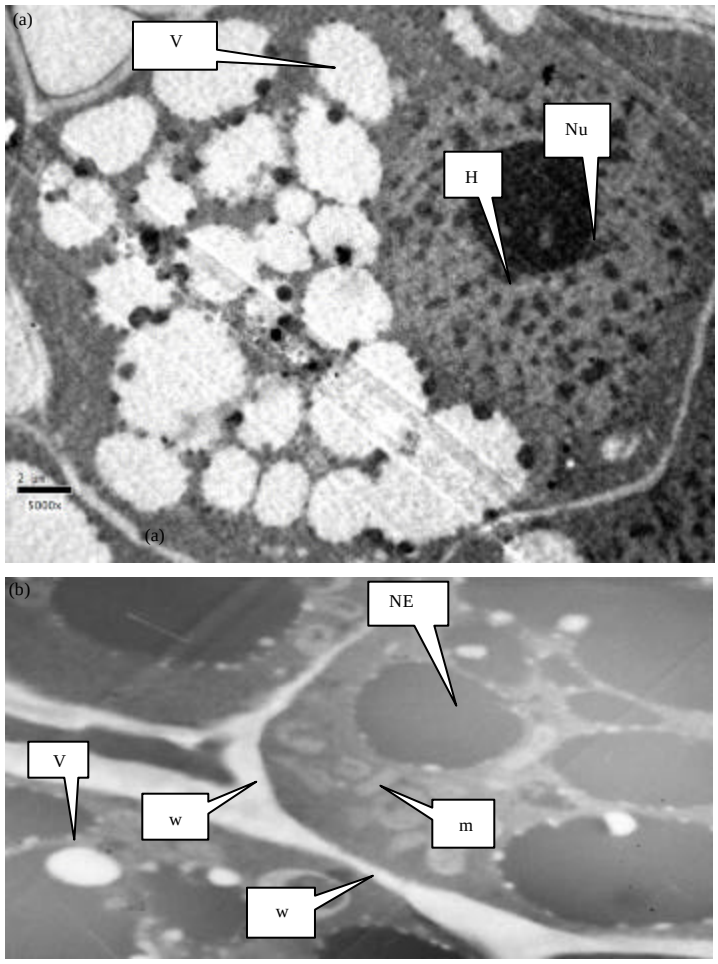

Fig. 3(a-b): TEM micrographs of ultra thin sections of treated (3 h) root tips of *Vicia faba* showing: (a) Big nucleolus (Nu) and (b) Abnormal mitochondria (m), H: Seprase heterochromatin patches inside the nucleoplasm, V: Vacuoles with electron-dense bodies, NE: Nuclear envelope without double membrane, w: Cell wall with abnormal thinking

are also observed inside the cell. The immature (meristematic) cells are always enclosed by a thin electron-lucent wall (Fig. 3b).

In treated specimens with narcissus extract after 6 h, an electron-lucent area is observed at the center of the nucleolus. In other cases, two nucleoli are detected inside the nucleus (Fig. 4a, b). The patches of heterochromatin are associated with the nuclear envelope (Fig. 4c).

In treated specimens with narcissus extract after 12 h, disorganized and collapsed cells are seen. The cell cytoplasm is disintegrated and become denser. The plasma membrane is moved away from the cell wall (Fig. 5). The appearance of oleosomes (lipid bodies) inside the more granulated cytoplasm is the characteristics phenomenon of this treatment. In addition, ruptured plasma membrane and collapsed nucleus are also detected (Fig. 5).

In the light of the results, these observations above may be due to the nucleotoxic action of the extracts or the disturbance of the formation of spindle fibers during cell division which leads to chromosomal aberrations. The ultra structural changes might occur with the other side beyond the

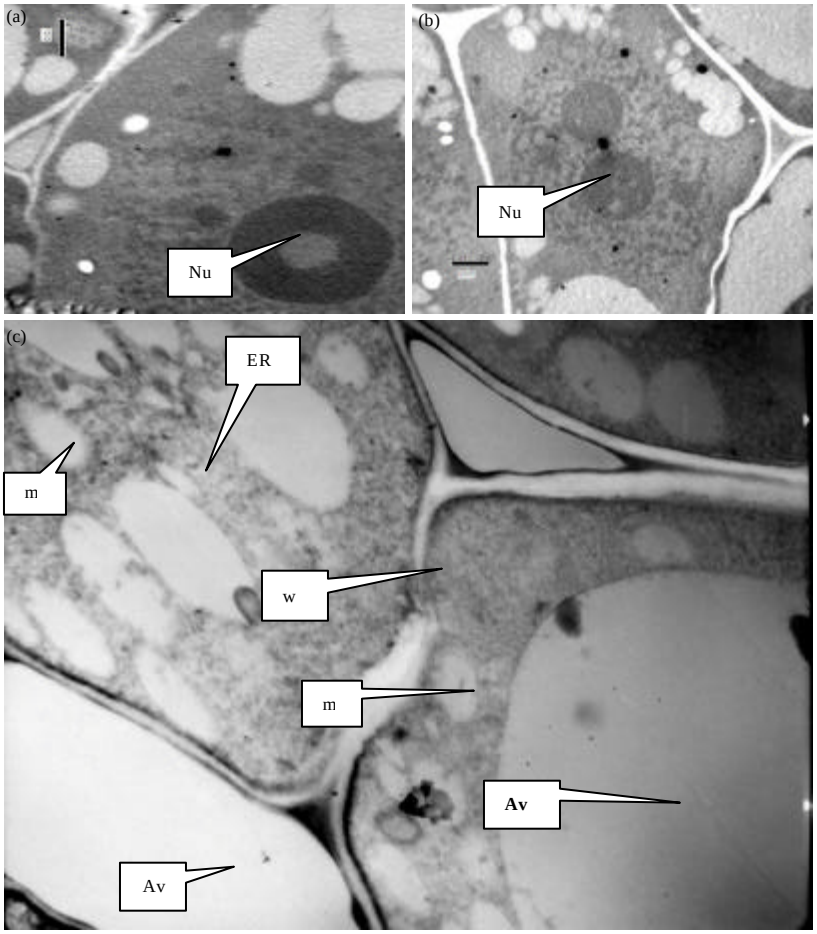

Fig. 4(a-c): TEM micrographs of ultra thin sections of treated (6 h) root tips of *Vicia faba* showing (a) An electron-lucent area in the center of the nucleolus (Nu) inside the nucleus (N), (b) Two nucleoli (Nu) inside the nucleus nuclear membrane without double membrane (ER) and (c) Ruptured cell wall (w), irregular ER, autophagic vacuole (Av) and abnormal shaped mitochondria (m)

nucleus and the division apparatus. Extreme swelling of some of the mitochondria accompanied with are duct ion of cristae, were observed in some of cells after treatments. Groups of mitochondria appeared to be empty of cristae and fused together. El-Shafey (1994) observed some apparently enlarged mitochondria in *Vicia faba* root tip cells after treatments with *Portulaca oleracea* plants extract. Furthermore, experiments have indicated that the Mitochondrial Transmembrane Potential (MTP) loss induced by opening of PTP is sufficient and necessary for apoptosis (Kroemer *et al*., 1997). Recently, the involvement of mitochondria in plant PCD revealed that mitochondrial oxidative burst and MTP changes are commonly involved in PCD of Arabidopsis (Yao *et al*., 2004; Zhang and Xing, 2008). Also, Ali, (2009) found that two plants extracts *S. bicolor* and *N. officinale* had less distractive to the mitochondria, dictyosomes and Endoplasmic Reticulum (ER).

The dependent increase in volume of dictyosome vesicles after narcissus extract treatments may reflect a contradictions effect on the function and storage and transport of substances synthesized

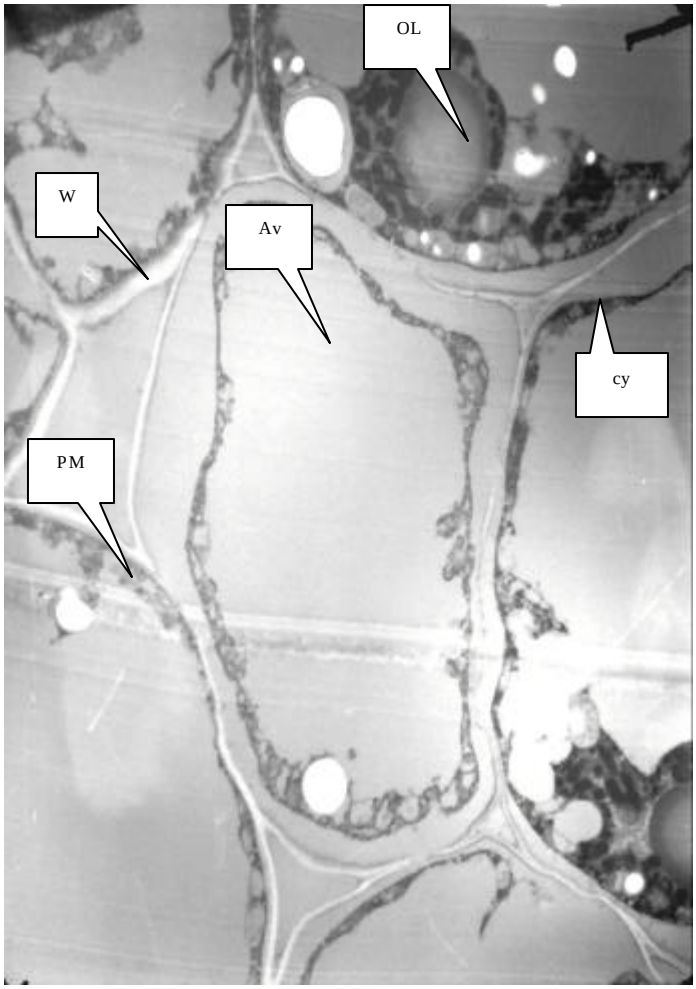

Fig. 5: TEM micrographs of ultra thin sections of treated (12 h) root tips of *Vicia faba* showing: collapsed cells with disintegrated cytoplasm, Plasma membrane (PM) moved away from the cell wall, Oleosome (OL) inside disintegrated cytoplasm (Cy), w: Ruptured cell wall, Av: Autophagic vacuole

by the cell. The induced formation of vacuoles by most treatment indicated that a real damage was exerted such a damage was demonstrated by Ambrose and Easty (1979) who stated that: "Cell injury involves an aspect of lysosomal function, known as autodigestion or autophagy, in which some of the contents of the cell itself are engulfed by the lysosome and broken down. When injury is due to starvation, this process enables the cell to use some of its own materials for the formation of essential substances no longer available from outside sources, without causing irreparable damage".

Vacuolization observed after treatment is thought to result from the fact that narcissus extract is a more destructive and comprehensive mutagen and is of a high-density solution. Increase observed in the number of nucleolus's in a nucleus and changing sizes depending on this can be directly related to the extract used for treatment. The evidence reviewed in this investigation and

formed works strongly suggest that cell walls, a first barrier stress, can immobilize narcissus extract, therefore prevent contact with the sensitive plasma lemma and cytoplasm components.

CONCLUSION

Present results indicate that *Narcissus tazetta* crude extract from the bulb was inhibitory to germination. The experiments showed that reduction in effect of *Narcissus tazetta* extract increased according to decomposition period length. Exposure to *Narcissus tazetta* crude extract prevents cells from properly entering into cell division. Significantly higher frequencies of cells with mitotic aberrations indicated the primary action of *Narcissus tazetta* extract to involve chromatin organization and mitotic spindles, leading to the induction of several abnormalities. Ultra-structural effects of Narcissus extract on the root meristem cell of *Vicia faba* plants. The nucleolus material was extruded from the nucleus into the cytoplasm. Also, disruption of nuclear membranes, chromatin material, mitochondrial cristae disappear, formation huge autophagic vacuoles and disintegration of organelles were observed. Taken together, the findings presented in this study strongly suggest that *Narcissus tazetta* may harbor biologically active products the natural properties of which may be exploited to create a successful bionatural herbicide.

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