POTENTIAL EFFECT OF SOME NATURAL FOOD ADDITIVES AGAINST MONOSODIUM GLUTAMATE-INDUCED GENOTOXICITY IN *Vicia faba*

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For many years, food additives have been added to all manufacture products and fast foods to preserve flavor or enhance its taste and appearance (Jinap and Hajeb, 2010). They provide the needed appeal for the foods that are not very flavorful (Löliger, 2000) and increase food intake in an institutionalized older population. Thus food additives increase intake of necessary vitamins, minerals and protein from food, which support and have a positive effect on immune status (Filer and Stegink, 1994; Schiffman, 1997).

Monosodium glutamate (MSG, also known as sodium glutamate) is one of the most abundant naturally occurring non-essential amino acids and can be produced commercially (Fernstrom and Garattini, 2000). It is considered as a source of energy for certain tissue and is important for glutathione synthesis as substrate. Today, MSG is considered to be the most common taste enhancer in the world, after salt and pepper. Sodium salt of glutamic acid (GLU) is one of six food additives that are admitted in the European Union, such as GLU (E620) and its sodium (E621), potassium (E622), calcium (E623), ammonium (E624) and magnesium (E625) salt. Consuming of these derivatives of glutamic acid differ from European and Asian countries people, where in European Union countries the mean intake ranges from 0.3 to 0.5 g/day, while in Asian countries people consume an average of 1.2-1.7 g/day (Bellisle, 2008).

Chinese restaurant syndrome is a collection of symptoms (flushing, tightness of the chest or difficulty in breathing) that some people suffer after eating Chinese food (Raif *et al*., 2000; Ubani, 2011). The food additive MSG was blamed, but it has not been proven to be the substance that caused this condition. Thus, the experiments were started to answer this question; Was MSG had a negative effects on human health? The answer still needs to be confirmed. Nakanishia *et al.* (2008) suggested that MSG safety profile should be re-examined and be potentially withdrawn from the food chain. They reported that injection of MSG in mice induced obesity and diabetes with steatosis and steatohepatitis resembling human NAFLD and NASH with pre-neoplastic lesions. Another experiment showed that MSG induced oxidative stress in rats (Mariyamma *et al*., 2009).

Chitosan is a natural polymer that

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has wide applications in food, biomedical and chemical industries (Shahidi *et al.*, 1999). Chitosan is a deacetylated product of chitin; the precursor of chitosan, with one of the polysaccharides having a free amino group at the C_2 position of the glucose residue of cellulose (Kim *et al.*, 1999). Shon *et al.* (2001) reported that chitosan oligosaccharide showed an inhibitory effect on the mutagenic activity of the cooked food mutagen. Moreover, Yoon *et al.* (2005) investigated the effects of chitosan oligosaccharide on mercury induced genotoxicity in mice using the micronuclei and chromosomal aberration tests. There was no significant difference between the untreated and experimental groups.

Several spices have antioxidant activities such as black pepper (*Piper nigrum* L.) (Shobana and Naidu, 2000; Suhaja *et al*., 2006), cumin (*Cuminum cyminum*) (Thippeswamy and Naidu, 2005; El-Ghorab *et al.*, 2010), chili pepper (*Capsicum annum* L.) (Sun *et al*., 2007; Mateos *et al*., 2013) and ginger (*Zingiber officinale*) (Shobana and Naidu, 2000; Stoilovaa *et al*., 2007). Also, Al-Qirim *et al*. (2008) and Arulmozhi *et al.* (2010) noted antioxidant properties in some plants such as *Solanim nigrium* (family: *Solanaceae*, commonly known as black nightshade; BNS), which have a free radical scavenging action and anticancer properties.

Vicia faba is an excellent genetic model commonly used as a biosensor to assess environmental pollutants, being frequently used in monitoring studies in this respect. However, this feature is not only due to the sensitivity to detect mutagens in different environments, but also to the possibility of assessing several genetic endpoints, which range from point mutations to chromosomal aberrations (Kanaya *et al.*, 1994).

The present study was aimed to evaluate the genotoxic effects of MSG as a food additive on the cytogenetical and molecular levels in *V. faba*. Moreover, the effect of adding some natural materials; such as chitosan, four different spices and three forms of black nightshade plant (BNS), in reducing the MSG-genotoxicity.

MATERIALS AND METHODS

Seed material

Seeds of *V. faba* L.; Sakha 1 variety, were kindly obtained from Food Legumes Research Section, Sakha Agricultural Research Station (SARS), Kafr El-Sheikh, Egypt.

Tested materials

Monosodium glutamate (MSG, CAS Number: 6106-04-3, Sigma-Aldrich) was used at a single concentration of 10 g/L according to Demirhan *et al.* (2015).

Chitosan (CAS Number: 9012-76-4) was obtained from CORNELL LAB Co., Cairo, Egypt.

Four spices (black pepper, cumin, chili pepper and ginger) were obtained

from local shops of herbalists at Tanta, Egypt. Also, three different forms of black nightshade plant (BNS) were used; either as BNS leaves immature and mature fruits. The tested materials of BNS were collected from a one field of Gharbia Governorate, Egypt.

All materials of chitosan, spices (black pepper, cumin, chili pepper and ginger), in addition to the three forms of BNS, were applied at a single concentration of 1% aqueous solution.

Experimental procedure and growth conditions

Vicia faba seeds of equal size were chosen and surface sterilized with 2.5% sodium hypochlorite for 3 min. Then, the seeds were rinsed with three changes of sterile distilled water and dried using sterile filter paper. The seeds were soaked in sterile distilled water for 1 h, and then thirty seeds were treated as described. The experiment was divided into ten groups. One group was left as a negative control in which distilled water was used, while the second one was used for treatment with 10 g/L of MSG and was considered as a positive control. The remaining eight treatments received 1% of each of chitosan, the four different spices and the three forms of BNS; in combination with MSG.

After soaking for 24 h, seeds were recovered in distilled water for two hours according to Pandey and Upadhyay (2007). Germination and seedling growth were carried out in a Randomized Complete Block Design with three replications. Ten seeds per replication were allowed to germinate and grow in a 15 cm diameter Petri dish lined with Whatman No. 1 filter paper moistened with distilled water. The dishes were incubated in the dark at 25°C and germination percentage was calculated after 48, 72 and 96 h as follow:

$$\text{Germination \%} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}}\ x\ 100$$

Seedling growth in terms of shoot and root length (cm) was measured after seven days from germination.

Cytological analysis

Root tips of *V. faba* germinated seeds (1.5-2 cm) of each replicate were cut and fixed in Carnoy's fixative solution (ethyl alcohol absolute and glacial acetic acid in the ratio of 3:1) for 24 h, then kept in 70% ethyl alcohol in refrigerator until used for cytological analysis.

Cytological preparations were carried out using 2% aceto-carmine stain as described by Darlington and La Cour (1976). Cells were examined under a light microscope for mitotic index, numbers and types of abnormalities by examination of at least 3000 cells per treatment (1000 cell/replicate). Mitotic index (MI) and percentage of abnormal cells were calculated using the following formulas:

$$\text{Mitotic index (MI)} = \frac{\text{Total dividing cells}}{\text{Total dividing and non dividing cells}} \times 100$$

$$\text{\% of abnormal cells} = \frac{\text{Total abnormal cells}}{\text{Total dividing cells}} \times 100$$

Genomic DNA extraction and RAPD-PCR procedures

Total genomic DNA was isolated from 150 mg of *V. faba* seedlings using CTAB-chloroform based method as described by Saghai-Maroof *et al.* (1984).

RAPD analysis was carried out using twelve arbitrary random primers which were purchased from Bio Basic Inc, Canada. The list of primers and their sequences are presented in Table (1). PCR amplification was performed using iNtRON's Master mix Solution (i-Taq^{TM}) in a volume of 10 µl reaction mixture containing 0.75 µL of 40 ng of genomic DNA, 0.75 µL of 20 µM primer, 5 µL of 2X Master mix Solution and 3.5 µL of sterile distilled water. Amplifications were carried out in a thermal cycler according to manufacture instructions as follow: the initial amplification program started with denaturation at 94°C for 2 min, followed by 45 cycles consisting of denaturation for 20 sec at 94°C, annealing for 30 sec at 31°C and extension for 30 sec at 72°C. The program was ended with a final extension step for 5 min at 72°C.

Amplification products were separated on 1.5% agarose gel, stained with ethidium bromide and visualized under ultraviolet light. A known DNA Ladder (Thermo Scientific O'GeneRuler DNA Ladder Mix, ready-to-use, Cat-no: SM1173) was run against the PCR products.

Data analysis

Germination, shoot and root lengths as well as cytological analysis were calculated in a Randomized Complete Block Design with three replicates. Obtained data were expressed as means ± standard error (SE). The data were subjected to One-Way Analysis of Variance; statistical package for social sciences (SPSS) software for windows version 20, followed by LSD test to compare the significance of differences between means. The results were considered significant at $P < 0.05$.

Genomic template stability (GTS) was calculated by the following equation: GTS (%) = (1-a/n) × 100; where: a is the number of polymorphic bands detected in each treated sample, and n is the number of total bands detected in the control. Polymorphism observed in RAPD profile included disappearance of a normal band and appearance of a new band in comparison to control RAPD profile (Atienzar *et al.*, 2002; Qari, 2010).

RESULTS AND DISCUSSION

Effect of treatments on seed germination and seedling growth of V. faba

Data presented in Table (2) revealed that the highest significantly differed germination percentages of *V. faba* were detected after 48 h in black pepper and cumin treatments, whereas the addition of chitosan was superior to the other treatments after 72 h. On the contrary, mature BNS fruits gave drastically negative effect on the germination throughout the experiment (48-96 h), while the other treatments had no effect after 96 h with

reference to negative and positive control.

From the aforementioned results, it could be reported that mature BNS fruits can be considered as a natural inhibitor for *V. faba* germination.

These results indicated that positive (MSG) and negative control did not differ significantly at 48 and 96 h for germination values, while treatment of MSG reduced germination value than negative control at 72 h. Satnam *et al.* (2009) tested the effect of MSG industrial wastewater (MSGW) on early growth of Chinese cabbage and maize by the seed germination bioassay. Their results indicated that; at MSGW concentrations below 1%, germination indices for both plant species were significantly higher than the control.

With respect to root and shoot lengths, results in Table (2) indicated that *V. faba* root length didn't differ significantly than the negative control by the application of black pepper, ginger and BNS leaves. The root length values were significantly increased by the application of MSG (positive control) and cumin. The highest value (3.11 cm) was observed for cumin treatment. The root length was decreased with chitosan, chili pepper, immature and mature BNS fruits. This result was in constant with that of seed germination, where the treatment of mature BNS fruits exerted a toxic effect on root length.

On the other hand; as presented in Table (2), no significant difference were recorded for *V. faba* shoot length for the different treatments compared to each other as well as negative and positive control.

Satnam *et al.* (2009) reported that the application of MSGW significantly increased the plant biomass yield at 5000 and 7500 l/ha, in addition, the biomass yield decreased as the MSGW dose increased. Moreover, Haghighi *et al.* (2015) reported that applying MSG wastewater with a high concentration of 17 amino acids and macro- and microelements improved the fresh weights of shoot and root as well as the protein content of lettuce.

Cytological effects on mitosis of V. faba root tips

The mitotic index (%) of *V. faba* seedlings treated with MSG and other combined treatments were presented in Table (3). The total number of proliferating cells and the number of cells at various mitotic stages of *V. faba* meristemic cells were scored in root tips.

Cytological analysis showed that the highest value of mitotic index (6.53%) was recorded for negative control. Application of MSG and all the combined treatments exhibited an effect on the mitotic index which significantly decreased compared to negative control. The lowest value of mitotic index (0.10%) was observed with the application of mature BNS fruits. This reduction in mitotic index could be explained as a result of inhibition of DNA synthesis at S-phase or blocking in G2 phase of the cell cycle, preventing cells from proceeding into prophase or depressing the mitotic phase following

prophase which is indicative of the induction of molecular changes in the genetic material, suggesting either DNA lesion, or interference with cell cycle (Sudhakar *et al.*, 2001; Baeshin and Qari, 2003). On the other hand, the results revealed that the application of black pepper, cumin and BNS leaves significantly increased mitotic index compared to their positive control (2.56%).

Chromosomal aberrations were scored in different mitotic stages of *V. faba* root tips and the cytological effect was estimated as the type and percentage of cells showing chromosomal abnormalities (Table 3 and Fig. 1). Results in Table (3) showed that the application of MSG caused significant increase (47.10%) in the percentage of abnormal cells in *V. faba* seedling compared to the negative control (4.23%). There is an agreement that MSG can lead to certain irreversible cytogenetic effects in plants and higher organisms. Adeyemo and Farinmade (2013) reported that MSG has potential genotoxic and cytotoxic effects in the root tip cells of *Allium cepa*. Moreover, Ataseven *et al.* (2016) investigated the genotoxic potential of MSG with six concentrations (250, 500, 1000, 2000, 4000 and 8000 μg/mL) in cultured human lymphocytes and alkaline comet assays in isolated human lymphocytes. They indicated that MSG significantly and dose dependently increased the frequencies of chromosome aberrations, sister-chromatid exchanges and micronucleus in all treatments and times, compared with control.

On the other hand, all the other treatments, caused significant increase in the percentage of abnormal cells compared to the negative control, except cumin treatment which did not differ significantly. Although the treatments of chitosan, black and chili pepper, ginger, BNS leaves and immature fruits increased the aberrations compared to the negative control, they decreased the percentage of abnormalities compared to their positive control (MSG). These results indicated that the application of spices and other plant materials was effective in reducing the side effects of MSG. Cumin treatment was the most effective in reducing percentage of abnormal cells of *V. faba* treated with MSG.

Thus, application of cumin improved mitotic activity in the cell and reduced the cytotoxic effects of MSG. These findings were in agreement with Aruna and Sivaramakrishnan (1992) who confirmed the anticancer properties of cumin and considered it an anticarcinogenic agent. Data obtained by Allahghadri *et al.* (2010) showed that cumin may be exploited as a natural antimicrobial and antioxidant agent.

As presented in Fig. (1) and summarized in Table (3), *V. faba* root tips revealed various types of chromosomal aberrations as a result of treatment with MSG and other combined treatments. The most frequent aberrations were micronuclei, c-metaphase, laggards, fragments, disturbed, bridges. Increase in the percentage of aberrations in root meristems indi-

cates genotoxic effects of the test chemicals (Smaka-Kincl *et al.*, 1996). Chromosomal aberrations might be induced by direct effect on DNA lead to chromosomal aberration. Moreover, chemical compounds could disturb the synthesis of DNA and protein or the translation of RNA, so that no materials relating to the chromosomal movement could be formed, and the chromosomal aberration occurred eventually. Any such irreversible DNA damages could lead to the chromosomal aberrations which are recognized as the validated biomarker of cancer risk in humans (Bonassi *et al.*, 2011; Ozden *et al.*, 2014).

Effect on RAPD profile and genomic stability

Genomic template stability test is a qualitative measurement of changes in RAPD profile. It is a highly sensitive parameter compared with the traditional indices. Atienzar and Jha (2006) reported that DNA alterations in the genome can clearly be shown by comparing DNA fingerprints from untreated and treated individuals with genotoxic agents.

RAPD technique was used to detect variations between DNA extracted from *V. faba* seedlings treated with MSG, all combined natural materials (except mature BNS fruits) and the control. Mature BNS fruits exerted the highest toxic effect on seed germination and root length as well as mitotic index compared to negative and positive control and other treatments. Therefore, no more *V. faba* seedlings treated with mature BNS fruits in combination with MSG were available for RAPD analysis.

RAPD profiles generated from *V. faba* DNA using twelve oligonucleotide primers were presented in Fig. (2). Profiles generated by these primers revealed differences between control and the different treatments, with relative changes in the number and the intensity of amplified DNA fragments.

Data presented in Table (4) show DNA variations induced in *V. faba* cells treated with different materials. Twelve random primers generated a total of 96 RAPD bands in negative control. Bands number ranged from two bands for primer OPA-20 to sixteen bands for primer OPB-11. The different treatments gave variable bands; compared to negative control, as reflected by changes in band intensity (increase/decrease), disappearance of bands and appearance of new bands.

An increase in band intensity was the major event arising in the patterns generated from *V. faba* DNA treated with MSG (positive control), black and chili pepper, ginger, BNS leaves and immature fruits. The treatments of immature BNS fruits and positive control showed the highest increase in band intensity (31 and 28 bands, respectevely). On the other hand, treatments of chitosan and cumin showed the highest disappearence of normal band compared to the negative control. The highest changes in bands number (both appearance of new bands or disappearance of normal bands) appeared in *V. faba* treated with black pepper and

cumin (21 bands for each), while the lowest change in bands number was recorded for positive control (12 bands) followed by the treatment of BNS leaves (13 bands).

Changes observed among RAPD profiles obtained from the control and the different treatments may be induced by direct and/or indirect interaction with genomic DNA. As revealed in literature, the damage caused to genomic DNA would induce the modification of the binding sites which can lead to alterations of electrophoretic PCR patterns (Atienzar and Jha, 2006; Rocco *et al.*, 2012). This notion supports that RAPD technique is a reliable and sensitive method that can identify a wide range of damaged DNA and genetic mutations (Atienzar and Jha, 2006) and therefore can be applied to genotoxicity and carcinogenesis studies. Cenkci *et al.* (2009) reported that RAPD technique has been commonly used for a variety of purposes and it has been successfully utilized in genotoxicity judgment of suspicious chemicals. Ataseven *et al.* (2016) investigated genotoxic effects of the MSG *in vitro* on human peripheral lymphocytes by analyzing the RAPD-PCR with arbitrary 10-mer primers. They demonstrate that MSG caused increase or decrease in band intensity and gain or loss of bands.

With respect to GTS (%), the differences in the RAPD profiles could be used to estimate reduction rates in GTS for each treatment. Data presented in Table (4) revealed considerable effect for the given treatments. All treatments caused reduction in GTS values compared to the negative control. The highest value of GTS (87.50%) was recorded for positive control, while treatments of black pepper and cumin exhibited the lowest value (78.12% for each).These results indicated that all the applied materials may interact with DNA causing genotoxic effect.

These results were consistent with the above described results of the effect of the tested materials on decreasing mitotic index and increasing the abnormal cells than the negative control which may occurs as a result of the induction of molecular changes in the genetic material. These findings were not consistent with Prakash and Gupta (2014) who suggested that the use of cumin in diet may reduce the risk of cancer and it can be considered as a helper in the therapy or the control of the cancer of colon, liver and prostrate. It has preventive properties against cancer as shown in their study of seven human cancer cell lines.

In conclusion, the results of the current study indicated that MSG which is frequently being used in the food industry possesses high genotoxic risks to *V. faba* cells. Thus, it can lead to certain irreversible effects in plants and even in higher organisms. Moreover, the application of spices and other plant materials was effective in reducing the deleterious effects of MSG. Black pepper and cumin were the most effective in reducing the genotoxicity of MSG on *V. faba* plants, although they lead to mutagenic effects as indicated by observing reduction in mitot-

ic index and the heritable changes in RAPD profiles and GTS. The outcome of the present investigations suggests that attention should be paid to estimate the toxic potential of the regularly used food additives and other chemicals in consumable items.

SUMMARY

Monosodium glutamate (MSG) is one of the most widely used flavor enhancers throughout the world. The objective of this study was to evaluate the genotoxic effect of MSG on *V. faba* seedlings. Moreover, the effects of adding some natural materials to MSG; e.g. chitosan, four different spices (black pepper, cumin, chili pepper and ginger) and three different forms of black nightshade plant; BNS (leaves, immature and mature fruits) were tested. Seeds of *V. faba* were treated with single concentration of MSG (10 g/L); alone (as a positive control) or combined with 1% aqueous solution of each of these natural additives. Results indicated that the treatment of MSG reduced germination value than negative control at 72 h. On the other hand, the use of black pepper and cumin at 48 h, in addition, chitosan at 72 h significantly increased seed germination compared to negative and positive control. The highest root length value (3.11 cm) was observed for cumin treatment. Exposure to MSG and combined treatments showed an inhibitory effect on cell division and caused a general decline in mitotic index. Additionally, all treatments caused significantly increase in the percentage of abnormal cells, except cumin which did not differ significantly from the negative control. By analyzing the RAPD-PCR with twelve arbitrary primers, all treatments caused slight reductions in genomic template stability (GTS) values compared to the negative control. The highest value of GTS (87.50%) was recorded for positive control, while treatments of black pepper and cumin exhibited the lowest value (78.12% for each). These results indicated that all the tested materials may interact with MSG causing genotoxic effects. In general, black pepper and cumin had the lowest genotoxic effects.

REFERENCES

Adeyemo, O. A. and A. E. Farinmade (2013). Genotoxic and cytotoxic effects of food flavor enhancer, monosodium glutamate (MSG) using *Allium cepa* assay. Afri. J. Biotechnol., 12: 1459-1466.

Allahghadri, T., I. Rasooli, P. Owlia, M. J. Nadooshan, T. Ghazanfari, M. Taghizadeh and S. D. A. Astaneh (2010). Antimicrobial property, antioxidant capacity, and cytotoxicity of essential oil from cumin produced in Iran. J. Food Sci., 75: 54-61.

Al-Qirim, T., M. Z. Syed, S. Moyad, S. Ghassan and B. Naheed (2008). Effect of *Solanum nigrum* on immobilization stress induced antioxidant defense changes in rat. Res. J. Biol. Sci., 3: 1426-1429.

Arulmozhi, V., M. Krishnaveni, K. Karthishwaran, G. Dhamodharan and S. Mirunalini (2010). Antioxidant and antihyperlipidemic effect of *Solanum nigrum* fruit extract on the experimental model against chronic ethanol toxicity. Pharmacogn Mag., 6: 42-50.

Aruna, K. and V. M. Sivaramakrishnan (1992). Anticarcinogenic effects of some Indian plant products. Food and Chem. Toxicol., 30:953-956 .

Ataseven, N., D .Yüzbaşıoğlu, A. Ç. Keskin and F. Ünal (2016). Genotoxicity of monosodium glutamate. Food and Chem. Toxicol., 91: 8-18.

Atienzar, F. A. and A. N. Jha (2006). The random amplified polymorphic DNA (RAPD) assay and related techniques applied to genotoxicity and carcinogenesis studies: a critical review. Mutat. Res., 613: 76-102.

Atienzar, F. A., P. Venier, A. N. Jha and M. H. Depledge (2002). Evaluation of the random amplified polymorphic DNA (RAPD) assay for the detection of DNA damage and mutations. Mutat. Res., 521: 151-163.

Baeshin, N. A. and S. H. Qari (2003). Combined genotoxic and cytotoxic effects of cadmium chloride and carbofuran in root meristems of *Vicia faba*. Saudia J. Biol. Sci., 10: 107-119.

Bellisle, F. (2008). Experimental studies of food choices and palatability responses in European subjects exposed to the umami taste. Asia Pac. J. Clin. Nutr., 17: 376-379.

Bonassi, S., R. El-Zein, C. Bolognesi and M. Fenech (2011). Micronuclei frequency in peripheral blood lymphocytes and cancer risk: evidence from human studies. Mutagenesis, 26: 93-100.

Cenkci, S., M. Yildiz, I. Cigerci, M. Konuk and A. Bozdag (2009). Toxic chemical induced genotoxicity detected by random amplified polymorphic DNA (RAPD) in bean (*Phaseolus vulgaris*) seedlings. Chemosphere, 76: 900-906.

Darlington, C. D. and L. E. La cour (1976). The Handling of Chromosomes, 6th ed. Allen and Unwin, London.

Demirhan, B. E., B. Demirhan, C. Sönmez, H. Torul, U. Tamer and G. Yentür (2015). Monosodium glutamate in chicken and beef stock cubes using high-performance liquid chromatography. Food Addit. Contam. Part B Surveill, 8: 63-66.

El-Ghorab, A. H., M. Nauman, F. M. Anjum, S. Hussain and M. Nadeem (2010). A comparative study on chemical composition and antioxidant activity of ginger (*Zingiber officinale*) and cumin (*Cuminum*

cyminum). J. Agric. Food Chem., 58: 8231-8237.

Fernstrom, J. D. and S. Garattini (2000). International symposium on glutamate. J. Nutr., 130 (Suppl 4): 891S-1079S.

Filer, L. J. and L. D. Stegink (1994). Report of the proceedings of the glutamate workshop. Crit. Rev. Food Sci. Nutr., 34: 159-174.

Haghighi, M., P. Fang and M. Pessarakli (2015). Effects of ammonium nitrate and monosodium glutamate in waste water on the growth, antioxidant activity, and nitrogen assimilation of Lettuce (*Lactuca sativa* L.). J. Plant Nutr., 38: 2217-2229.

Jinap, S. and P. Hajeb (2010). Glutamate. Its applications in food and contribution to health. Appetite, 55: 1-10.

Kanaya, N., B. S. Gill, I. S. Grover, A. Murin, R. Osiecka, S. S. Sandhu and H. C. Andersson (1994). *Vicia faba* chromosomal aberration assay. Mutat. Res., 310: 231-247.

Kim, Y. H., H. S. Bom, K. Y. Kim, H. K. Kim and J. Y. Kim (1999). Inhibitory effect of chitosan on the milk transfer of radiostrontium from contaminated mice to their sucklers. Kor. J. Chitin Chitosan, 4: 15-18.

Löliger, J. (2000). Function and importance of glutamate for savory foods. J. Nutr., 130: 915S-920S.

Mariyamma, T., K. S. Sujatha and G. Sisilamma (2009). Protective effect of *Piper longum* L. on monosodium glutamate induced oxidative stress in rats. Indian J. Exp. Biol., 47: 186-192.

Mateos, R. M., A. Jiménez, P. Román, F. Romojaro, S. Bacarizo, M. Leterrier, M. Gómez, F. Sevilla, L. A. Río, F. J. Corpas and J. M. Palma (2013). Antioxidant systems from pepper (*Capsicum annuum* L.): involvement in the response to temperature changes in ripe fruits. Int. J. Mol. Sci., 14: 9556-9580.

Nakanishia, Y., K. Tsuneyamaa, M. Fujimotoc, T. L. Salungaa, K. Nomotoa, J. L. Ana, Y. Takanoa, S. Iizukae, M. Nagatae, W. Suzukie, T. Shimadae, M. Aburadae, M. Nakanof, C. Selmig and M. E. Gershwinh (2008). Monosodium glutamate (MSG): A villain and promoter of liver inflammation and dysplasia. J. of Autoimmunity, 30: 42-50.

Ozden, S., D. Yüzbasloglu, F. Ünal and M. Ozel (2014). The determination of possible genetic damage to women undergoing *in vitro* fertilization due to infertility caused by the male factor. Food Chem. Toxicol., 74: 294-300.

Pandey R. M. and S. Upadhyay (2007).

Impact of food additives on mitotic chromosomes of *Vicia faba* L. Caryologia, 60: 309-314.

Prakash, E. and D. K. Gupta (2014). Cytotoxic activity of ethanolic extract of *Cuminum cyminum* Linn against seven human cancer cell line. Universal J. Agri. Res., 2: 27-30.

Qari, S. H. M. (2010). DNA-RAPD fingerprinting and cytogenetic screening of genotoxic and antigenotoxic effects of aqueous extracts of *Costus speciosus* (Koen.). JKAU: Sci., 22: 133-152.

Raif, S. G., A. Beiser, C. Ren, R. Patterson, P. A. Greenberger, L. C. Grammer, A. M. Ditto, K. E. Harris, M. A. Shaughnessy, P. R. Yarnold, J. Corren and A. Saxon (2000). Review of alleged reaction to monosodium glutamate and outcome of a multicenter double-blind placebo-controlled study. J. Nutr., 130: 1058S-1062S.

Rocco, L., G. Frenzilli, G. Zito, A. Archimandritis, C. Peluso and V. Stingo (2012). Genotoxic effects in fish induced by pharmacological agents present in the sewage of some Italian water- treatment plants. Inc. Environ. Toxicol., 27: 18-25.

Saghai-Maroof, M. A., K. M. Soliman, R. A. Jorgensen and R. W. Allard (1984). Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc. Natl. Acad. Sci. USA, 81: 8014-8018.

Satnam, S., Pd. Rekha, A. B. Arun and Y. Chiu-Chung (2009). Impacts of monosodium glutamate industrial wastewater on plant growth and soil characteristics. Eco-logical Engineering, 35: 1559-1563.

Schiffman, S. S. (1997). Taste and smell losses in normal aging and disease. J. Am. Med. Assoc., 278: 1357-1362.

Shahidi, F., J. K. V. Arachchi and Y. J. Jeon (1999). Food applications of chitin and chitosans. Trends Food Sci. Technol., 10: 37-51.

Shobana, S. and K. A. Naidu (2000). Antioxidant activity of selected Indian spices. Prostaglandins, Leukotrienes and Essential Fatty Acids (PLEFA), 62: 107-110.

Shon, Y. H., Y. M. Ha, T. R. Jeong, C. H. Kim and K. S. Nam (2001). Modifying action of chitosan oligosaccharide on 2-amino- 3,8-imethylimidazo [4,5-*f*] quinoxaline (MeIQx)-induced mutagenesis. J. Biochem. Mol. Biol., 34: 90-94.

Smaka-Kincl, V., P. Stegnar, M. Lovka and J. M. Toman (1996). The evaluation of waste, surface and ground water quality using the *Allium* test procedure. Mutat. Res., 368: 171-

179.

Stoilovaa, I., A. Krastanova, A. Stoyanovab, P. Denevc and S. Gargovaa (2007). Antioxidant activity of a ginger extract (*Zingiber officinale*). Food Chem., 102: 764-770.

Sudhakar, R., N. Gowda and G. Venu (2001). Mitotic abnormalities induced by silk dyeing industry effluents in the cells of *Allium cepa*. Cytologia, 66: 235-239.

Suhaja, M., J. Rácováa, M. Polovkab and V. Brezová (2006). Effect of γ-irradiation on antioxidant activity of black pepper (*Piper nigrum* L.). Food Chem., 97: 696-704.

Sun, T., Z. Xu, C. T. Wu, M. Janes, W. Prinyawiwatkul and H. K. No (2007). Antioxidant activities of different colored sweet bell peppers (*Capsicum annuum* L.). J. Food Sci., 72: S98-S102.

Thippeswamy, N. B. and K. A. Naidu (2005). Antioxidant potency of cumin varieties-cumin, black cumin and bitter cumin- on antioxidant systems. Eur. Food Res. Technol., 220: 472-476.

Ubani, L. U. (2011). Preventive therapy in complimentary medicine. Publs: Xlibris (July 5, 2011), 770 pages.

Yoon, H. J., H. S. Park, H. S. Bom, Y. B. Roh, J. S. Kim and Y. H. Kim (2005). Chitosan oligosaccharide inhibits 203$HgCl_2$-induced genotoxicity in mice: micronuclei occurrence and chromosomal aberration. Arch. Pharm. Res., 28: 1079-1085.

Table (1): List of random amplified polymorphic DNA (RAPD) primers and their nucleotide sequence.

Primer code	Sequence (5`→3`)
OPA-09	GGGTAACGCC
OPA-14	TCTGTGCTGG
OPA-20	GTTGCGATCC
OPB-01	GTTTCGCTCC
OPB-05	TGCGCCCTTC
OPB-06	TGCTCTGCCC
OPB-07	GGTGACGCAG
OPB-10	CTGCTGGGAC
OPB-11	GTAGACCCGT
OPB-12	CCTTGACGCA
OPB-14	TCCGCTCTGG
OPB-17	AGGGAACGAG

Table (2): Impact of MSG combined with chitosan, different natural spices and some forms of BNS on germination (%), root and shoot lengths (cm) of *V. faba*.

Treatment	Germination (%)			Root length (cm)	Shoot length (cm)
	48 h	72 h	96 h		
Negative control	63.33±10.85^{ab}	96.66±3.33^{ab}	96.66±3.33^{a}	2.24±0.19^{bc}	1.58±0.12^{a}
Positive control (MSG)	53.33±6.66^{ab}	86.66±4.22^{abc}	96.66±3.33^{a}	2.62±0.24^{ab}	1.35±0.10^{a}
Chitosan	60.00±5.16^{ab}	100.00±0.00^{a}	100.00±0.00^{a}	1.74±0.18^{cd}	1.64±0.10^{a}
Black pepper	70.00±6.83^{a}	96.66±3.33^{ab}	100.00±0.00^{a}	2.29±0.18^{bc}	1.59±0.10^{a}
Cumin	70.00±6.83^{a}	96.66±3.33^{ab}	100.00±0.00^{a}	3.11±0.32^{a}	1.72±0.12^{a}
Chili pepper	43.33±9.54^{abc}	76.66±6.15^{bc}	90.00±4.47^{a}	1.68±0.19^{cd}	1.30±0.13^{a}
Ginger	36.66±12.02^{bc}	73.33±9.88^{c}	90.00±4.47^{a}	1.96±0.30^{bc}	1.65±0.13^{a}
BNS leaves	40.00±8.94^{bc}	70.00±10.00^{c}	83.33±9.54^{a}	1.89±0.37^{bc}	1.50±0.09^{a}
Immature BNS fruits	46.66±6.66^{ab}	86.66±6.66^{abc}	86.66±6.66^{a}	1.53±0.18^{cd}	1.56±0.11^{a}
Mature BNS fruits	16.66±13.08^{c}	30.00±11.25^{d}	33.33±9.88^{b}	0.92±0.34^{d}	1.50±0.00^{a}

Values in each column followed by the same letter(s) are not significantly differ at 0.05 probability level.

Table (3): Mitotic index, percentage of mitotic phases, types and percentage of abnormalities of *V. faba* root tip cells treated with MSG combined with chitosan, different natural spices and some forms of BNS.

Treatment	No. of examined cells	No. of dividing cells	No. of abnormal cells	Mitotic phase (%)				Chromosomal aberration (%)							Mitotic index (%)	Abnormalities (%)
				Prophase	Metaphase	Anaphase	Telophase	Micronucleus	C-metaphase	Laggard	Fragment	Disturbance	Stickiness	Bridge		
Negative control	3082	203	6	64.04	10.34	11.33	14.29	1.97	0.00	0.00	0.00	0.98	0.00	0.00	6.53±1.81^{a}	4.23±3.24^{c}
Positive control (MSG)	3495	89	39	74.16	5.62	13.48	6.74	10.11	19.10	6.74	4.49	1.12	0.00	2.25	2.56±0.35^{bc}	47.10±14.39^{a}
Chitosan	3677	123	5	66.66	11.38	8.13	13.82	0.81	0.00	0.81	0.81	1.63	0.00	0.00	3.20±0.98^{bc}	6.73±4.55^{bc}
Black pepper	3274	162	13	68.51	12.35	6.79	12.35	3.09	2.47	1.23	0.62	0.00	0.62	0.00	4.93±1.33^{ab}	6.96±2.13^{bc}
Cumin	3301	138	7	48.55	13.77	11.59	26.09	4.35	0.00	0.00	0.72	0.00	0.00	0.00	4.23±0.76^{ab}	4.86±2.40^{c}
Chili pepper	3318	77	14	61.03	11.68	14.29	12.98	14.29	1.30	1.30	1.30	0.00	0.00	0.00	2.36±0.83^{bc}	13.13±10.23^{bc}
Ginger	3447	101	11	49.50	15.84	13.86	20.79	2.97	1.98	2.97	2.97	0.00	0.00	0.00	3.03±0.58^{bc}	11.26±7.98^{bc}
BNS leaves	3484	138	52	63.04	15.94	5.07	15.94	31.88	4.35	0.72	0.00	0.00	0.00	0.72	3.90±1.21^{ab}	30.70±10.38^{ab}
Immature BNS fruits	3333	73	12	60.27	17.81	6.85	15.07	2.74	8.22	1.37	0.00	4.11	0.00	0.00	2.23±0.23^{bc}	17.33±6.47^{bc}
Mature BNS fruits	3200	1	0	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10±0.10^{c}	0.00±0.00^{c}

Values within columns followed by the same letter(s) are not significantly differing at 0.05 probability level.

Table (4): Changes in DNA-RAPD profile of *V. faba* treated with MSG combined with chitosan, different natural spices and some forms of BNS.

Primer	No. of Bands in negative control	Positive control (MSG)				Chitosan				Black pepper				Cumin				Chili pepper				Ginger				BNS leaves				Immature BNS fruits			
		a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d
OPA-09	11	0	0	4	0	1	6	1	4	1	2	1	4	3	1	3	0	4	2	5	0	4	0	5	0	3	1	1	0	5	2	5	0
OPA-14	4	0	0	4	0	0	0	0	0	1	3	1	0	0	0	2	0	0	0	1	0	0	1	0	1	0	0	2	0	0	1	2	0
OPA-20	2	0	0	2	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	2	0	0	1	0
OPB-01	5	0	2	0	2	0	0	2	0	0	0	1	0	0	0	2	0	1	0	2	0	1	0	2	1	0	0	2	1	0	0	2	0
OPB-05	8	0	1	0	4	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OPB-06	4	0	0	1	1	0	0	0	1	1	1	3	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
OPB-07	8	0	0	6	0	0	2	0	2	0	1	4	0	0	2	0	0	0	0	2	0	0	0	0	0	0	0	6	0	1	0	7	0
OPB-10	9	0	0	2	0	0	1	0	0	0	2	0	1	0	2	0	1	0	0	1	0	1	0	4	0	0	0	4	0	0	1	4	0
OPB-11	16	1	5	1	2	1	3	2	2	1	3	2	1	0	8	1	3	1	2	5	1	0	5	2	3	1	2	2	1	1	3	5	0
OPB-12	10	0	0	2	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1	0	0	1	1	0	0	0	0	2	0	0	2	0
OPB-14	8	0	1	1	1	1	1	1	0	0	1	0	0	1	1	0	0	1	1	0	0	0	1	0	0	0	4	0	0	0	2	0	0
OPB-17	11	2	0	5	0	0	1	0	0	3	1	4	0	2	1	3	0	3	0	4	0	3	0	3	0	1	0	0	1	2	0	3	0
Total	96	3	9	28	10	3	14	6	9	7	14	18	7	6	15	13	5	10	5	21	1	9	9	17	5	6	7	17	7	9	9	31	0
a+b		12				17				21				21				15				18				13				18			
GTS %		87.50				82.29				78.12				78.12				84.37				81.25				86.46				81.25			

a: appearance of new band, b: disappearance of normal band, c: increase in band intensity, d: decrease in band intensity, a+b: polymorphic bands, GTS: genomic template stability.

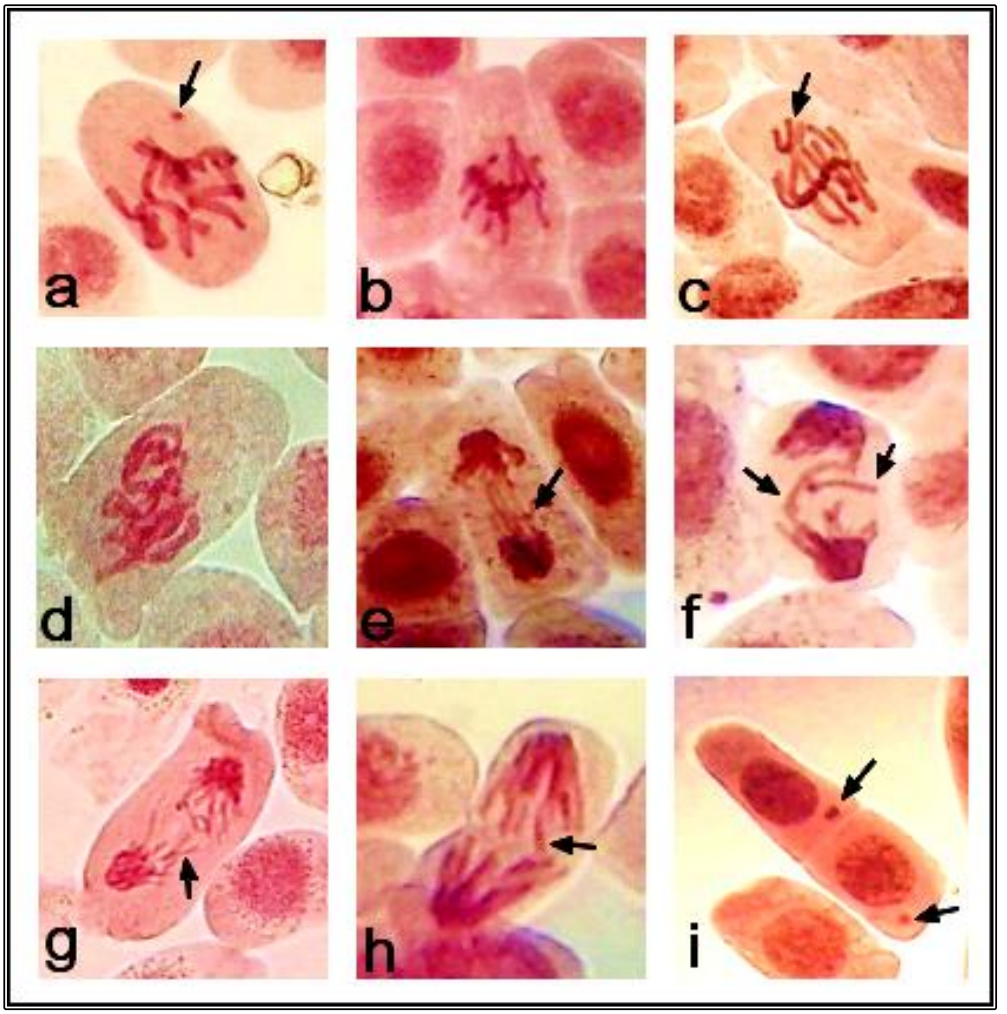

Fig. (1): Types of abnormalities observed in *V. faba* root tips treated with MSG and other combined natural materials. a) C-metaphase with fragment, b) Disturbed metaphase, c) Metaphase with laggard, d) Sticky prophase, e) Multiple bridges, f) Telophase with single bridge and laggard, g) Anaphase with chromosome breakage, h) Anaphase with laggard i) Micronucleus.

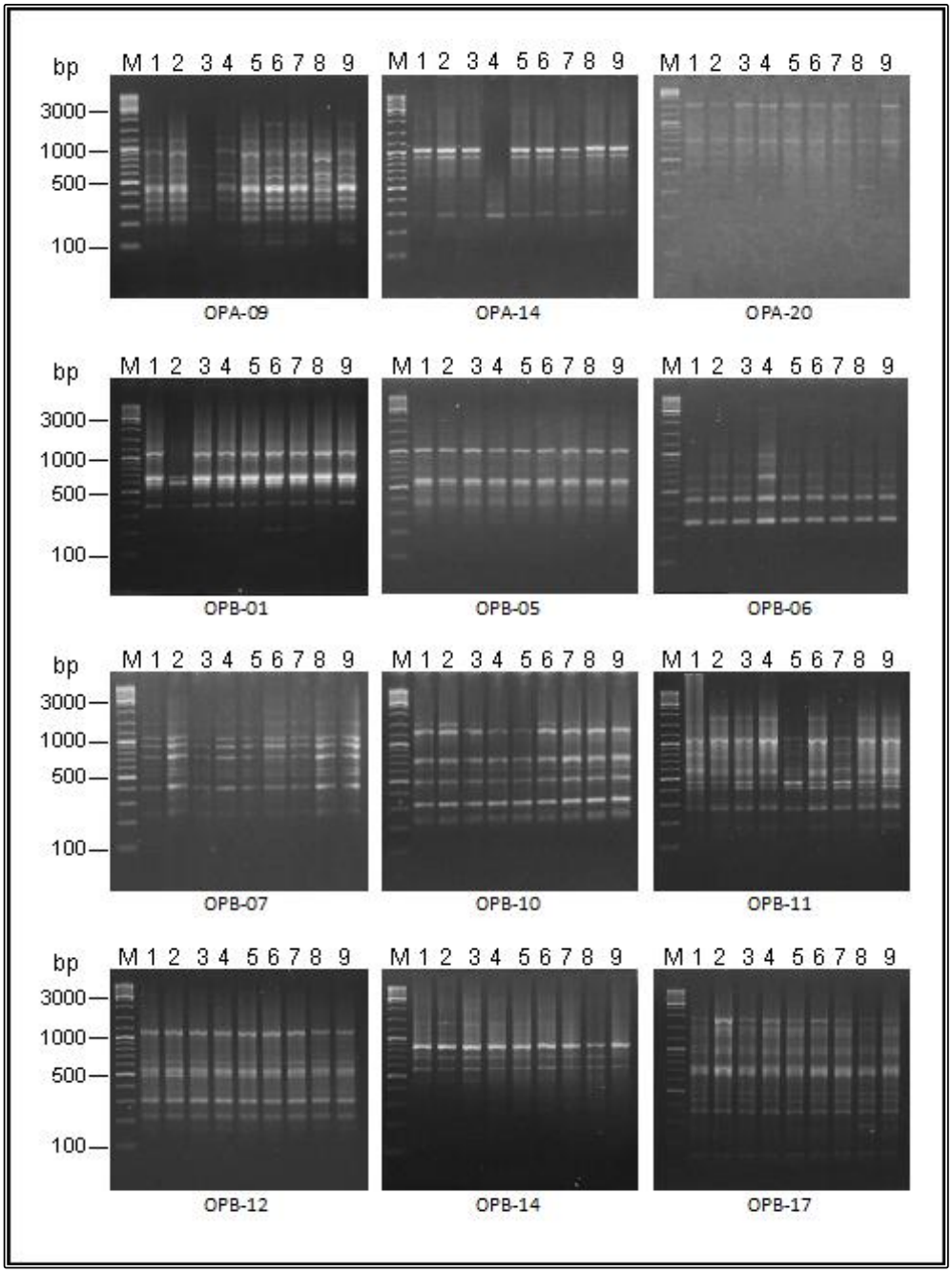

Fig. (2): RAPD profiles of genomic DNA extracted from *V. faba* seedlings treated with MSG (positive control) and other combined natural materials using twelve primers. Lane M: DNA marker, lane 1: negative control, lane 2: positive control, lanes 3, 4, 5, 6, 7, 8 and 9: treatments of chitosan, black pepper, cumin, chili pepper, ginger, BNS leaves and immature fruits, respectively.