J. Indian bot. Soc. 63 : 69—74, 1984.

MUTAGENIC EFFECT OF MALATHION IN BARLEY[1]

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ABSTRACT

The cytological effects of malathion are explored in barley and compared with those of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). The chromosomal abnormalities noticed at metaphase include fragments, ring chromosomes, sticky chromosomes, lagging chromosomes and bridges. A few cells with increased number of chromosomes were also noticed. Univalents and multivalents were noticed at metaphase-I. Anaphase-I and subsequent stages of meiosis were also irregular. In the M_1 generation, chlorophyll mutants were observed.

INTRODUCTION

We are screening the mutagenicity of pesticides employing chromosome aberrations (mitotic and meiotic) and chlorophyll mutations in barley as the parameters (Grover and Tyagi, 1979, 1980 a, b). The present report deals with the mutagenic effects of malathion.

MATERIALS AND METHODS

Barley (*Hordeum vulgare* L.) grains were treated with malathion (S-1, 2-bis (emoxycarbonyl) emyl 0,0-dimethyl phosphorodithioate) nitro-N- and a known chemical mutagen N-methyl-N'-nitro-N-nitrosogua-nidine (MNNG) as a positive control. The procedure of Wuu and Grant (1967) was adopted for treatment. The seeds were presoaked in water for 16 h at 25°C and treated for 4h with various concentrations of malathion (0.05%, 0.1%, 0.25 %, 0.5%, and 1%). The preparations were made in phosphate buffer (pH 7.0). During the treatment, the flasks were continuously shaken on SH_2 horizontal shaker (Ganson Pvt. Ltd., Bombay). The grains were treated with a non-lethal concentration of MNNG (0.01 %) for 4h. A supplementary treatment of MNNG (0.01%) for 4h to each group of malathion treated grains and a 4h treatment of each dose of malathion to MNNG treated grains, was also administered.

The effects of each treatment were also estimated on grains germination, chromosomal changes and chlorophyll mutations.

Grain germination: After each treatment, the grains were thoroughly washed in running water and put in 10 cm petri plates lined with blotting sheet and kept in incubator at 25°C. The germination count was made after 72 h. The emergence of coleoptile was taken as the criterion of germination. The experiment was run in triplicate and repeated twice. Two hundred grains per

1. Accepted for publication on November 11, 1982.

treatment after thoroughly washing in running water, were sown in 1 m xl m soil beds.

Cytological studies:

Mitotic : For mitotic studies, the grains were germinated as mentioned above. The root tips were taken at an interval of 24 h and treated with 0.0029 M 8-hydroxyquinoline for 2h at 15°C. After this treatment, the root tips washed with distilled water for 6-8 times and then fixed in freshly prepared Farmer's fluid (1 glacial acetic acid : 3 ethyl alcohol) and squashes were prepared according to the method of Darlington and LaCour (1962) employing aceto-orcein stain. Approximately 250 cells from at least ten root tips were scored to determine the frequency of aberrant cells. Care was taken to exclude incomplete cells so as not to have any confusion regarding an aneuploid cell. The slides were scored at random. The experiment was repeated and the resulting data were pooled to calculate mean percentage frequencies of anomalies.

Meiotic : For meiotic analysis, the suitable inflorescences were fixed in Carnoy's (6:3:1) solution for at least 24 h. Anthers were smeared in aceto-carmine per schedule of Darlington and LaCour (1962). The analysis was made at metaphase-I, anaphase-I, telophase- I and II. Wherever possible, analysis was made from approximately 300-400 PMCs from 5 different plants of each treatment.

Chlorophyll mutants : Seeds from each plant of M_1 were collected separately. Seeds from 7-10 randomly selected families were sown according to one plant-one row method and chlorophyll mutants were screened at seedling stage which were identified and classified according to Gustafsson (1940).

RESULTS

Germination : The effects of malathion on the seed germination are shown in Fig. 1. A reduction in percentage of seed germination with increasing concentrations of pesticide was noted.

Mitosis : The squash preparations from root tips from control material revealed 14 chromosomes at metaphase. The subsequent stages were normal. No instance of chromosome aberration was found.

The treated root tips revealed aberrant chromosomes at metaphase and subsequent stages were also anomalous. Chromosomal aberrations include sticky chromosomes, despiralization, fragments, ring chromosomes, aneuploid and polyploid cells, laggards and chromatin bridges.

The frequency of aberrations is shown in figure 2. The maximum frequency of 22.33% of aberrant cells was noticed with 1% of malathion. An increase in aberration frequency was noticed following supplementary treatment of MNNG.

Meiosis : In untreated plants, seven bivalents were consistently observed at diakinesis and metaphase-I. The chromosomes separated regularly at anaphase and the subsequent stages were normal. Meiotic analysis of malathion treated plants carried out at metaphase-I revealed the presence of univalents and quadrivalents. Rarely, bivalents of unequal size indicating deletions were also noticed. The mean pairing varied with different concentrations. The irregularities at anaphase-I include unequal separation, laggards and chromatin bridges. Rarely, micronuclei were also seen at telophase-II. The failure

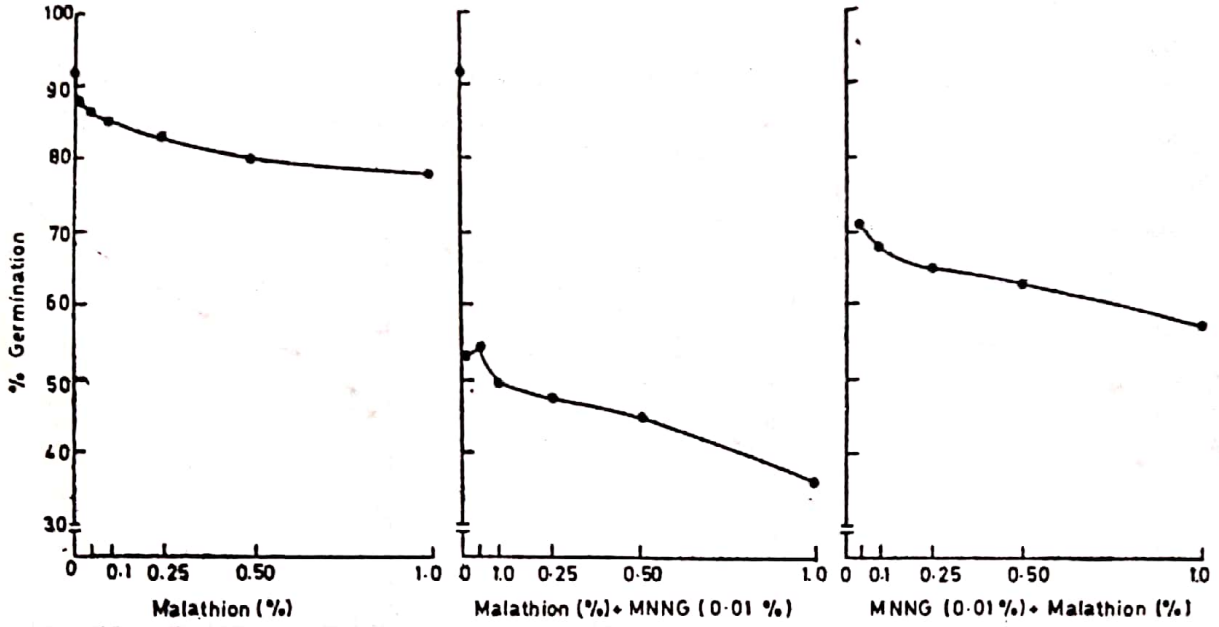


Fig. 1. Relationships between germination and varicus concentrations of malathion and MNNG.

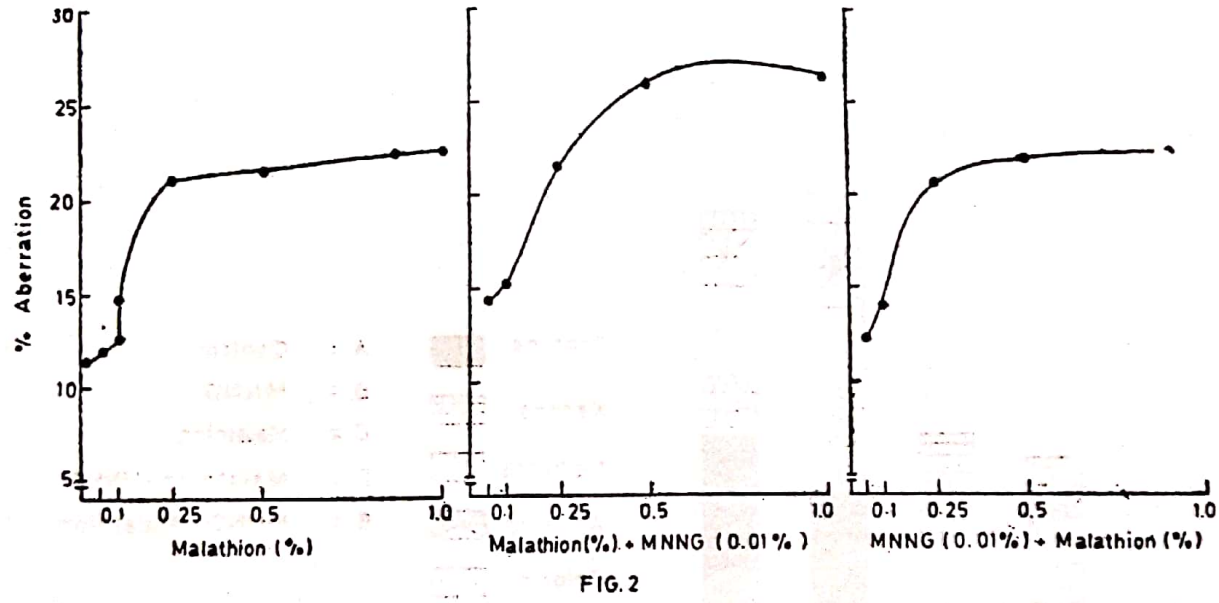


Fig. 2. Relationships between chromosomal aberration (mitotic) and various concentrations of malathion and MNNG.

of cytokinesis was also noticed in almost all the treatments. The frequency of chromosomal aberrations is shown in Fig. 3. No qualitative difference was noticed in the combined treatment of malathion and MNNG. The supplementary treatment of MNNG enhanced the aberration frequency.

Chlorophyll mutants: The spectrum of chlorophyll mutants includes xantha, tigrina, maculata, alboviridis, chlorina and viridoxantha (Fig. 4).

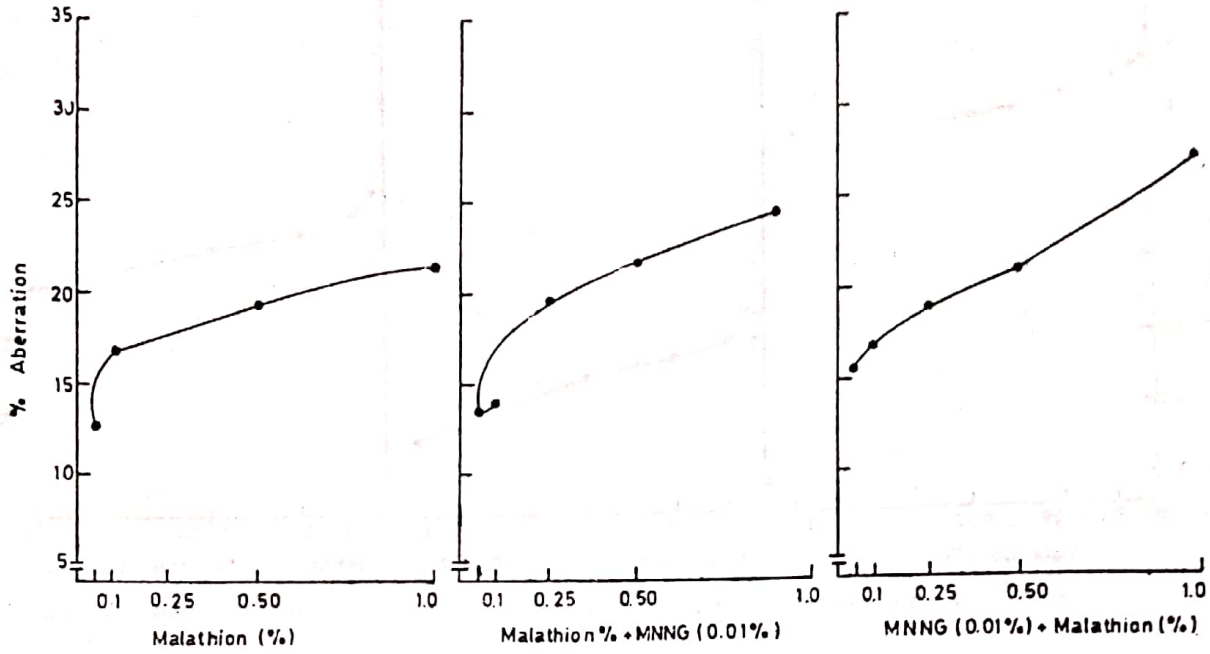
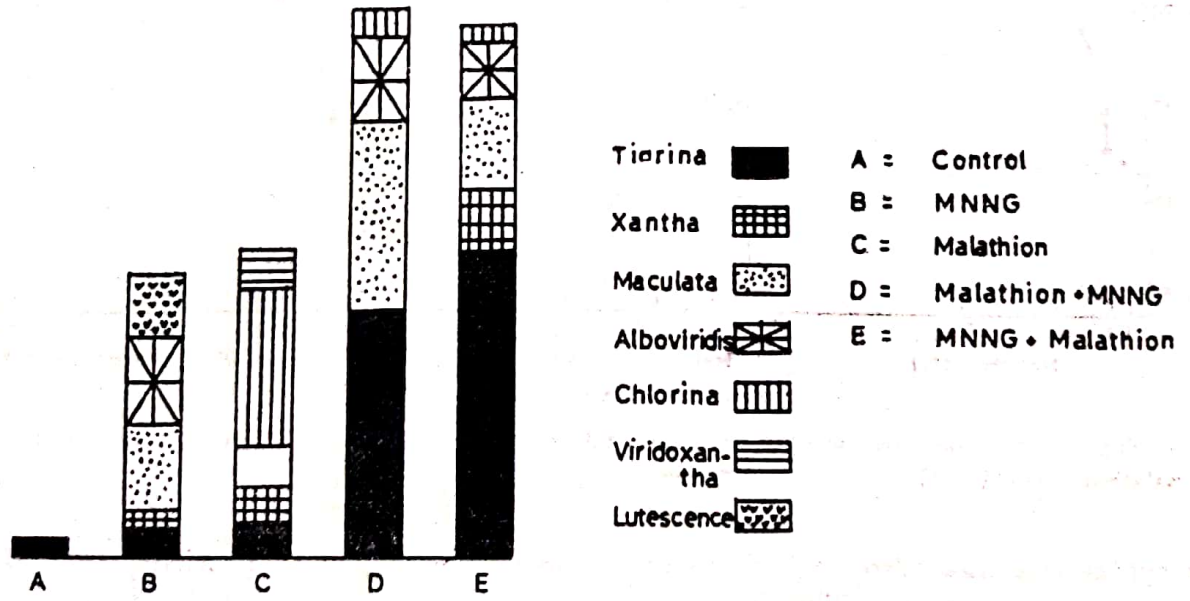


Fig. 3. Realtionships between aberrant pollen mother cells and various concentrations of malathion and MNNG.



ig 4 Chlorophyll Mutants spectrum induced by MNNG and malathion and in combination with each other in M_2 generation

Fig. 4. Chlorophyll mutants spectrum induced by MNNG and malathion and in combination with each other.

DISCUSSION

The reduction in the germination following malathion treatment is in conformity with other such studies (Wuu and Grant, 1966: George *et al.*, 1970). The combined treatment revealed that the action of malathion and MNNG is of synergistic in nature. It has been suggested that endogenous growth regulators play an important role in the germination of seeds and there exists a "balance" between promoters and inhibitors (Aman, 1968). It is probable that such a "balance" is disturbed following treatment with malathion.

The chromosomal aberrations noticed following treatments include fragments, increased number of chromosomes, chromatin bridges and laggards. Pesticides induced chromosomal aberration is known in a number of cases (Grover and Tyagi, 1980a,b). The increased number of chromosomes ascribcable to induced disturbances in the synthesis of proteins, is in accord with the observations of Skorpuska (1975) who reported similar result in *Pisum* following treatment with phenyl carbamates. The presence of both single and paried bridges suggests the occurrence of both chromatid and chromosome breakage (Sax, 1940). This observation is in accord with similar observations made by a few workers (Das *et al.*, 1978; Grover and Tyagi, 1980a; Oku, 1976 ; Wuu and Grant, 1966). The presence of micronuclei may be attributed to the exclusion of acentric fragments during nucleus formation, (Clowes, 1964, Ravindran, 1971).

The occurrence of an appreciable degree of univalents at metaphase-I, their random distribution on the spindle, non-disjunction and the formation of micronuclei seems to be the outcome of some disturbances during pairing of homologous chromosomes. Grover and Tyagi (1980b) noticed pesticide induced disturbed meiosis in barley. It seems, univalents arise from partial or complete lack of homologue chromosome pairing. Such partial or complete lack of chromosome pairing may be attributed to various causes such as gene mutation, chromosome breakage, the influence of environment and cryptic structural changes (Jain and Basak, 1965; Maguiri, 1966). In the treated plants, multivalents were encountered in low frequency, which may be due to low frequency or of recovery of cells with a few translocated segments. Other types of abnormalities noticed are the laggards, bridges, fragments and spindle inhibition. Amer and Ali (1968, 1974) noticed stickiness, lagging chromosomes and anaphase bridges in PMCs following treatment with phenols and herbicides. Such abnormalities following pesticide treatment have also been noticed by other workers (Wuu and Grant, 1967; Grover and Tyagi, 1980b).

Bridges at meiotic anaphase suggest the possibility of chromosome having inverted segments. However, as most of the bridges were unaccompanied by fragments, the probability of the formation of bridges due to inversion is remote. Bridges may also result due to the failure of terminalization of chiasmata in a few cells.

The combined treatment altered the chromosomal aberration frequency.

The authors are not aware of any study undertaken to unravel the interaction of mutagen and pesticides. The present result may be attributed either to some synergistic reaction between MNNG and malathion or both of these

may be acting on the same or similar site for its radiomimetic action.

Chlorophyll mutants: The detailed studies carried out to estimate the chlorophyll mutants induced by pesticides are very limited. Recently, Grover and Tyagi, (1979) reported the induction of albina, xantha, and tigrina mutants induced by a few pesticides. The present study revealed that tigrina and maculata were the most frequent mutants. The variable frequency at different doses suggests that action of pesticide in induction of chlorophyll mutants is random and there may be a number of sites, whose mutability may lead to a distinct kind of mutant.

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