DISCUSSION

In view of the harmful effects of pesticides and food additives, which are being consistently recorded, it is essential that toxic effects of these chemicals should be evaluated under variety of conditions. This study deals with two mutagens, chloroacetic acid (CAA) and 2,4-Dichlorophenoxyacetic acid (2,4-D). CAA is used as herbicide and for treatment of plantar warts, it is also used as preservative and drying agent for curing hay. As for hazards posed to human beings, at least two deaths have been reported due to exposure to CAA (Zeldenrust, 1951; Mann, 1969). 2,4-D is a common systemic herbicide used in the control of broad leaf weeds. It is the most widely used herbicide in the world, and the third most commonly used in North America (ITF, 2006). 2,4-D can cause low growth rates, reproduction problems, change in behavior or death in non-tagged species (SCC, 2005).

Though new and sophisticated techniques are being developed, several conventional techniques still provide useful information on toxicity of suspected compounds. Three of such techniques, which have been widely used, are:

i) \textit{In vivo} bone marrow chromosomal aberrations (CAs)

ii) Micronucleus (MN) assay and,

iii) Sperm abnormalities (SPA)
In the present study, we evaluated the mutagenic potential of CAA and 2,4-D in bone marrow cells (BMCs) of *Rattus norvegicus* employing these techniques; *i.e.* CA and MN assays, with the polychromatic erythrocytes (PCEs) being part of the latter assay.

Only few studies have focused attention on the mechanism of micronucleus (MN) formation. Evans et al. (1959) showed that when the root tips of *Vicia faba* were subjected to fast neutrons and gamma rays exposure, induction of chromosomal aberrations (CAs) resulted in micronuclei (MN) formation. Schmid (1976) showed that MN are formed due to chromosomal breaks and spindle inhibition. It is generally accepted that clastogenic mutagens induce micronuclei formation which are break-away part of chromosome fragments, or of lagging chromosomes (Schmid, 1975). Heddle and Carrano (1977) have worked out theoretical aspects of the relationship between CAs and MN formation.

Two sub-lethal concentrations of CAA and 2,4-D were given to rats during present investigations after 1st and 7th day which caused chromatid/chromosomal breaks, gaps, exchanges, multiple aberrations and micronuclei formation in the BMCs. It is evident that in addition to an apparent interference during chromosome condensation due to chromosome breakage, fragmentation and
disintegration, which demonstrates the clastogenic potential, both of these chemicals also cause spindle poisoning (Sharma et al., 2000).

In the present study after treating *R. norvegicus* with CAA and 2,4-D, a dose and duration-dependent increase in CA and MN formation in PCEs were recorded. Our data demonstrate that 2,4-D is more damaging mutagen than CAA, since the maximum frequency of chromosomal aberrations after 7th day with 2,4-D had the values of 12.76±0.9 mean (%) frequency as compared to 6.22±0.7 mean (%) frequency with CAA. Similar conclusion can be made from the data on micronuclei assay as the maximum value of 8.24±0.9 mean/1000 PCEs was obtained for 2,4-D, while the value for CAA was 5.68±0.6 mean/1000 PCEs. In both cases, significant differences were observed after 7th day but after 1st day with 2,4-D only.

As per our results, CAA is more mutagenic than the related compound TCA, since CAA caused significant damage in BMCs of *R. norvegicus* at a concentration of 0.80 mg/100 gm b.wt., *i.e.* 08 mg/kg, while 500 mg/kg of TCA was required in case of mice to produce the mutation of this magnitude (Bhunya and Behera, 1987). In other words, taking the parameters used here, CAA is >60 times mutagenic than TCA. The results also show that CAA and 2,4-D cause rapid decrease in the values of CAs and MN in BMCs after the *C. sinensis* intake concurrently with the chemicals following an initial increase observed after 1st and
7th day of treatment, which may be due to the reasons outlined in the preceding paragraph.

Present study suggests that 2,4-D is clastogenic to *R. norvegicus* and a number of reports support the genotoxic potential of 2,4-D. These studies employ a wide range of experimental techniques which show the genotoxicity of 2,4-D (Charles et al., 1999; Amer and Aly, 2001; Madrigal-Bujaidar et al., 2001; Ateeq et al., 2002; Holland et al., 2002; Farah et al., 2004; Zeljezic and Garaj-Vrhovac, 2004). Madrigal-Bujaider et al. (2001) reported that oral administration 50, 100 and 200mg/kg of 2,4-D in somatic cells results in formation of a significant sister chromatid exchange (SCEs) with high doses which was manifested in a dose-dependent manner. In our observations, we evaluated that 2,4-D damaged the chromosomal DNA with two sub-lethal doses by using CAs and MN for one and seven days consecutively. The dose level was very low (33.3mg / 100gm b. wt.) than the study of Madrigal-Bujaider et al. (2001) when administered intraperitoneally. The extent of damage will depend on the availability of reactive intermediates of the mutagenic compounds generated during their metabolism in the target tissues or cells. 2,4-D and its possible genotoxic effects reported on human lymphocytes *in vitro* by CA analysis and MN assay (Zeljezic and Garaj-Vrhovac, 2004). In an earlier study, a significant increase in the percentage of CAs in bone marrow and spermatocytes cells were observed after oral administration of 2,4-D for three and five consecutive days (Amer and Aly, 2001).
The sperm abnormality (SPA) is a sensitive and reliable parameter and is widely used to identify germ cell mutagen (Wyrobek and Bruce, 1978; Giri et al., 2002a, b; Nahas and Ashwany, 2004; Roy et al., 2008). Wyrobek et al. (1983) reported that chemicals induced SPA also proved to be carcinogenic. In the present study, intraperitoneal administration of two sub-lethal doses of CAA and 2,4-D produced genotoxic effects in rats as revealed from enhanced frequencies of SPA. Effects of 2,4-D on sperm have been identified in studies of both exposed people and laboratory animals (Amer and Aly, 2001; Swan, 2003; US EPA, 2004). A study found that 2,4-D levels were five times as high as those found in men with about average sperm count (Swan, 2003). In addition, EPA lists a variety of effects on male sex organs that include atrophy of the testes, degeneration of sperm producing tissues and decrease numbers of sperms in testes (US EPA, 2004). 2,4-D also caused an increase in number of abnormal sperms in a study of National Research Centre (Amer and Aly, 2001). A recent finding also suggested that organophosphorus pesticides can reduce the number of spermatozoa which showed greater susceptibility to DNA denaturation in samples from agricultural workers (Sanchez-Pena et al., 2004).

Cytotoxic drugs suppress spermatogenesis in mammals (Wyrobek et al., 1983) by causing death of developing germ cells in seminiferous tubules (Lu and Meistrich, 1979). The cytotoxicity was further evident in terms of increased
incidences of SPA and mortality. CAA and 2,4-D affected the morphogenesis of spermatozoa at all dose-levels and all sampling times, indicating that there was interference in the metamorphosis of germ cells into mature structurally perfect sperms. SPA significantly increased in a dose and time-dependent manner only after 7 days and not on one day treatment, possibly because of the cytotoxicity of these herbicides. The results from this study suggest that the drug is absorbed from peritoneal cavity and reaches the germ cells. The formation of abnormal sperm indicates the ability of CAA and 2,4-D to produce exogenous factors when accumulated in germ cell pool, induce alterations in sperm morphology mainly by point mutation (Chauhan et al., 2000) but also by other mechanisms (Styrna et al., 1991; Rubes et al., 1998). Small alteration in testicular DNA (Tophman, 1980), or the interference of the test substance with the genetically controlled differentiation of sperm cells (Bruce et al., 1974; Rai and Vijaylakshmi, 2001) are some cause of the SPA.

Bhunya and Das (1987) injected single doses of 12.5, 25 and 50 mg/kg CAA intraperitoneally into groups of 3 male Swiss mice. After 35 days an increased number of malformed sperms was found in the two highest dose groups while in our investigation, we induced lower dose level (0.24 and 0.80 / 100gm) of CAA for a short period of 1 and 7 days and found positive results after 7 consecutive days of treatment in the form of SPA. It is also documented that certain chemicals including CAA and 2,4-D, are germ cell mutagens affecting
specific gene loci in spermatogonial cells thereby increasing the percentage of SPA (Soares et al., 1979; Letz, 1990; Amer and Aly, 2001; Donya, 2002; Bhunya and Behera, 1987; Bhunya and Das, 1987). It is further stated that sperm cell morphology is genetically controlled by numerous autosomal and sex-linked genes (Krazonowska, 1976). Hence, formation of abnormal sperm population in the present study is very likely due to mutagenic effects of CAA and 2,4-D induced ROS on specific gene loci of germ cell chromosomes involved in the maintenance of the normal sperm structure. The present results of the sperm morphology test are in consensus with that of an earlier report (Bhunya and Das, 1987; Amer and Aly, 2001), in which CAA and 2,4-D affected the morphology of sperm of rats but in different manner. In the conclusion, the results of this study indicate that 2,4-D and CAA are cytotoxic and genotoxic to germ cells in the male rat.

Rats treated with both of these chemicals showed a conspicuous decrease in aberrant type cells and micronuclei and sperm abnormalities after 1\textsuperscript{st} and 7\textsuperscript{th} day of treatment when \textit{C. sinensis} was used simultaneously with CAA and 2,4-D. Such a decrease has been attributed to elimination of metabolites from the body, repair of damaged genetic material, removal of cell chromosomes with damaged genetic material, or caused by reduction in the level of test compound due to its conversion to other products (Tates and Natarajan, 1976; Bhunya and Behera, 1987).
C. sinensis (Green tea) has well recognized antimutagenic properties against several mutagens (Kada et al., 1985; Kuroda and Hara, 1999; Siess et al., 2000). Extracts or other purified preparations of phenolic rich foods offer antioxidant, antibacterial, anti-inflammatory, antiviral, antimutagenic, anti-carcinogenic, vasodilatory, and neuroprotective properties (Middleton et al., 2000; Scalbert et al., 2005; Soobrattee et al., 2005, 2006). There is a compelling interest in understanding the effect of the polyphenol/flavonoid content within human foods such as leafy vegetables, edible root crops, grapes, apples, mangoes, onions, cocoa, corn, buckwheat, redwine, and tea because of the potential health benefits from these compounds (Santos-Buelga and Scalbert, 2000; Steinberg et al., 2003; Scalbert et al., 2005; Zaveri, 2006; Schroeter et al., 2006). Regarding possible genotoxicity or carcinogenicity effects, catechins and/or C. sinensis extracts have been shown to be chemopreventive with respect to various mutagens or carcinogens, in both in vitro and in vivo systems (Arimoto et al., 2003; Iwai et al., 2005; Aruoma et al., 2006). It has been postulated that the antioxidant properties of catechins constitute their main mode of action in this chemopreventive effect.

CA assays of C. sinensis catechins (GTC) with reactive oxygen species (ROS) scavengers demonstrated that H$_2$O$_2$ is responsible for the in vitro clastogenic potential of GTC (Roy et al., 2003). Available information indicates that H$_2$O$_2$ is generated in the presence of GTC due to the pro-oxidant property of
catechins. In short, catechins reduce oxygen to form $\text{O}_2^-$ and then further reduce this to form $\text{H}_2\text{O}_2$ (Katiyar and Mukhtar, 1996; Gupta et al., 2002; Edenharder and Grunhage, 2003; Roy et al., 2003). In our results, it is observed that the *C. sinensis* aqueous leaves extract (ALE) decreased the frequency of CAs in BMCs when administered with CAA and 2,4-D simultaneously. *C. sinensis* was not produced any change in the frequency of chromosomal aberrations, micronuclei and sperm abnormality than control when induced alone.

The number of micronuclei in the animals influenced by a GTC’s most effective catechin, epigallocatechin gallate (EGCG) alone did not differ from that of the control group. The formation of micronuclei and DNA damage in the form of comet tail length during single cell gel electrophoresis was significantly suppressed by EGCG in a dose-dependent manner (Roy et al. 2003). In the present investigation, results also showed that the green tea ALE decreased the number of micronuclei in bone marrow cells and SPA in epididymis with CAA and 2,4-D, simultaneously.

Another aim of the present study was to evaluate the neuro-biochemical and neuro-behavioral changes by two sub-lethal doses of the CAA (0.24, 0.80mg/100gm) and 2,4-D (19.98, 33.30mg/100gm) after 1 and 7 consecutive days. We were able to demonstrate a time- and dose-dependent effect of CAA and
2,4-D on cerebellum, cerebrum and brain stem using lipid peroxidation (LPO), catalase (CAT), superoxide dismutase (SOD), total sulfhydryl group (TSH) and reduced glutathione (GSH), for neuro-biochemical effect, and to examine the neuro-behavioral effect; we used open field behavior, elevated plus maze and rota rod test.

The mechanism of toxicity of many compounds concerns the formation of reactive oxygen species (ROS), including superoxide anion, hydrogen peroxide, superoxide radical and hydroxyl radical. The compounds under discussion are capable of reacting with proteins, nucleic acids, lipids and/or molecules that lead to changes in their structure and finally to cell damage (Gwoździński, 2000; Mates, 2000). However, a majority of cells possess defense mechanisms against the potential harmful effects of ROS. These defenses include superoxide dismutase, which converts its substrate, superoxide anion, into hydrogen peroxide, and also catalase, which converts hydrogen peroxide into water and oxygen. Extra- and intracellular substances that are antioxidative in nature prevent overproduction of radicals and protect the organism against propagation of peroxidative reactions (Kulikowska-Karpińska and Moniuszko-Jako-Niuk, 2004). Pesticides may induce oxidative stress by generating free radicals, thus causing lipid peroxidation (LPO). Increased LPO and oxidative stress can affect the activities of protective
enzymatic antioxidants that have been shown to be the sensitive indicators of increased oxidation reactions.

As per the results, rate of LPO was increased with CAA and 2,4-D at 1st to 7 days daily treatment, which showed the high level of Malondialdehyde (MDA) in cerebrum, cerebellum and brain stem in comparison to control rats. There is still lack of information about 2,4-D while CAA does not have even a single report on this kind of work, and this is the first study which shows the alterations in these parameters in brain parts. Many reports are available on the production of free radicals by these compounds on mammalian and non-mammalian systems but on the tissues other than brain which indicate the active participation in LPO. LPO is a potential endogenous source for DNA residues modification that plays role in carcinogenesis (Chung et al., 1996). 2,4-D can bind to certain phospholipids and disturb physical interactions in membrane, which probably increases the availability of lipids to peroxidation (Pogosyan et al., 1984). The induction of LPO in pea microsomes may be a result of the production of free radicals (e.g. •CCl3, Cl3CO•, Cl3COO •) formed during metabolism of carbon tetrachloride in endoplasmatic reticulum with participation of cytochrome P-450 (Pogosyan et al., 1984). 2,4-D caused lipid peroxidation as well as the increase in membrane fluidity at the 16 carbon atom of fatty acids and also hemolysis in human erythrocytes (Duchnowicz et al., 2002, 2003). Suwalsky et al. (1996) showed that
2,4-D disturbs phospholipid bilayer structure. It has also been suggested that peroxidation of synaptic endings modifies the lipid content of synaptoplasmic membranes. This consequently leads to severe disturbances in the function of neurotransmitter uptake systems and depolarization-dependent calcium channels (Dabrowiecki et al., 1985).

The enhanced LPO may also be due to marked depletion of GSH content of brain, which acts as one of the guarding factors against oxidative stress. It is reported that GSH depletion may lead to an increased LPO, possibly due to the lowering of the cellular defense system against endogenous toxic intermediates (Younes and Siegers, 1981). The present evidence of neuronal defense against H$_2$O$_2$, which is the most toxic molecule to the brain, is mediated primarily by the glutathione system (Dringen, 2000). Therefore, it is reasonable to expect that the decrease of TSH, GSH may be a risk factor for enhancement of LPO, because of their protecting effect against free radical damage (Zaidi and Banu, 2004).

Exogenous supplementation of antioxidants has been reported to exert protective effect in various pathological states in which free radicals are involved (Ozcan et al., 2004). In our study simultaneous treatment of C. sinensis reduced the oxidative stress markers associated with CAA and 2,4-D exposure in different parts of brain on albino rats. LPO decreased while TSH and GSH levels increased
significantly on treatment with *C. sinensis*. The significant reduction in LPO clearly demonstrates that *C. sinensis* protects the different parts of brain against CAA and 2,4-D induced oxidative damage.

GTC scavenge a wide range of free radicals, including the most active hydroxyl radical, which may initiate LPO. Therefore, catechins may decrease the concentration of lipid free radicals and eliminate initiation and propagation of LPO. They can protect brain from LPO, deamination induced by oxidative stress. This is evidenced by the fact that *C. sinensis* extract may decrease LPO markers in the liver, serum and brain (Skrzydlewska et al., 2002). Due to the protective effects of the *C. sinensis* extract on the central nervous system (CNS) tissue expressions, the decreased level of LPO products has been reported (Rice-Evans et al., 1996). Preventive nature of *C. sinensis* has also been reported against ethanol induced LPO in liver and brain (Ostrowska et al., 2004), oxidative stress in plasma and erythrocytes (Coimbra et al., 2006). It has been earlier reported that consumption of *C. sinensis* within a balanced controlled diet improve the overall antioxidative status and protection against oxidative damage in humans (Erba et al., 2005). Our results are in favour of these available studies which are showing the antioxidative nature of *C. sinensis* against free radical generation and/or oxidative damage.
The LPO inhibition and TSH, GSH elevation property of these adaptogens were observed in our study. The findings of present investigation suggest that *C. sinensis* when given orally with CAA and 2,4-D simultaneously for the same duration in which TSH and GSH levels were elevated, brings about a significant decrease in LPO level. It suggests that *C. sinensis* protection may be mediated through modulation of cellular antioxidant levels.

In our study, exposure of CAA and 2,4-D significantly decreased the levels of SOD and CAT in cerebrum, cerebellum and brain stem indicating depletion of antioxidant resources in albino rats to induce free radicals. Oxidative stress inhibited SOD activity in various regions of brain in rats (Shukla et al., 1987), which is consistent with the results observed in this study. A remarkable decrease after treatment with these chemicals in SOD activity detection may be responsible for the formation of superoxide radical during chemical treatment. It has been reported that production of radicals in the brain is due to catecholamine metabolism such as dopamine and norepinephrine (Venarucci et al., 1999), and elevated catecholamine levels may undergo autooxidation, in which electrons are generated that in turn can produce ROS (Carpagano et al., 2003). Therefore to neutralize ROS, the body mainly uses enzymatic copper, zinc-superoxide dismutase (Cu, Zn-SOD), catalase (CAT) and selenium-dependent glutathione peroxidase (Se-GSH-Px) and non-enzymatic antioxidants, like reduced glutathione.
(GSH). Antioxidant enzymes are considered as primary defense mechanisms that protect biological macromolecules from oxidative damage. Our data indicated that CAA and 2,4-D induced a significant inhibition of SOD. The possible reason for this finding could be the decreased amount of this enzyme caused by enhanced LPO in this condition (Chaudiere and Ferrari-Iliou, 1999). After CAA and 2,4-D treatment, SOD activity is significantly decreased exacerbating neuronal cell damage. This endangerment consistently correlates with disruption of energy pathways and low energy availability. SOD is the first of the scavenger enzyme series to ameliorate the damage caused in the cells by free radicals (Slater, 1984). Formation of superoxide radicals after chemical stress has already been reported previously (Naqvi and Hasan, 1992). In the present investigation decrease in SOD activity detection may be responsible for the formation of superoxide radicals.

CAT activity suggests that it attenuates the excessive formation of ROS. The inhibition rate of CAT is dependent upon the rate at which H$_2$O$_2$ is generated or added. H$_2$O$_2$ and free radical by product (hydroxyl radical) have been strongly implicated in neuronal degeneration induced by monoamine neuron toxins such as 6-hydroxydopamine or 5,7 dihydrotryptamine (Allis and Cohen, 1977). The decline in CAT can be attributed to ineffective scavenging of H$_2$O$_2$ resulting in increased H$_2$O$_2$ levels, which can react with O$_2$ to give OH radical and thus increased LPO. The reduction in CAT activity is either due to its increased
degradation or decreased synthesis, and may also be due to the simultaneous increase in peroxidase activity. Also it might be attributed to the decreased ability of CAT to detoxify peroxides with CAA and 2,4-D, and inactivation of CAT by free radicals. SOD and CAT both are scavenger enzymes that are reported to work together to eliminate toxic free radicals (Fridovich, 1981).

Experimental studies have shown that phenolic compounds, particularly flavonoids and catechins are important antioxidants and superoxide scavengers. Their scavenging efficiency depends on the concentration of phenol, the number and location of the hydroxyl groups (Benavente-Garcia et al., 2002). Earlier workers have reported the antioxidant properties of flavonoids from different sources. A relation between the antioxidant property and radiation protection by flavonoids has been reported (Shimo et al., 1996).

To reduce or suppress the chemical effects, aqueous *C. sinensis* leaves extract was used simultaneously with CAA and 2,4-D and alone. The most effective polyphenol (catechin) of *C. sinensis* is epigallocatechin gallate (EGCG) in the inhibition of free radical induced injury. It has been proved to penetrate into the cerebral parenchyma through the blood brain barrier in an animal experiment (Suganuma et al., 1998) and therefore due to the presence of this polyphenol, *C. sinensis* can be possibly developed as an effective neuroprotective antioxidant.
Catechins contain free radical scavenging properties and act as biological antioxidants. It has been demonstrated that they can scavenge both superoxide and hydroxyl radicals (Guo et al., 1996; Nanjo et al., 1999), and 1,1 diphenyl-3-picrylhydrazyl radical (Zhao et al., 2001), carbon centre free radicals, singlet oxygen and lipid free radical (Zhao et al., 2001). In addition, after the oxidation of catechins, due to their reaction with free radicals, a dimerised product is formed, which has been shown to have increased superoxide scavenging and iron chelating potential (Yoshino et al., 1999). So, *C. sinensis* protect antioxidant enzymes (SOD, CAT) from CAA and 2,4-D induced alterations in CNS of male rats. Declined SOD activity in different parts of brain of treated rats was brought back to nearby a normal level with simultaneous administration of *C. sinensis* with CAA and 2,4-D. Significant elevation of SOD and CAT activities with *C. sinensis* are also supported by earlier researchers (Young et al., 2002; Erba et al., 2005; Skrzydlewska et al., 2005).

The chemical exposures may cause serious health hazards in human and animal populations, it is somewhat surprising that animal testing to examine the neurotoxic effects has not been initiated on a wider scale. In response to this lack of information, there has been an increase in regulatory activity aimed at developing strategies for testing of neurotoxicity at animal level, which includes behavioral end points (US EPA, 1991; Winneke, 1992; OECD, 1992; OECD,
Neurobehavioral parameters were evaluated with two sub-lethal doses of CAA and 2,4-D induced anxiety, locomotor, exploratory and motor coordination behavioral test, like elevated plus maze (EPM), open field test (OFT) and rota rod test (RRT).

Chemicals can influence the neurobehavioral profile of the organism and complex, interactive mechanisms have been proposed for these effects. Many studies have indicated the changes in behavioral and biochemical characteristics in depressed patients. In the present study, we found that exposure of CAA (0.24, 0.80mg/100gm) and 2,4-D (19.98, 33.30mg/100gm) for one and seven days consecutively on rats appeared to have behavioral deficit including suppressed EPM activity, OFT activity, and interfering the motor coordination by RRT to stay on revolving rod. The toxic effects on motor function were shown essentially by the loss of rotarod performance which might be due to muscular weakness (Duffard and Duffard, 1996). There are several mechanisms by which a toxicant can affect performance in a behavioral situation, e.g., by altering the processing and integration of sensory input, etc.

These chemicals induced alterations which were recovered with the treatment of aqueous leaves extract (ALE) of *C. sinensis* when used with CAA and 2,4-D simultaneously (50 mg/kg/day for 1 and 7 days, daily).
Both EPM and OFT have been used very effectively to assess neurobehavioral profile of animals under the influence of anxiogenic/anxiolytic agents (Oliveria and Palermo-Neto, 1993; Carobrez and Bertoglio, 2005). In the EPM, increased aversion of open arms are indicative of enhanced anxiety state and our results indicated that CAA and 2,4-D caused reduction in the number of entries (%) and time spent in open arms (%) in EPM activity. Similarly in the OFT, exposure to CAA and 2,4-D induced behavioral alterations as evidenced by decrease in ambulation, preening and rearing, which can satisfactorily be explained due to increased anxiety of fear (Weyers, 1994) and that these differences may depend upon differences within the mesolimbic dopamine system (Stöhr et al., 1998). These chemicals affected the motor coordination in RRT which decreased the staying ability due to muscular weakness on rotating rod in comparison to control rats. Our results are in agreement with earlier investigations (Oliveria and Palermo-Neto, 1993; Novack and Zwolshen, 1983; Cartmell et al., 1991; Weyers, 1994; Rozas and Labandeira-Gracia, 1997; Stöhr et al., 1998). These chemicals induced alterations which were recovered with the treatment of ALE of C. sinensis when used with CAA and 2,4-D simultaneously after 1 and 7 days.