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EFFECT OF HERBAL AND SYNTHETIC INSECTICIDES ON THE DNA CONTENT OF (*BRASSICA CAMPESTRIS L.*) MUSTARD PLANT

Bharati Bhusana Rath[1] and Upendra Prasad Tripathy[2]*

[1]PGT Botany, Govt. (SSD) Higher Secondary School, Ramagiri, Gajapati, Odisha, Pin-761016.
[2]Pulp & Paper Research Institute, Jaykaypur, Rayagada, Odisha, (India), Pin-765017.

***Corresponding Author: Upendra Prasad Tripathy**

Pulp & Paper Research Institute, Jaykaypur, Rayagada, Odisha, (India), Pin-765017.

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ABSTRACT

The main aim of this research work is to assess the effect of natural and synthetic insecticide on DNA content of *Brassica Campestris* (mustard). For the experimental study, two biocides, Neem oil and Carbosulfan 25% E.C. (Marshal) were taken. Neem oil is a natural bio-pesticide whereas Carbosulfan 25% E.C (Marshal) is synthetic one. The treatment of doses i.e., 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0 mg/lit.is taken on 7, 14, 21, 28, 35 days growing plants. The plant growth rate with its DNA contents of leaves as well as roots were identified. It is observed the natural insecticides (Neem oil) has a little effect on the DNA modification in leaves as well as roots as compared with the synthetic insecticides. Again, Pollution of environment by using synthetic insecticide is one of the major problems now-a-days.

KEYWORDS: Brassica campestris, Neem oil, Carbosulfan 25% E.C. (Marshal).

INTRODUCTION

Agricultural pest are serious competitors of man for food and their population density must be regulated below the economic injury level. In agroecosystem pest control can be done by using synthetic chemical pesticide.[1] However due to the development of resistance in many pest species and concerns about environment pollution pressure to eliminate synthetic chemical pesticide several pest management programmes are continuously mounting.[2] Hence, there is a need of alternative control methods which is effective and eco-friendly. Biological control offers an excellent alternative to pesticides and forms the backbone of modern concept of integrated pest management. In recent years several repellents have been used in wildlife management.[3] But due to the toxic manifestation of synthetic compounds now attention is being focused on photochemical as they pose little environmental risk because they do not bio accumulate and show specific biological activity. Mustard (*Brassica Campestris L.*) belongs to the family Cruciferae or Brassicaceae is a Rabi oil seed crop cultivated by the farmers of India.[4,5] The panel of National Research Council, USA considered *Azadirachta indica* to be one of the most promising of all plants which may eventually benefit every person on this planet. The extract of Neem has been used as antifeedant, repellent and anti-fertility agent. Nimbecidine (Neem oil-based repellent) manufactured and has been certified as a totally safe pesticide by the Central Insecticide Board, Government of India.[6] Besides Neem several other plant parts or extracts are used as a bio pesticide. The population of India, growing at annual compound growth rate of 2.1 percent is expected to be 1.546 million by the year 2020. Assuming that 27 percent of the population would be under 15 years of age which would require half the adult intake of edible oil. The oil yielding crops rank at present is next to pulses in the crop status. Mustard seed contains 30 to 45% oil, and it is used for cooking and burning purposes. The oil cake is also used as cattle feed and manure. The productivity of mustard is 1013 kg/hectare in the last decade. In India, in the year 1997-98, mustard production was 49.4 lakh tons which drastically reduced from the previous year's production i.e., 69.4 lakh tons. Pesticides affect man, animal, plant, soil, air as well as the whole living biota. Toxicity towards the aquatic flora and fauna is an important criterion for a pesticide to be considered as a hazardous pollutant. It also causes Physiological damages, biochemical changes, pathological disorders, resistance to biochemical degradation, high ecological specificity & biological magnification in the different trophic levels of the food chain. Another fact is that pesticide is generally applied to the soil, plant, water bodies and human settlements.[7] There would be least environmental contamination if these falls exactly on the target organisms and get degraded to harmless compounds. But this does not happen. A little percentage of the pesticide hit the target and the other portion drift into the environment thus affecting the ecosystem. These pesticides not only affect the plant but also affect harm to the consumers and cause phytotoxicity to the plants. Deng H. (2016) reported on the relation sheep between nuclear DNA-content of

sperm cells and timing events in the cell cycle of *Brassica Campestris L*.[8] Warasy A. A (2021) reported on karyomorphological analysis of two varieties of *Brassica Campestris L.* from Bangladesh.[9] These residues in the soil also affect the micro-organisms by reducing their activity temporarily and inhibit their role in the breakdown of pesticides in the soil. Three aspects of pesticide problem appear to be particularly important: Effects on man, domestic animals which may take up pesticides mostly through contaminated food and feeds. Effects on wildlife, where certain species can severely be affected by adsorption or accumulation of pesticides through food weds, disturbing the balance of ecosystem in nature and effects on plants which take up pesticides through roots and through leaf surfaces. According to a report "Death in the grass of pesticides" published in Hindustan Times Dec, 30, 1992, pesticides spread most widely in the environment through migration. They are washed off from the crops into the water, enter water bodies, penetrate with fodder, the organisms of animals and hence the food stuffs. Organophosphates like Ethion, Fenthion, Dimethote. Trithion, Monocrotophos, Diarinon, Dursban, Phosdrin, Thionaien, Chlorpyrifos, Phosmet, Parathion, Malathion and Metasystor inhibit the production of cholinesterase at the junction between adjoining nerve cells with the result that cholinesterase breaks down acetyl choline secreted by nerve cell axons excessive accumulation of acetyl choline interferes with the nerve impulse transmission. Kamunhukamwe T et. al. (2022) reported the effect of Neem-bio-pesticide and synthetic insecticide on the population of FAW larvae, level of leaf damage and maize grain yield in comparison with untreated control plants. They observed that Neem bio pesticide and synthetic insectides resulted in significantly higher yield compared to untreated plant.[10] The organophosphorus insecticides such as Parathion, Malathion and TEPP may be absorbed by the lungs, eye membrane and skin in toxic amounts. This excessive absorption leads to large accumulation of acetyl choline in the body disturbing the normal functioning of blood. The chlorinated hydrocarbon insecticides, now among the world most widely distributed synthetic chemicals are contaminating a substantial part of the biosphere. They are dispersed throughout the environment in currents of air and water.[11] Their movement and widespread distribution throughout the world are explained by their solubility characteristics and chemical stability and especially their tendencies to absorb an organic matter to be transported in air droplets and to become concentrated in food transfers from plants to herbivores to carnivores. The chlorinated hydrocarbons are seriously degrading biotic communities in many parts of the world. They have been shown to destroy larval stages of valuable aquatic food organisms and to reduce photosynthesis of marine phytoplankton. Some examples of chlorinated hydrocarbons are DDT (Dichloro-diphenyl-Trichloro ethane) methoxy-chloro, dieldrin, dicofol, endosulfan etc. DDT was the first of a long line of insecticide based on hydrocarbons with chlorine atom replacing some hydrogen atoms. It was observed that at higher concentration of DDT in body parts and blood of human beings cause anxiety, tension, cancer, mutations, stress reactions congenial in uteri malformations and impotency.[12-15] It affects the central nervous system which leads to death. It dissoloves in lipids and accumulates in the body fats. At lower level, it causes cancer, high blood pressure and cirrhosis of liver. Carbonate compounds are rapidly detoxified and excreted, so that their risk to warm blooded animals is less than the other agents. They are degraded rapidly in the environment, so persistence is not a problem. They are, however, a danger to many useful insects, especially to honeybees. Some examples of carbonate compounds are carbary), aldicarb, methoxmy (Lannate) etc. Chronic accumulation of pesticides plays an important role such as liver and kidney malfunctioning, secretion of excess of aninoacids in human blood and urine blood abnormalities as well as electroencephalogram deformation of brain tissues. Insecticides such as aldrin, dieldrin, chlordane, endosulfan, heptachlor and gemaxine are reported to affect the wildlife by changing their metabolic activities and body chemistry.[16-22] Chlorophenoxy acid compounds such as 2, 4-D affect embryos while triazones may cause mutagenic effects in animals. Organophosphate pesticides cause extreme muscular weakness, tremors and dizziness in poisoned animals. Depletion in respiratory metabolism dispersion of melanin pigment (blackening of the body colour), decrease in DNA, RNA and protein are the most important findings of the pesticide study. Fiaz K et. al. (2014) reported that heavy metal toxicity of Pd/Cd decreases the yield of *Brassica Campestris L.*[23]

MATERIAL AND METHODS

In this present investigation pure line seeds of mustard were collected and two bio pesticides i.e., Neem oil and Carbosulfan 25% E.C. (Marshal) were used in this research.

Brief Description of Neem Oil

Neem (*Azadirachta indica*) that belongs to Meliaceae family has universally been accepted as a wonder tree because of its diverse utility. Multi directional therapeutic uses of Neem have been known in Indian since Vedic times. Besides its therapeutic efficacies, Neem has already established its potential as a source of naturally occurring insecticides, pesticides and agrochemicals. Since the report on the isolation of nimbin from Neem seed oil by Siddiqui in 1942, more than 140 compounds have been isolated so far from different parts of Neem tree. An indica contains at least 35 biologically active principles of which Azadirachtin is the most active ingredients and is present predominantly in the seed, leaves and other part of Neem tree. Like Neems antibacterial and antiviral properties its antifungal properties are often a given among scientist in India and other Asian nations where most of the current resource are being conducted. Reports were completed before 1992 to indicate that compounds in Neem help to control fungi which causes athletes foot ringworm as well as

fungi that may affect plants. Neem cake, Neem leaves (poweder or aqueous extract), Neem oil and extracts of Neem seeds or Kernels have been found to control phyto pathogenic fungi on many plants such as tomatos, rice, cotton, soya, grapes, wheat, beans, roses and cucumbers. Carbosulfan 25% E.C. (marshal) is a broad spectrum and contact insecticide based on the active ingredient, carbosulfan. This formulation can be used for the control of caterpillar and sucking pests of rice and chillies. The root knot nematode melodidogyne incognita is a serious pest of vegetables. An attempt was made by the management of root knot nematode by using varied application (soil application, seed soaking and foliar spray) i.e., 1000ppm with half recommended dose of carbofuran 3G (1.0 kg /ha) gave highest crop yield and reduced the no. of galls per plant.

Preparation and Mode of Application of Test Chemicals

The common recommended doses, of different concentrations of the test chemicals were prepared by using distilled water as solvent. In this piece of investigation, 2 bio pesticides i.e., Neem oil and Carbosulfan 25% E.C. (Marshal) were used, the preliminary concentrations were 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 ml/lit. were used.

DNA Extraction Method

The residue after extraction as suspended in 1ml of 5% (V/V) TCA to remove the nucleotide and kept in refrigerator for 15 min. Then centrifuged at 5000x g for 20 min at 0˚C and the supernatant was discarded. This process was repeated 2 times. Then to the residue 5ml. of 10% TCA was added for nucleic acid extraction and boiled in a water bath for 10-20 min cooled and then centrifuged. The DNA content of TCA extract after cooling was determined by diphenylamine reagents by the method of Schneider.

RESULTS AND DISCUSSION

Effects of Neem oil on DNA Content in Mustard Seedlings

The maximum content of DNA of the control plant were 0.406 ± 0.09 mg/g, 0.491± 0.06 mg/g, 0.628 ± 0.12 mg/g, 0.713 ± 0.15mg/g and 0.821 ± 0.17 mg/g observed on 7, 14, 21, 28 and 35 days respectively in mustard seedling in fresh weight. However, seedling treated with 5.0 ml/lit concentration of Neem oil solution, the minimum content of DNA were 0.128 ± 0.03 mg/g, 0.194 ± 0.04 mg/g, 0.296 ± 0.05 mg/g, 0.328 ± 0.06 mg/g and 0.497 ± 0.07 mg/g observed on 7, 14, 28, 35 days respectively on fresh weight. All other concentration of Neem oil showed intermediate values. Values are in mean ± S.D. of ten samples values of r calculated as -0.951, -0.959, -0.958, -0.961, -0.968 on 7, 14, 21, 28, 35 days respectively on shoot of mustard. The crop selected for the work is Brassica campestris var. Pusa bold preserved in the laboratory for experimental purpose. Impact of two bio pesticides i.e., Neem oil and carbosulfan 25% E.C. (Marshal) study on the DNA contents involve following changes. The lower concentration of the biocides caused higher rate of DNA estimation as compared to the higher concentrations natural pesticides are eco-friendly whereas synthetic pesticides cause serious undesirable side effects resulting in the change in photosynthetic efficiency, biochemical disorders and genetic damages. It was reported that there are about 1005 species of plant exhibiting properties of insecticides, 384 with antifeedant, 297 with repellent, 27 with attractant and 31 species with growth inhibitory properties. The most used botanicals (in which the allele-chemicals present are used as pesticide) are Azadirachta indica, Pongamia glabra, Madhuca indica etc. Allelo-chemicals present in these plants are also being used to control nematodes. In biological control measures of pest population, natural enemies play a key role. Conservation and employing biological suppressing agents such as predators like aphidilions, ground beetles, lady bird beetles, spharphide flies, spiders, entomophillic nematodes, toads, frogs, and birds' parasitoids can attack and kill the pests. Synthetic pesticides are harmful to the environment, and it causes several disorders in plants as follows.

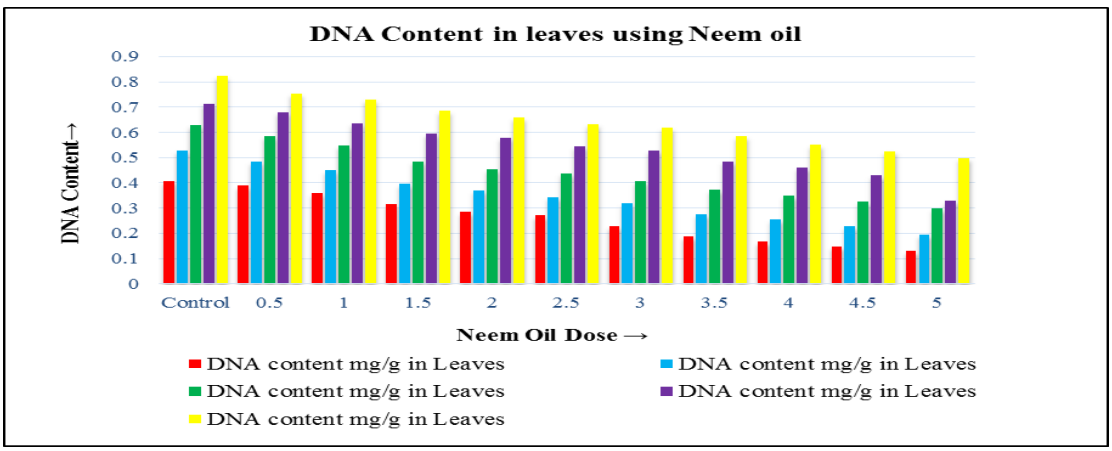

Figure 1: DNA Content (mg/lit.) in leaves of *Brassica Campestris* (Mustard) using Neem oil as Insecticides.

Significance level calculated P≤ 0.001 with degree of freedom 20. Likely the maximum content of DNA of the control set in the root of mustard plant were 0.382±0.08mg/g, 0.491±0.06mg/g, 0.574±0.09mg/g. 0.668±0.12mg/g and 0.785±0.14mg/g observed on 7,14,21,28 and 35 days respectively on fresh weight. Similarly root seedling treated with 5.0ml/lit concentration of Neem oil solution the minimum content of DNA was 0.106±0.02mg/g, 0.157±0.01mg/g, 0.254±0.03mg/g, 0.356±0.04mg/g and 0.453±0.06mg/g observed on 7,14,21,28 and 35 days on fresh weight. Values are in mean ±S.D. of ten samples. All other concentration of Neem oil showed intermediate values. Value of r calculated as -0.943, -0.955, -0.965, -0.968 and -0.978 on 7,14,21,28 and 35 days respectively on root of mustard. Significance level calculated P≤0.001 with degree of freedom 20.

Figure 2: Germination, Growth pant and flowering plant of *Brassica Campestris* (Mustard).

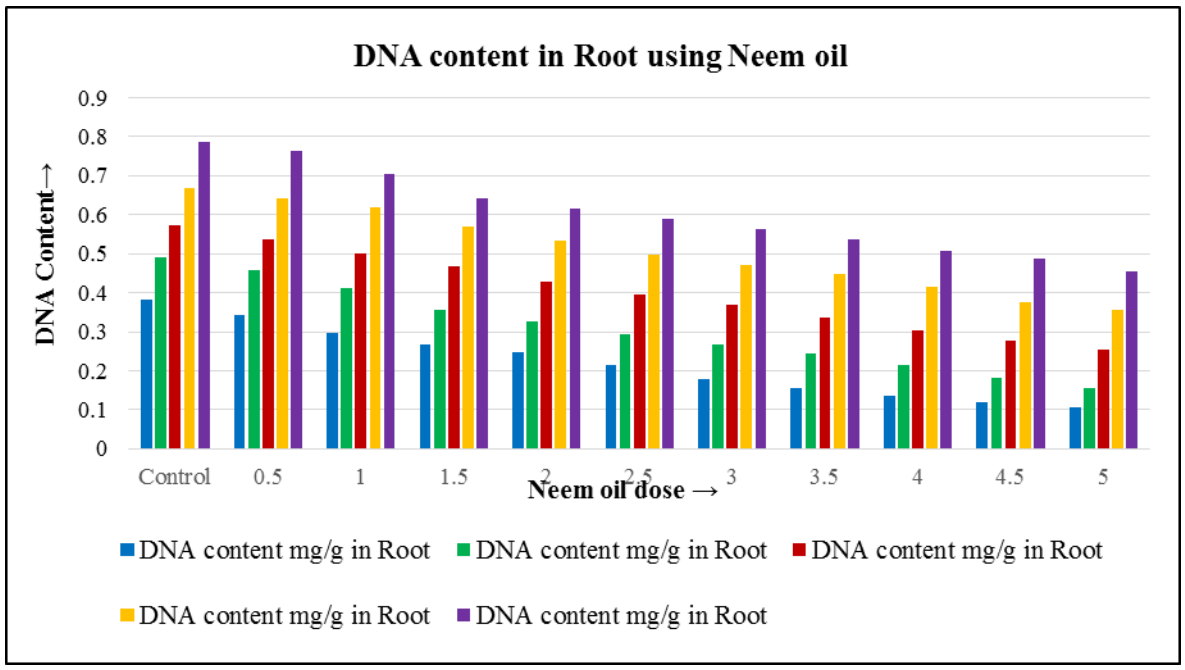

Figure 3: DNA Content (mg/lit.) in Roots of *Brassica Campestris* (Mustard) using Neem oil as Insecticides.

Effects of Carbosulfan 25% E.C. (*Marshal*) on DNA Content in Mustard Seedlings

The maximum content of DNA of the control plant were 0.406 ± 0.09 mg/g, 0.491± 0.06 mg/g, 0.628 ± 0.12 mg/g, 0.713 ± 0.15mg/g and 0.821 ± 0.17 mg/g observed on 7, 14, 21, 28 and 35 days respectively in mustard seedling in fresh weight. However, seedling treated with 5.0 ml/lit concentration of Carbosulfan 25% E.C. (Marshal) solution, the minimum content of DNA were 0.073 ± 0.01 mg/g, 0.154 ± 0.02 mg/g, 0.251 ± 0.04 mg/g, 0.348 ± 0.07 mg/g and 0.459 ± 0.08 mg/g observed on 7, 14, 28, 35 days respectively on fresh weight. All other concentration of Neem oil showed intermediate values. Values are in mean ± S.D. of ten samples values of r calculated as -0.956, -0.961, -0.962, -0.967, -0.971 on 7, 14, 21, 28, 35 days respectively on shoot of mustard. Significance level calculated P≤ 0.001 with degree of freedom 20. Likely the maximum content of DNA of the control set in the root of mustard plant were 0.382±0.08mg/g, 0.491±0.06mg/g, 0.574±0.09mg/g. 0.668±0.12mg/g and 0.785±0.14mg/g observed on 7,14,21,28 and 35 days respectively on fresh weight. Similarly root seedling treated with 5.0ml/lit concentration of Neem oil solution the minimum content

of DNA was 0.058±0.01mg/g, 0.119±0.01mg/g, 0.216±0.03mg/g, 0.315±0.05mg/g and 0.412±0.06mg/g observed on 7,14,21,28 and 35 days on fresh weight. Values are in mean ±S.D. of ten samples. All other concentration of Neem oil showed intermediate values. Value of r calculated as -0.961, -0.966, -0.969, -0.972 and -0.977 on 7,14,21,28 and 35 days respectively on root of mustard. Significance level calculated P≤0.001 with degree of freedom 20.

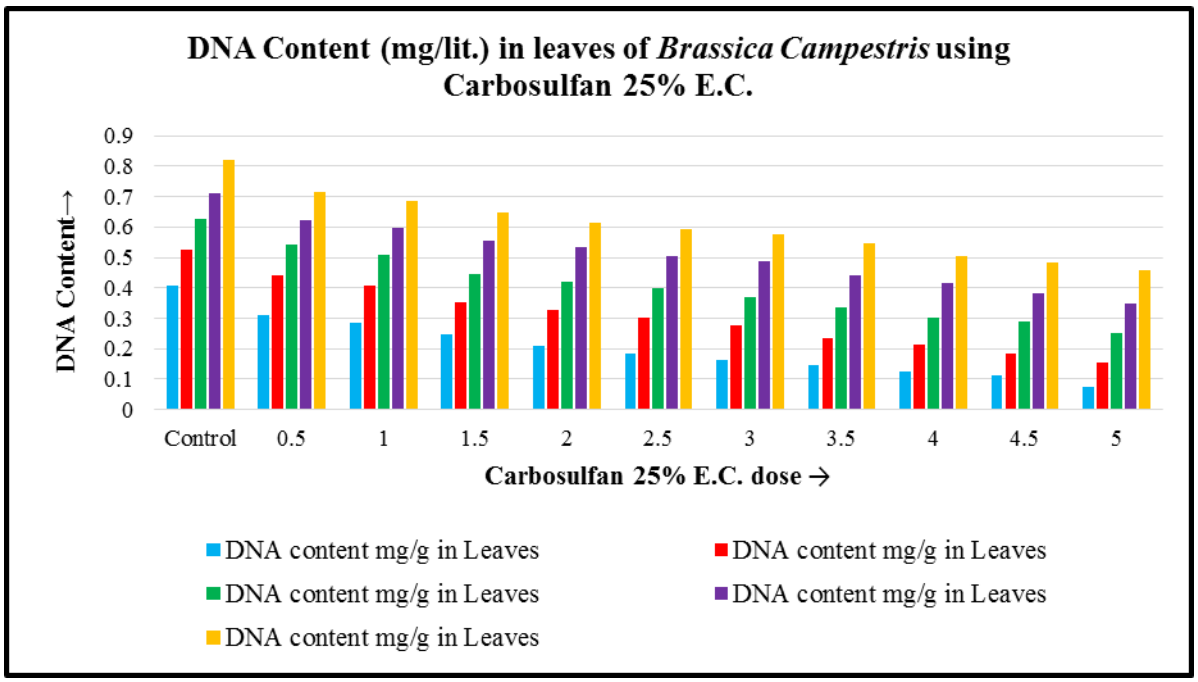

Figure 4: DNA Content (mg/lit.) in leaves of *Brassica Campestris* (Mustard) using Carbosulfan 25% E.C. (*Marshal*) as Insecticides.

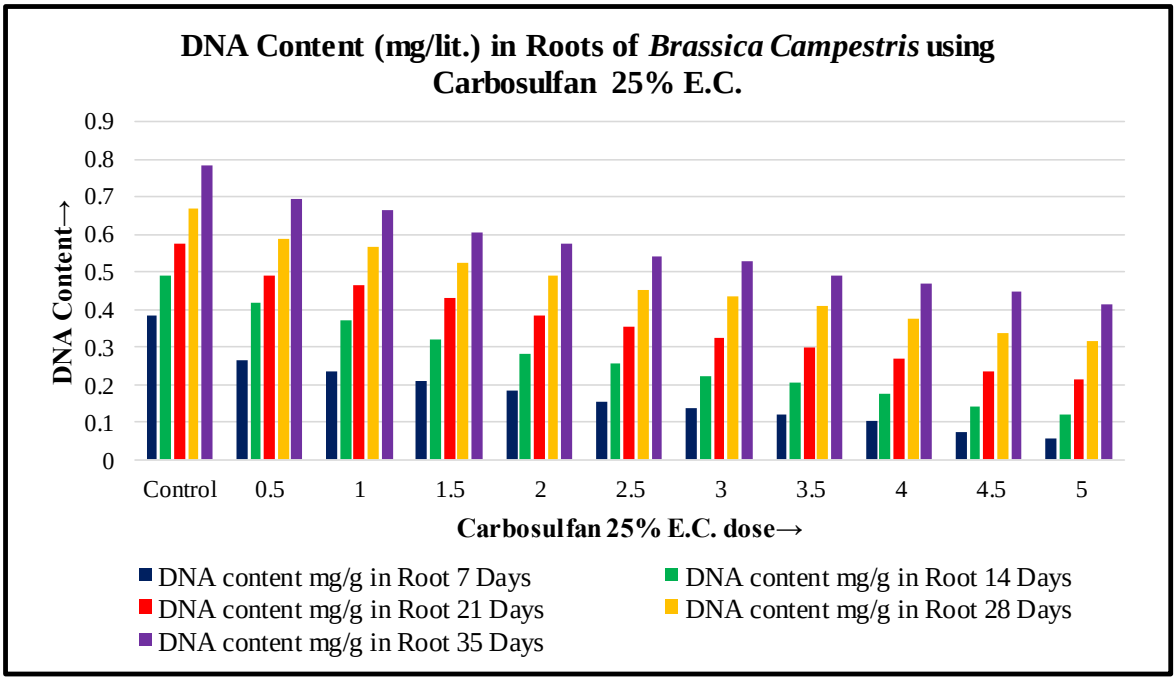

Figure 5: DNA Content (mg/lit.) in Roots of *Brassica Campestris* (Mustard) using Carbosulfan 25% E.C. (*Marshal*) as Insecticides.

Table 1: Illustration of pesticides and their sites of action.

Site of action	Pesticide (s)
Nervous system	Organophosphorus compounds, N-methyl or N.N. dimethyl carbamates, pyrethroids most organochlorine compounds nicotine and chlorodime form and related compounds.
Respiration	Arsenicals, copper compounds and those which can form capper chelates, oxathin carboxanidides, dinitroaniline herbicides (secondary site of action), dinitrophenols, pentachlor phenol, tri-substituted organ tins, hydroxyl benzonitrials, rofenone hydrogen cyanide, phosphine.
Photosynthesis	Herbicides straight chains, substituted and cyclic ureas, triazines, acylanilides phenylcarbanates, triazinones, phenolic herbicides, nitrodiphenylethers
Cell growth of development	Benzimidazoles and related compounds, dicarboxamides, N-phenyl carbamates, dinitroanilines, phosphoramidates, sulphonlylureas, maleic hydrazide, juveline hormones and analogues and precocenes
Bio synthesis	Acylalanines, hymexazole, cycloheximide, pyridazinones, aminotriazote thiocarbamates, imidazoles, triazoles, pyrimidines, dichlobenil, diflubenzuron, glyphosphate, ethirimol, and tricyclazole.
Non-specific	Mercury compounds compounds sodium fluoride, captan type fungicides, petroleum and tar oils, long chain guanidine fungicides, chloroacetanilides, chlorinated short chain aliphatic carboxylates, alkyl bis-dithiocar-bamates chlorthaloni.

CONCLUSION

Many different pesticides are continuously used in the agriculture for killing pests, insects, fungus, bacteria, rodents, weeds etc. under different brand name and these pesticides are sprayed in agricultural field's mass spray in a particular dose as recommended by the manufacturing companies. This dose(concentration) is selected after very careful scrutiny in such a way that it will not kill the crop plants, the economic target system but can kill the pest, insect, pathogen, fungus or bacteria. It is not true that these pesticides have no effect on the crop plants. These pesticides show several changes and damages in the DNA modification. In the present research it is observed that the bio pesticides have a little effect in the DNA modification as compared with synthetic pesticides.

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