International Journal of Trend in Scientific Research and Development (IJTSRD)
Volume 4 Issue 2, February 2020 Available Online: www.ijtsrd.com e-ISSN: 2456 – 6470

Impact of Some Biocides on Chlorophyll and Enzymatic Activities of Rice Plants

Rath B. B.[1], Adhikary S. P.[2]

[1]Department of Botany, RHSS, Ramgiri, Gajpati, Odisha
[2]Department of Botany, Aska Science College, Aska, Ganjam, Odisha

ABSTRACT

Agricultural productivity has increased substantially in the last half century due in part to the introduction and expanded use of agricultural chemicals. Pesticides continue to be a significant and growing component of modern rice technology. The relative importance of pesticides has increased despite the availability of alternatives to exclusive chemical pest control such as varietal resistance and integrated pest management (IPM). The evaluation of toxicological impact of pesticides in the tropical paddy has been evaluated through estimation of chlorophyll content and enzymatic activities (peroxidase and polyphenol oxidase). The seedlings are the crucial stage of the plant, so, the experimental study evaluated 14 days old seedling's chlorophyll content and enzymatic activities influenced by different biocides (neem oil, carbosulfan and Oxadiargyl). The experimental results found that all concentrations of (0.5, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0 ml/l) neem oil, carbosulfan and oxadiargyl decreased the percentage of chlorophyll content and enzymatic activities (peroxidase and polyphenol oxidase) of 14 days old seedlings. Among three biocides, neem oil was found less toxic towards the test cultivar followed by carbosulfan and oxadiargyl. The experimental studies concluded that neem oil is good for pest management. Farmer's should be well trained on proper application and implementation of integrated pest management strategies on rice field by which it restore sustainability of ecosystem and increased productivity.

KEYWORDS: *Biocide, peroxidase, polyphenol oxidase, carbosulfan and oxadiargyl*

How to cite this paper: Rath B. B. | Adhikary S. P. "Impact of Some Biocides on Chlorophyll and Enzymatic Activities of Rice Plants" Published in International Journal of Trend in Scientific Research and Development (ijtsrd), ISSN: 2456-6470, Volume-4 | Issue-2, February 2020, pp.143-148, URL: www.ijtsrd.com/papers/ijtsrd29923.pdf

IJTSRD29923

INRODUCTION

Agricultural productivity has increased substantially in the last half century due in part to the introduction and expanded use of agricultural chemicals. More recently, however, some agricultural practices including those associated with pesticide and fertilizer use have been viewed as having a major impact on the ecosystem and as being sources of environmental pollution and human health problems. Concern with the potential linkages among human health, environmental quality, and agricultural productivity reflects the growing demand by the general public and the international donor community for researchers to recognize the social benefits and social costs of agricultural technology and to address the long-run sustainability of existing agricultural practices. Pesticides continue to be a significant and growing component of modern rice technology. The relative importance of pesticides has increased despite the availability of alternatives to exclusive chemical pest control such as varietal resistance and integrated pest management (IPM). While Asia's elite are becoming increasingly concerned about the adverse long-term effects of pesticides on the environment and human health, little scientific research has been done to address this issue. Many pesticides commonly sold in Asia, extremely hazardous category I and II chemicals, are either banned or severely restricted for use in the developed world even when used with high levels of protection. In Asia, these chemicals are used with minimal protection, and the opportunities for increasing farmer safety are small. Rice is an important crop in southern part of Odisha. About 70 % people depend on cultivation of rice for their livelihood. To maximize rice yields, sustainable management of the crop is needed to control the main pests that limit rice growth and development. It is necessary to use biocides and pesticides. Neem oil act as biocides due to its active ingredient azadirachtin substances with pesticidal properties are found in all parts of the neem tree. Azadirachtin consists of more than 25 different but closely related compounds act as pesticides which are currently available. Azadirachtin can act as a feeding deterrent against a number of insect pests including beetles (Rath and Adhikary, 2018). Adults are not killed by the growth regulating properties of azadirachtin but mating and sexual communication may be disrupted which results in reduced fecundity (Schmutter, 1990). Neem oil works in a number of different ways. The oil forms a coating on the insect's body, blocking the breathing openings and suffocating the insect. It also has a repellent effect on certain insects and mites. Neem oil prevents the germination and penetration of some fungal spores.

Carbosulfan shows a good agronomic efficiency, this insecticide has environment restrictions due to the risks of contamination. One problem frequently associated to the carbofuran is the death of forest animais if they contaminated with it. Dietrich *et al.,* (1995) found similar problems for the carbofuran granules applied in field crops in Swiss. According to Crepeau and Kuivila (2000), the continuous presence of carbofuran in water channels and rivers might cause severe, sub-lethal or chronic damages to

aquatic organisms. Gupta (1994) observed that carbofuran is toxic to micro crustaceous and vertebrates of a same food chain. Norberg-King *et al.,* (1991) affirm that carbofuran is a toxic insecticide to the aquatic wildlife. The use of carbofuran in agriculture is prohibited in some countries (Anonymous, 1996a; Anonymous, 1996b; James, 1995) due to its eco-toxicity. The carbofuran persistence in the paddy water and in soil solution has been studied in tablelands and channels, because carbofuran is degraded mainly by hydrolysis (Seiber *et al.,* 1978; Tejada and Magallona, 1985 and Trevizan *et al.,* 2002). Tejada and Magallona (1985) demonstrated that carbosulfan was not detected in samples of paddy water and soil solution collected few hours after its application. Hydrolysis is the main path of carbosulfan to carbofuran degradation, its first metabolite, but in some cases, th is latter might present longer persistence in a low pH environment (Ramanand *et al.,* 1991). Other environmental factors, such as temperature variation, might directly influence the carbosulfan degradation process (Sahoo *et al.,* 1990).

Oxadiargyl acts as protoporphyrinogen IX oxidase inhibitor. It is pre-emergence and early post-emergence herbicide active on broad-leaved weeds (Amaranthus, Bidens, Chenopodium, Malva, Monochoria, Polygonum, Portulaca, Potamogeton, Raphanus, Solanum, Sonchus, Rotala), grasses (Echinochloa, Leptochloa, Brachiaria, Cenchrus, Digitaria, Eleusine, Panicum and wild rice) and annual sedges, in rice, upland crops (sunflower, potato, vegetables and sugar cane) and perennial crops (fruit trees and citrus). Soil/Environment DT50 (lab., aerobic) 18-72 d (20-30 °C), forming two major metabolites (one of which is herbicidal) which are, in turn, steadily degraded, resulting in mineralisation to CO2 and a soil-bound residue. Oxadiargyl dissipates rapidly from water into the sediment phase and is readily degraded under anaerobic conditions. Strongly adsorbed to soil (Koc 1000-3000); oxadiargyl and its two major soil metabolites show low mobility in 4 soil types and are unlikely to leach. Field results were consistent: DT50 9-25 d, mean DT90 90 d; for oxadiargyl and its two major metabolites, DT50 was 9-31 d, DT90 65-234 d; >95% of oxadiargyl residues remained in the top 10 cm of soil, and no residues were found below 30 cm. Oxadiargyl is a potential herbicide in crops such as lettuce and capsicums (Dickmann *et al.,* 1997).

Oxidative state of the host plants has been associated with HPR to insects (He *et al.,* 2010 and Zhao *et al.,* 2009) which results in production of ROS that are subsequently eliminated by antioxidative enzymes. POD constitutes one such group of enzymes, which scavenges the ROS besides having other defensive roles. PODs are an important component of the immediate response of plants to insect damage (Usha Rani and Jyothsna, 2010, War *et al.,* 2011 and Gulsen *et al.,* 2010). PODs are monomeric hemoproteins distributed as soluble, membrane-bound, and cell wall-bound within the cells, and are widely spread in plants and include several isozymes whose expression depends on tissue, developmental stage, and environmental stimuli (He *et al.,* 2010 and Gulsen *et al.,* 2010). A number of process are regulated by PODs that have direct or indirect role in plant defense, including lignification, suberization, somatic embryogenesis, auxin metabolism, and wound healing (He *et al.,* 2010, Heng-Moss *et al.,* 2004 and Sethi *et al.,* 2009). Role of PODs in plant resistance to insect pests has been studied in various plant systems (War *et al.,* 2011 and Gulsen *et al.,* 2010). Production of phenoxy and other oxidative radicals by the PODs in association with phenols directly deters the feeding by insects and/ or produces toxins that reduce the plant digestibility, which in turn leads to nutrient deficiency in insects with drastic effects on their growth and development (Chen *et al.,* 2009 and Zhang *et al.,* 2008). In addition, PODs have been reported to have direct toxicity in guts of herbivores (Zhu-Salzman *et al.,* 2008). PODs have been purified and characterized from many plants where they were induced in response to insect attack (He *et al.,* 2010, Gulsen *et al.,* 2010 and Stout *et al.,* 2009).

The PPOs are important enzymes in plants that regulate feeding, growth, and development of insect pests, and play a leading role in plant defense against the biotic and abiotic stresses (He *et al.,* 2010 and Bhonwong *et al.,* 2009). PPOs can function in following ways: a) PPO-generated quinones could alkylate essential amino acids, decreasing plant nutritional quality, (b) quinones may produce oxidative stress in the gut lumen through redox cycling, and (c) quinones and ROS produced by phenolic oxidation, could be absorbed and have toxic effects on herbivores. The PPOs are metallo-enzymes that catalyze the oxidation of monophenols and *o*-diphenols to quinones, which are highly reactive intermediate compounds that readily polymerize, and react with nucleophilic side chain of amino acids and crosslink proteins, thereby reducing the availability of such proteins, and affect the nutritional quality of the food (Bhonwong *et al.,* 2009 and Zhang *et al.,* 2008). Under acidic conditions, quinones form semiquinone radicals that in turn give rise to ROS, while under basic conditions; quinines react with cellular nucleophiles Bhonwong *et al.,* 2009). Quinines are more toxic to plant herbivores than the original phenols (Bhonwong *et al.,* 2009). In addition to their role in digestibility and palatability of plant tissues, melanin formation by PPOs increases the cell wall resistance to insects and pathogens (Zhao *et al.,* 2009). Induction of PPO activity under abiotic and biotic stresses and by treatment with compounds related to the octadecanoid pathway makes it an important tool in plant resistance against different stresses (He *et al.,* 2010 and Bhonwong *et al.,* 2009). The PPO genes are differentially induced by signaling molecules and injury due to wounding, and pathogen, or insect infestation (Bhonwong *et al.,* 2009 and Zhao *et al.,* 2009). Correlation between induction of PPO activity and insect fitness has been reported in many plants including tomato and lettuce (Bhonwong *et al.,* 2009 and Sethi *et al.,* 2009). Although PPOs accumulate in leaves, roots, stems and flowers of the plants, young tissues with greater vulnerability to insect attack exhibit greater induction. One of the important aspects of HPR against insects is the disruption of insect's nutrition. The enzymes that impair the nutrient uptake by insects through formation of electrophiles includes peroxidases (PODs), polyphenol oxidases (PPOs), ascorbate peroxidases, and other peroxidases by oxidizing mono- or dihydroxyphenols, that lead to the formation of reactive o-quinones, which in turn polymerize or form covalent adducts with the nucleophilic groups of proteins due to their electrophilic nature (e.g., -SH or e-NH_2 of Lys) (Bhonwong *et al.,* 2009, Gulsen *et al.,* 2010 and Gill *et al.,* 2010).

Investigation of these pesticides is of the utmost importance for a better understanding of their dynamics in the environment. The literature available the effect of neem oil, carbosulfan and Oxadiargyl on chlorophyll content and enzymatic activities (peroxidase and polyphenol oxidase) of

rice crop is very scanty hence the aim of this study was undertaken to evaluate the impacts of biocides on chlorophyll contents and enzymatic activities of 14 day's old seedlings.

METHODS AND MATERIALS

Pure line seeds of above mentioned cultivars of rice were procured from Regional Agricultural Research Station, Odisha University of Agriculture and Technology (O.U.A.T), Brahmapur. Bio-pesticides i.e. neem oil, Carbosulfar 25%E.C. (Marshal) and Topstar (Oxadiargy1 80% w.p.) were used in this present piece of investigation.

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 the preliminary concentrations 0.5, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0 ml/l or gm/kg of all the bio-pesticides i.e. Neem oil, carbosulfan 25% E.C. (Marshal) and Topstar (oxadiargy1 80% W.P.) were prepared.

Experimental schedule:-

Seed germination and seedling growth experiments of test plant were conducted in the laboratory condition and study the vegetative growth parameters and chlorophyll contents.

METHODOLOGY

Surface Sterilization

All the glass wares including seeds have been properly sterilized. Prior to germination, all the seeds were surface sterilized by treating with 5% concentration of sodium hypochloride solution for 12 minutes and then thoroughly washing it with distilled water for 3 to 4 times to remove completely the sodium hypo-chloride solution. Glass materials, absorbent cotton, filter papers and vermiculites were properly sterilized and used for germination of test plant rice seeds.

GERMINATION BED

Clear and sterilized petridishes were divided into triplicate for each concentration of test chemical and control for experimental purpose. Ten ml of different concentrations of neem oil, carbosulfan 25% E.C. (Marshal) and Topstar (oxadiargyl 80% W.P.) i.e. 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0 ml/l were applied to different petridishes (10 cm in size)@ 50 test plant seeds per dish lined with blotting paper. Control set of petridishes were prepared by taking same amount of water. Watering was allowed in all petridishes at an interval of 12 hr with 10 ml of water.

GERMINATION CHAMBER

Specially designed germination chamber was meant to study the vigoursity of germination of test seeds under different pesticides i.e. treatment with scheduled concentration of test pesticides. Light is an inhibitory factor for the process of the germination, hence precaution were taken to make the germination chamber light proof throughout the course of investigation.

The duration of germination experiment limited to 72 hr. for first observation of germination was recorded after 12 hr. treatment and subsequently at an interval 12 hr. duration the germination strategy of scheduled i.e. 72 hr. Emergence of radical through the coleoptiles extending the length of 0.1 cm was considered as the standard of germination.

SEEDLING CHAMBER

A special type of chamber has been designed for the growth of germination seed designated as seedling chamber. The chamber is partitioned by wooden plates to keep the petridishes. Besides maintaining of thermo-stability, Photo-stability has been allowed with 2000 ± 200 lux of fluorescent light throughout the course of seedling growth. Temperature constancy maintained at 25^0 C during the course of seedling growth investigations.

Petridishes with germination seeds were transferred to the seedling chamber. Growth of radical and plumule of the germinating seeds were allowed for seven days. Throughout the period of seedling growth aeration under the laboratory conditions was kept intact. Random collection of days old seedlings was made from the seedling chamber for the different vegetative parameters and chlorophyll content.

The collected seedlings were washed properly with water and separated root and shoot portion. The separated shoot and root portions were soaked with blotting paper and measured length and fresh weight. The shoot and root were dried in a woven with 60 ^{o} centigrade and measured dry weight.

Extraction and estimation of chlorophyll:

In order to measure the chlorophyll content in leaves, ten seedlings of 14 days old were taken randomly from each petridish of both control and treated a sets, leaves were separated from seedlings, washed thoroughly, dried the surface water by soaking on a blotting paper, cut in to small pieces and weighted for 50 mg. then this weighted samples were homogenized with 1-2 ml. of 80% ethanol(v/v) with mortar and pestle and the homogenate were centrifuged at 3000 xg for 20 minutes at 28± 10c. The pellets were again homogenized with 80% ethanol and centrifugation was repeated till colorless pellets obtained which were further used for other chemical analysis. All the supernatants were pooled together and a total volume of 10ml. was prepared with 80% ethanol. The optical density (O.D) of the extract was measured with help of Elico digital spectrophotometer (model CL-27) at 663 and 645 nm, taking 80% ethanol as blank. Estimations were calculated following the formulae suggested by Arnon (1949).

$$\text{Total Chl.} = \frac{20.2\times(O.D.at645nm)+8.02\times(O.D.at663nm)}{1000\times W}\times V$$

mg/g (fresh weight)

Where V= Total volume of extract in C.C
W= Weight of samples in gram
And O.D. = Optical density of the extract

Extraction and Assay of Peroxidase

In order to measure the peroxidase content in leaves, ten seedlings of 14 days old were taken randomly from each petridish of both control and treated a sets, leaves were separated from seedlings, washed thoroughly, dried the surface water by soaking on a blotting paper, cut in to small pieces and weighted for 50 mg. then this weighted samples were homogenized with 4ml. 0.1m phosphate buffer (pH=7.0) with a pre-chilled mortar and paste and homogenate was centrifuged at 15,500 × g for 30 minutes at 4^{0C} and the supernatant was used as the source peroxidase.

Peroxidase activity was assayed following the modified method of Kar and Mishra (1976). Assay mixture for peroxide contained 2ml of 0.1m phosphate buffer (pH=7.0), 1ml. 0.01 M pyrogallol, 1 ml 0.0005M hydrogen peroxide and 1ml well diluted enzyme extract. The reaction was stopped by adding 1ml. 2.0 ml sulphuric acid after 5 minutes incubation at 25 at 25^0C and the amount of purpurogallin

was estimated by the absorbance at 420 nm. Peroxidase activity was expressed in absorbency units.

Extraction and Assay of Polyphenyl Oxidase

Polyphenyl oxidase extraction was similar to that of Peroxidase. Polyphenyl oxidase activity was assayed by a modified method of Kar and Mishra (1976). Assay mixture contained 2ml. 0.1m phosphate buffer (pH=7.0), 1ml 0.01M Pyrogallol and 1ml diluted enzyme extract. The reaction was stopped by adding 1ml 2.0m sulphuric acid after 5 minutes incubation at 25^0C and the amount of purpirogallin formed was estimate by measuring the absorbance at 420 nm. Polyphenol oxidase activity was expressed in absorbency unit.

RESULT

CHOROPHYLL CONTENTS IN RICE SEEDLINGS

Effect of neem oil

The different concentrations of neem oil considerably checked the chlorophyll contents of rice seedling. The seedling of control set exhibited maximum chlorophyll - a contents 3.04 ± 0.06 mg at 14 days. However the chlorophyll - a contents of rice seedling exhibited 2.68 ± 0.06mg with treatment of 0.5 ml./lit. concentration of neem oil at 14 days. The chlorophyll - a contents 0.89 ± 0.02 mg in 14 days old rice seedlings were found in treatment with 5 ml./lit. concentration of neem oil. All other concentration of neem oil shows intermediate values.

The seedling of control set exhibited maximum chlorophyll - b contents 0.89 ± 0.02 mg at 14 days. However the chlorophyll - b contents of rice seedling exhibited 0.83 ± 0.02 mg with treatment of 0.5 ml./lit. concentration of neem oil at 14 days. The chlorophyll-b contents 0.37 ± 0.01 mg in 14 days old rice seedlings were found in treatment with 5 ml./lit. concentration of neem oil. All other concentration of neem oil shows intermediate values.

The seedling of control set exhibited maximum total chlorophyll contents 3.93 ± 0.09 mg at 14 days. However the total chlorophyll contents of rice seedling exhibited 3.51 ± 0.08 mg with treatment of 0.5 ml/lit. concentration of neem oil at 14 days. Total chlorophyll contents 1.26 ± 0.03 mg in 14 days old rice seedlings were found in treatment with 5 ml/lit. concentration of neem oil. All other concentration of neem oil shows intermediate values (Table - 1).

The correlation coefficient values were calculating between the concentration of neem oil and chl.-a, chl.-b and total chl. trough out the observation. Significance values $p \leq 0.001$ were calculated taking degree of freedom 20 for the different concentration of biocide. The values were r = -0.978, r= - 0.967 and r = - 0.976 for chl.-a., chl. b and total chl. respectively at $p \leq 0.001$ level of significance (Table - 1).

Effect of Carbosulfan 25% E.C.(Marshal)

All the concentration of Carbosulfan 25% E.C. (Marshal) considerably checked the chlorophyll contents of rice seedlings. The seedling of control set exhibited maximum chlorophyll - a contents 3.04 ± 0.06 mg at 14 days. However the chlorophyll - a contents of rice seedling exhibited 2.46 ± 0.05 mg with treatment of 0.5 ml/lit. concentration of Carbosulfan 25% E.C. (Marshal) at 14 days. Chlorophyll - a contents 0.76 ± 0.02 mg in 14 days old rice seedlings were found in treatment with 5 ml./lit. concentration of Carbosulfan 25% E.C. (Marshal). All other concentration of Carbosulfan 25% E.C. (Marshal) shows intermediate values.

The seedling of control set exhibited maximum chlorophyll-b contents 0.89 ± 0.02 mg at 14 days. However the chlorophyll - b contents of rice seedling exhibited 0.68 ± 0.02mg with treatment of 0.5 ml/lit. concentration of Carbosulfan 25% E.C. (Marshal) at 14 days. The chlorophyll-b contents 0.27 ± 0.01 mg in 14 days old rice seedlings were found in treatment with 5 ml/lit. concentration of Carbosulfan 25% E.C. (Marshal). All other concentration of Carbosulfan 25% E.C. (Marshal) shows intermediate values.

The seedling of control set exhibited maximum total chlorophyll contents 3.93 ± 0.09 mg at 14 days. However the total chlorophyll contents of rice seedling exhibited 3.14 ± 0.07mg with treatment of 0.5 ml/lit. concentration of Carbosulfan 25% E.C. (Marshal) at 14 days. Total chlorophyll contents 1.03 ± 0.02 mg in 14 days old rice seedlings were found in treatment with 5 ml/lit. concentration of Carbosulfan 25% E.C. All other concentration of Carbosulfan 25% E.C. (Marshal) shows intermediate values (Marshal) (Table - 2).

The correlation coefficient values were calculating between the concentration of Carbosulfan 25% E.C. (Marshal) and chl.-a, chl.-b and total chl. trough out the observation. Significance values $p \leq 0.001$ were calculated taking degree of freedom 20 for the different concentration of biocide. The values were r = -0.953, r= - 0.974 and r = - 0.958 for chl.-a., chl. b and total chl. respectively at $p \leq 0.001$ level of significance (Table - 2).

Effect of Topstar (Oxadiargyl 80% W.P.)

The different concentrations of Topstar (Oxadiargyl 80% W.P.) considerably checked the chlorophyll contents of rice seedlings. The seedling of control set exhibited maximum chlorophyll - a contents 3.04 ± 0.06 mg at 14 days. However the chlorophyll –a contents of rice seedling exhibited 2.39 ± 0.05 mg with treatment of 0.5 ml/lit. concentration of Topstar (Oxadiargyl 80% W.P.) at 14 days. The chlorophyll-a contents 0.68 ± 0.02 mg in 14 days old rice seedlings were found in treatment with 5 mg./lit. concentration of Topstar (Oxadiargyl 80% W.P.). All other concentration of Topstar (Oxadiargyl 80% W.P.) shows intermediate values.

The seedling of control set exhibited maximum chlorophyll - b contents 0.89 ± 0.02 mg at 14 days. However the chlorophyll –b contents of rice seedling exhibited 0.66 ± 0.02mg with treatment of 0.5 ml/lit. concentration of Topstar (Oxadiargyl 80% W.P.) at 14 days. Chlorophyll - b contents 0.23 ± 0.01 mg in 14 days old rice seedlings were found in treatment with 5 ml/lit. concentration of Topstar (Oxadiargyl 80% W.P.). All other concentration of Topstar (Oxadiargyl 80% W.P.) shows intermediate values.

The seedling of control set exhibited maximum total chlorophyll contents 3.93 ± 0.09 mg at 14 days. However the total chlorophyll contents of rice seedling exhibited 3.05 ± 0.07mg with treatment of 0.5 mg/lit. concentration of Topstar (Oxadiargyl 80% W.P.) at 14 days. Total chlorophyll contents 0.91 ± 0.02 mg in 14 days old rice seedlings were found in treatment with 5 ml./lit. concentration of Topstar (Oxadiargyl 80% W.P.). All other concentration of Topstar (Oxadiargyl 80% W.P.) shows intermediate values (Table - 3).

The correlation coefficient values were calculating between the concentration of Topstar (Oxadiargyl 80% W.P.) and chl.-a, chl.-b and total chl. trough out the observation. Significance values $p \leq 0.001$ were calculated taking degree of freedom 20 for the different concentration of biocide. The

values were r = -0.961, r= - 0.952 and r = - 0.969 for chl.-a., chl. b and total chl. respectively at p≤0.001 level of significance (Table - 3).

CHANGES IN ENZYME ACTIVITIES

A number of enzymes are induced or suppressed in response to biocides. In the present study an attempt has been made to assess the changes in peroxidase and polyphenol oxidase.

Peroxidase content

Effect of neem oil

The different concentrations of neem oil considerably checked the peroxidase contents of rice seedling. The 14 days old seedling of control set exhibited maximum peroxidase contents in leaves was 1.19 ± 0.32 mg. However the peroxidase contents of rice seedling exhibited 1.06 ± 0.27 mg with treatment of 0.5 ml./lit. concentration of neem oil at 14 days. The peroxidase contents 0.62 ± 0.06 mg in 14 days old rice seedlings were found in treatment with 5 ml/lit. concentration of neem oil. All other concentration of neem oil shows intermediate values.

The seedling of control set exhibited maximum peroxidase contents in roots was 1.26 ± 0.41 mg at 14 days. However the peroxidase contents of rice seedling exhibited 1.19 ± 0.39 mg with treatment of 0.5 ml/lit. concentration of neem oil at 14 days. The peroxidase contents 0.76 ± 0.09 mg in 14 days old rice seedlings were found in treatment with 5 ml/lit. concentration of neem oil. All other concentration of neem oil shows intermediate values (Table - 1).

The correlation coefficient values were calculating between the concentration of neem oil and peroxidase of leave and root trough out the observation. Significance values p≤0.001 were calculated taking degree of freedom 20 for the different concentration of biocide. The values were r = -0.961 and r= - 0.968 for peroxidase activities of leave and root of 14 days old seedling of test rice plant respectively at p≤0.001 level of significance (Table - 1).

Effect of Carbosulfan 25% E.C. (Marshal)

The different concentrations of Carbosulfan 25% E.C. (Marshal) considerably checked the peroxidase contents of rice seedling. The 14 days old seedling of control set exhibited maximum peroxidase contents in leaves was 1.19 ± 0.32 mg. However the peroxidase contents of rice seedling exhibited 0.92 ± 0.25 mg with treatment of 0.5 ml/lit. concentration of Carbosulfan 25% E.C. (Marshal) at 14 days. The peroxidase contents 0.45 ± 0.06 mg in 14 days old rice seedlings were found in treatment with 5 ml/lit. concentration of Carbosulfan 25% E.C. (Marshal). All other concentration of Carbosulfan 25% E.C. (Marshal) shows intermediate values.

The seedling of control set exhibited maximum peroxidase contents in roots was 1.26 ± 0.41 mg at 14 days. However the peroxidase contents of rice seedling exhibited 1.07 ± 0.36 mg with treatment of 0.5 ml/lit. concentration of Carbosulfan 25% E.C. (Marshal) at 14 days. The peroxidase contents 0.57 ± 0.13 mg in 14 days old rice seedlings were found in treatment with 5 ml./lit. concentration of Carbosulfan 25% E.C. (Marshal). All other concentration of Carbosulfan 25% E.C. (Marshal) shows intermediate values (Table - 2).

The correlation coefficient values were calculating between the concentration of Carbosulfan 25% E.C. (Marshal) and peroxidase of leave and root trough out the observation. Significance values p≤0.001 were calculated taking degree of freedom 20 for the different concentration of biocide. The values were r = -0.972 and r= - 0.968 for peroxidase activities of leave and root of 14 days old seedling of test rice plant respectively at p≤0.001 level of significance (Table - 2).

Effect of Topstar (Oxadiargyl 80% W.P.)

The different concentrations of Topstar (Oxadiargyl 80% W.P.) considerably checked the peroxidase contents of rice seedling. The 14 days old seedling of control set exhibited maximum peroxidase contents in leaves was 1.19 ± 0.32 mg. However the peroxidase contents of rice seedling exhibited 0.88 ± 0.23 mg with treatment of 0.5 ml./lit. concentration of Topstar (Oxadiargyl 80% W.P.) at 14 days. The peroxidase contents 0.41 ± 0.07 mg in 14 days old rice seedlings were found in treatment with 5 ml/lit. concentration of Topstar (Oxadiargyl 80% W.P.). All other concentration of Topstar (Oxadiargyl 80% W.P.) shows intermediate values.

The seedling of control set exhibited maximum peroxidase contents in roots was 1.26 ± 0.41 mg at 14 days. However the peroxidase contents of rice seedling exhibited 1.04 ± 0.34 mg with treatment of 0.5 ml/lit. concentration of Topstar (Oxadiargyl 80% W.P.) at 14 days. The peroxidase contents 0.53 ± 0.13 mg in 14 days old rice seedlings were found in treatment with 5 ml./lit. concentration of Topstar (Oxadiargyl 80% W.P.). All other concentration of Topstar (Oxadiargyl 80% W.P.) shows intermediate values (Table - 3).

The correlation coefficient values were calculating between the concentration of Topstar (Oxadiargyl 80% W.P.) and peroxidase of leave and root trough out the observation. Significance values p≤0.001 were calculated taking degree of freedom 20 for the different concentration of biocide. The values were r = -0.967 and r= - 0.959 for peroxidase activities of leave and root of 14 days old seedling of test rice plant respectively at p≤0.001 level of significance (Table - 3).

Polyphenol Oxidase

Effect of the Neem oil

The different concentrations of neem oil considerably checked the polyphenol oxidase contents of rice seedling. The 14 days old seedling of control set exhibited maximum polyphenol oxidase contents in leaves was 1.65 ± 0.56 mg. However the polyphenol oxidase contents of rice seedling exhibited 1.57 ± 0.53 mg with treatment of 0.5 ml/lit. concentration of neem oil at 14 days. The polyphenol oxidase contents 1.08 ± 0.18 mg in 14 days old rice seedlings were found in treatment with 5 ml./lit. concentration of neem oil. All other concentration of neem oil shows intermediate values.

The seedling of control set exhibited maximum polyphenol oxidase contents in roots was 1.43 ± 0.48 mg at 14 days. However the polyphenol oxidase contents of rice seedling exhibited 1.32 ± 0.46 mg with treatment of 0.5 ml/lit. concentration of neem oil at 14 days. The polyphenol oxidase contents 0.81 ± 0.14 mg in 14 days old rice seedlings were found in treatment with 5 ml./lit. concentration of neem oil. All other concentration of neem oil shows intermediate values (Table - 1).

The correlation coefficient values were calculating between the concentration of neem oil and polyphenol oxidase of leave and root trough out the observation. Significance values p≤0.001 were calculated taking degree of freedom 20 for the different concentration of biocide. The values were r = -0.972 and r= - 0.976 for polyphenol oxidase activities of

leave and root of 14 days old seedling of test rice plant respectively at p≤0.001 level of significance (Table - 1).

Effect of Carbosulfan 25% E.C.(Marshal)

The different concentrations of Carbosulfan 25% E.C. (Marshal) considerably checked the polyphenol oxidase contents of rice seedling. The 14 days old seedling of control set exhibited maximum polyphenol oxidase contents in leaves was 1.65 ± 0.56 mg. However the polyphenol oxidase contents of rice seedling exhibited 1.42 ± 0.45 mg with treatment of 0.5 ml/lit. concentration of Carbosulfan 25% E.C. (Marshal) at 14 days. The polyphenol oxidase contents 0.86 ± 0.12 mg in 14 days old rice seedlings were found in treatment with 5 ml./lit. concentration of Carbosulfan 25% E.C. (Marshal). All other concentration of Carbosulfan 25% E.C. (Marshal) shows intermediate values.

The seedling of control set exhibited maximum polyphenol oxidase contents in roots was 1.43 ± 0.41 mg at 14 days. However the polyphenol oxidase contents of rice seedling exhibited 1.17 ± 0.29 mg with treatment of 0.5 ml/lit. concentration of Carbosulfan 25% E.C. (Marshal) at 14 days. The polyphenol oxidase contents 0.63 ± 0.08 mg in 14 days old rice seedlings were found in treatment with 5 ml/lit. concentration of Carbosulfan 25% E.C. (Marshal). All other concentration of Carbosulfan 25% E.C. (Marshal) shows intermediate values (Table - 2).

The correlation coefficient values were calculating between the concentration of Carbosulfan 25% E.C. (Marshal) and polyphenol oxidase of leave and root trough out the observation. Significance values p≤0.001 were calculated taking degree of freedom 20 for the different concentration of biocide. The values were r = -0.964 and r= - 0.961 for polyphenol oxidase activities of leave and root of 14 days old seedling of test rice plant respectively at p≤0.001 level of significance (Table - 2).

Effect of Topstar (Oxadiargyl 80% W.P.)

The different concentrations of Topstar (Oxadiargyl 80% W.P.) considerably checked the polyphenol oxidase contents of rice seedling. The 14 days old seedling of control set exhibited maximum polyphenol oxidase contents in leaves was 1.65 ± 0.36 mg. However the polyphenol oxidase contents of rice seedling exhibited 1.38 ± 0.43 mg with treatment of 0.5 ml/lit. concentration of Topstar (Oxadiargyl 80% W.P.) at 14 days. The polyphenol oxidase contents 0.79 ± 0.14 mg in 14 days old rice seedlings were found in treatment with 5 ml./lit. concentration of Topstar (Oxadiargyl 80% W.P.). All other concentration of Topstar (Oxadiargyl 80% W.P.) shows intermediate values.

The seedling of control set exhibited maximum polyphenol oxidase contents in roots was 1.43 ± 0.48 mg at 14 days. However the polyphenol oxidase contents of rice seedling exhibited 1.14 ± 0.27 mg with treatment of 0.5 ml/lit. concentration of Topstar (Oxadiargyl 80% W.P.) at 14 days. The polyphenol oxidase contents 0.57 ± 0.09 mg in 14 days old rice seedlings were found in treatment with 5 ml/lit. concentration of Topstar (Oxadiargyl 80% W.P.). All other concentration of Topstar (Oxadiargyl 80% W.P.) shows intermediate values (Table - 3).

The correlation coefficient values were calculating between the concentration of Topstar (Oxadiargyl 80% W.P.) and polyphenol oxidase of leave and root trough out the observation. Significance values p≤0.001 were calculated taking degree of freedom 20 for the different concentration of biocide. The values were r = - 0.958 and r= - 0.956 for polyphenol oxidase activities of leave and root of 14 days old seedling of test rice plant respectively at p≤0.001 level of significance (Table - 3).

DISCUSSION

Seedling establishment is considered as one of the crucial stage, after germination, where various morphogenesis activities are established along with changes in physiological and biological processes. During this period the seedlings try to become self established by the development of root and shoot systems. The growth and development of shoot and root are controlled by various metabolic activities carried out in leaves, hence changes in total chlorophyll contents and enzymatic activities in leaves were considered important parameters of seedling establishment.

All the concentration of neem-oil, marshal (carboslfan 25% E.C) and Top star (Oxadiargyl 80% W.P.) considerably reduced the seedling growth due to interaction of phytotoxins with photophosphorylation pathway and inhibition of light activated mg $^{+2}$ ATPase activities (Moreland,1980). Though the carbohydrate synthesis mainly depends on the efficacy of photosynthetic processes, the accumulation of carbohydrate in plants is directly proportional to the amount of chlorophyll present in the leaves.

The decrease in various parameters of seedling growth in the test crop is might be due to controlling of enzyme activities responsible for synthesis of plant growth regulators like GA_3 and IAA that control shoot and root growth. Butler (1973) and Mishra and Adhikary (1986) reported that plant growth regulators, at lower concentrations induce synthesis of macromolecules resulting better seedling growth whereas higher concentration act as inhibitors.

Abdl Baki and Anderson (1972) suggested that many physiological and bio-chemical processes such as respiration fatty acid synthesis, protein synthesis, oxido-reduction reaction and several enzyme activities are associated with seedling vigor.

Vegetative growth of the plant body also depends on different environmental factors such as change in temperature, humidity, photoperiod and application of different agrochemicals (Bremner, 1969b and Cannel, 1979). It is quite common the most visible tillers die without producing grains and there may be many buds which never reach the visible stage (Davidson, 1964), Vergara et al (1966) opinion that plant height is prime importance while working on the crop plants. Cleland (1964) suggested that gibberellins play important role on the growth of plants. Jones and Phillips (1966) suggested that in many plants highest level of gibberellins occur in apices of stem and root, young leaves, embryos and endosperm of developing seeds.

The application of different synthetic pesticides, herbicide and other biocides is a common and modern agricultural practice by the farmers for better yields, which more or less selectively interfere with growth of plant species. Further these chemical have a high toxicity on wide range of plants while others are very resistance to degradation in the environment and accumulate in the plants creating problems faster than they can solved. Moore (1965) suggested that all the biocides are not hazardous but most of them are toxic. A vast reports are available on effect of biocides several crop

plants such as soya bean (Hsia and Cao, 1978), ragi (Kumar and Khan, 1982 and Acharya, 1994). Further due to advancement of biotechnologies, pesticides of plant origin (biological pesticide or biopesticides active) are developed and recorded for use. Neem oil is one of such biological derived pesticide having pesticidal active indegredients "Limonoids" which include azadiractuin, nimbocinal, epinimbocinal, nimbin, solamin and meliontriol. There are limonoid compound contain complex of active ingredients which act on crop pests by its larvicidal activities, disrupts, the growth process of insects, repellant or antifeedant to insect and act afectively against many fungal diseases like smut, rust, powdery mildew etc because of the above features neem oil is approved by the integrated pest management (IPM). As insects and pathogens die by the application of biocides, there is no guarantee that crop plants affected. These days may be significant in first generation but certainly these are physiologically are genetically hazardous as the plants and animals are more or less similar at molecular or sub-molecular level. Based on the results obtained by the application of different concentration of neem oil, carbosulfan and oxadiargyl on 14 days old seedlings of test cultivar the discussion are described below.

Chlorophyll content of 14 days old seedling of rice were significantly reduced by all concentrations of neem oil, carbosulfan and oxadiargyl which might be due to inhibiting and/or checking of protein, nucleic acids and carbohydrate synthesis. Such type of results have been reported by Kohli *et al.,(*1988) on green gram, Padhy *et al.*,(1992) on sesame, Jayakumar *et al.,*(1990), Panda(1994) on ground nut by the eucalyptus globulus leaf leachate which contain allelochemics like phenolics , terpenoid and flavonoid which are more or less similar in structure and function of neem oil and other biocides. Further, it was reported that influence of biopesticide in relation to cell multiplication to auxin production in cells, influence of auxins on translocation of carbohydrates from source to sink are needed for better understanding on physiological and biochemical changes that occurs during seedling growth. Such type of related allelochemics present in neem oil might have suppressed various metabolic activities resulting decrease in seedling growth.

The decreased in height of plant suggests that the metabolites might have synthesized less and could not properly trans-located from source to sink. Murty *et al.,* (1973) suggested that leaf thickness or specific leaf weight has significance correlation with photosynthetic rate per unit area in rice. Yoshida and Ahn (1968) observed that carbohydrate content of grain is largely depends on the amount of photosynthates formed after flowering.

All the concentration of neem-oil, marshal (carboslfan 25% E.C) and Top star (Oxadiargyl 80% W.P.) considerably reduced the peroxidase and polyphenol oxidase in 14 days old seedling due to change the stoichiometry of enzymes structure by interaction of different biocides. Most of the biocides are polyphenolic compounds with its derivatives. Above critical concentration of neem-oil, marshal (carboslfan 25% E.C) and Top star (Oxadiargyl 80% W.P.) are interrupted the metabolic pathways of oxidation of phenolic compounds through its hydroxylation reactions to monophenols to *o*-diphenol and the oxidation of *o*-diphenol to *o*-quinone with different reactive oxygen species. Peroxidase is an enzyme which is responsible for scavenging of hydrogen peroxide. This enzyme has received maximum attention to study the toxicity of plants. PPO is a dicopper-containing enzyme. Several studies have reported the involvement of PPO in the oxidation of the polyphenols from plants. PPO activity can be monitored by oxygen consumption or spectrophotometrically using a variety of substrates such as pyrogallol, pryocatechol, 4-methylcatechol, 3, 4-dihydroxyphenylacetic acid, 4-tert-butylcatechol and chlorogenic acid. PPO shows high activity with diphenols. Two kinds of reactions generated by PPO are the hydroxylation of monophenols to *o*-diphenol and the oxidation of *o*-diphenol to *o*-quinone.

The related activity of PPO and POD is due to the generation of hydrogen peroxide during the oxidation of phenolic compounds in PPO-catalyzed reactions (Francisco and Juan Carlous, 2001, Khann and Robinson, 1994 and Sugai and Tadini, 2006). The catalytic reactions of the oxidative enzymes, POD and PPO, have been studied in fruits and vegetables for many years. Both enzymes have some common substrates, but each also has its specific substrates (Banci, 1997, Onsa *et al.,* 2000 & 2004, Furumo, 2008). Their common substrates are diphenolic products. A binding of ligand and protein may result in the activation or the inhibition of the enzyme (Furumo, 2008 and Altschul *et al.,* 1997). PPO has a slightly smaller binding pocket than POD. Therefore, the number of binding amino acid residues was observed. Interaction energy of benzoic compounds showed high affinity to grape ascorbate peroxidase and polyphenol oxidase. Peter and John, (1996) reported that 2, 3-dihydroxybenzoic acid showed no inhibitory effect whereas 2, 4-dihydroxybenzoic acid was a strong polyphenol oxidase inhibitor. The inhibitor 3, 4,5-trihydroxybenzoic acid has high affinity with both enzymes. The series of monohydroxybenzoic acids (*m*-, *o*-, *p*-hydroxybenzoic acid) have high affinities with grape polyphenol oxidase with lower negative interaction energy values than those with peroxidase. Other compounds, including 2,3-dihydroxybenzoic acid, 3,4-dihydroxybenzoic acid, *o*-hydroxybenzoic acid, and *m*-hydroxybenzoic acid, can be used as common inhibitors for both enzymes. The present experimental results suggest that different biocide viz. neem-oil, marshal (carboslfan 25% E.C) and Top star (Oxadiargyl 80% W.P.) might have inhibited peroxidase and polyphenol oxidase activities of 14 days rice seedling at their respective critical concentration is due to its polyphenolic derivatives and high affinity to above test chemicals studied. PODs are monomeric hemoproteins distributed as soluble, membrane-bound, and cell wall-bound within the cells, and are widely spread in plants and include several isozymes whose expression depends on tissue, developmental stage, and environmental stimuli (He *et al.,* 2010 and Gulsen *et al.,* 2010). A number of processes are regulated by PODs that have direct or indirect role in plant defense (He *et al.,* 2010). The PPOs are metallo-enzymes that catalyze the oxidation of monophenols and *o*-diphenols to quinones, which are highly reactive intermediate compounds that readily polymerize, and react with nucleophilic side chain of amino acids and crosslink proteins, thereby reducing the availability of such proteins, and affect the nutritional quality of the food (Bhonwong *et al.,* 2009 and Zhang *et al.,* 2008). Since no adequate reports are available on the effect of different biocide viz. neem-oil, marshal (carboslfan 25% E.C) and Top star (Oxadiargyl 80% W.P.) on any crop plants, no definite correlations and conclusions can be drawn for which in-depth research at molecular level is highly essential.

Conclusion

The chlorophyll content of leaves was significantly inhibited at different concentration of biocide viz. neem-oil, marshal (carboslfan 25% E.C) and Top star (Oxadiargyl 80% W.P.) and exhibited a positive correlation with chlorophyll content and different test chemical studied. The impact of neem-oil, marshal (carboslfan 25% E.C) and Top star (Oxadiargyl 80% W.P.) on peroxidase and polyphenol oxidase also exhibited same trend as it found in chlorophyll content. The experimental data noticed that among three test biocides neem oil was found less inhibitory effect on chlorophyll content, peroxidase and polyphenol oxidase followed by carbosulfan and Oxadiargyl. Hence, awareness should be created among the paddy farmer on successful implementation of integrated pest management strategies for more productivity of crop and eco-friendly agriculture.

REFERENCES

[1] Abdul Baki and Anderson, J. D. (1941). Physiological and biochemical deterioration of seeds. In: *Seed Biology,* 3: 283 – 315.

[2] Acharya, B. (1994). Studies on the effect of certain agrochemicals on seed germination, growth and yield of ragi (*Eleusine coracana Gaertn.*). Ph.D. Thesis, Berhampur University, Berhampur, Orissa, India.

[3] Altschul, S. F., Madden, T.L., Schaffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman, D.J., Gapped, BLAST and PSI-BLAST (1997). A new generation of protein database search programs. *Nucl. Acids Res.,* 25: 3389–3402.

[4] Anonymous, (1996a). Canada to ban two forms of carbofuran. *Eur. Chem. News*., 65: 27.

[5] Anonymous, (1996b). Canada imposes carbofuran ban. *Eur. Chem. News,* 65: 39.

[6] Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts. Poly phenol Oxidase in Beta vulgar. *Plant physiol*., 24: 1-15

[7] Banci, L. (1997) Structural properties of peroxidases. *J. Biotechnol*. 53: 253–263.

[8] Bhonwong, A., Stout, M. J., Attajarusit, J., Tantasawat, P. (2009). Defensive role of tomato polyphenol oxidases against cotton bollworm (*Helicoverpa armigera*) and beet armyworm (*Spodoptera exigua*). *J Chem Ecol*., 35: 28–38.

[9] *Bremner, P. M. (1969 b). Growth and yield of 3 varities of wheat with particular reference to the influence of unproductive tillers.

[10] Butler, G. C. (1973). Principles of Ecotoxicology Scope - 12, John wiley and sons, Chisester, New York, Brisbane: 350

[11] Cannell, R. D., Gales, K, Snydon, R. M. and Suhail, B. A., (1979). Effect of short term water logging on the growth and yield of pea (*Pisum sativum*). *Ann. Appl. Biol.,* 93: 327 – 335.

[12] Chen, Y., Ni, X., Buntin, G. D. (20090. Physiological, nutritional, and biochemical bases of corn resistance to foliage-feeding fall armyworm. *J. Chem. Ecol*., 35: 297–306

[13] *Cleland: Role of endogenous auxin. *Physiol. Plant*. 17: 126 – 135.

[14] Crepeau, K. L., Kuivila, K. M. (2000). Rice pesticide concentrationsin the Colusa Basin Dr<! In and the Sacramento River, California, 1990- 1993. *J. Environ. Qual*., 29: 926-935.

[15] Davidson, J. L. (1964). Some effects of leaf-area control on the yield of wheat. *Aust. J. Agric. Res.,* 16: 721 – 731.

[16] Dickmann, R., Melgarejo, J., Loubiere, P. and Montagnon, M. (1997). Oxadiargyl: A new novel herbicide for rice and sugar cane. Proceedings of the Brighton Crop Protection Council Conference - Weeds 1, 51-7. (Brighton Crop Protection).

[17] Dietrich, D. R., Schmid , P., Zweifel, U., Schlatter, Ch., JenniEiermann,S., Bachmann, H., Bühler, U., Zbinden, N. (1995). Mortality of birds of prey following field application of granular carbofuran: a case study. *Arch. Environ. Contam. Toxicol*., 29: 140- 145.

[18] Francisco, A.T-B., Juan, Carlos E. (2001). Phenolic compounds and related enzymes as determinants of quality in fruits and vegetables. J. Sci. Food Agric., 81: 853–876.

[19] Furumo, N. C., Furutani, S. (2008). A simple method for assaying total protein, polyphenol oxidase and peroxidase activity from 'Kaimana' Litchi chinensis Sonn. *J. Haw. Pac. Agric.,* 15.

[20] Gill, R.S., Gupta, K., Taggar, G. K., Taggar, M.S. (2010). Role of oxidative enzymes in plant defenses against herbivory. *Acta Phytopathol Entomol Hung.*, 45: 277–90.

[21] Gulsen, O., Eickhoff, T., Heng-Moss, T., Shearman, R., Baxendale, F., Sarath, G. (2010). Characterization of peroxidase changes in resistant and susceptible warm-season turfgrasses challenged by *Blissus occiduus. Arthropod-Plant Interact*, 5–55.

[22] Gupta, R.C. (1994). Carbofuran toxicity. *J. Toxicol. Environ. Health.* 43: 383--418.

[23] He, J., Chen, F., Chen, S., L. G., Deng, Y., Fang, W. (2011). Chrysanthemum leaf epidermal surface morphology and antioxidant and defense enzyme activity in response to aphid infestation. *J Plant Physiol.,* : 168-687.

[24] Heng-Moss, T. M., Sarath, G., Baxendale, F., Novak, D., Bose, S., Ni, X. (2004). Characterization of oxidative enzyme changes in buffalograsses challenged by *Blissus occiduus. J. Econ. Entomol.*, 97: 1086–1095.

[25] Hsia, C. P. and Kao, C. H. (1978). Soybean leaf disc senescence. Hormone action and hormon membrane permeability reaction. In: The Hindu Survey of Indian agriculture pp: 61 - 65: In plant physiology, Kalyani Public, New Delhi, 572 – 582. In: Proc. Int. Symp. on Current problems on Fruit and Vegetables. *Los. Bonos. Loguna*, 60: 273-277.

[26] James, P. C. (1995). Interna1izing externalities: granu lar carbofuran use on rapeseed in Canada. *Eco. Econ.,* 13: 181-1 84.

[27] Jaya Kumar, M., Eyimi, M. and Pannierselvan, S. (1990). Alleolopathic effect of E. Globulus Labill on ground nut and corn. Comp. *Physiol. Ecol*., 15: 109 - 113.

[28] *Jones, R. L. and Phillips, D. R. (1966). Organs of gibberellin synthesis in light grown sun-flower plants.

[29] Kar, M. and Mishra, D. (1976). Catalase, peroxidase and polyphenol oxidase activities during rice leaf senescence. *Plant physiol.,* 57: 315 – 319.

[30] Khan, A.A., Robinson, D.S. (1994). Hydrogen donor specificity of mango isoperoxidases. *Food Chem*., 49: 407–410.

[31] Kohil, R. K., Kaur, K. and Kumari, A. (1988). Inhibition of seed germination of Vigna ambelata in response to Eucalyptus globules allelochemicals. In: proceedings of intl. cong. Plant physiol. Society of plant physiol. And biochem., New Delhi: 15-50.

[32] Kumar, K. B. and Khan, P. A. (1982). Effect of insecticides oxydemeton methyl and Dimenthoate on chlorophyll retention and hydrogen peroxide utilization in Ragi (*Eleusine coracana Gacrth CV PR 202*) leaves during senescence. *Indian J. Expt. Biol.,* 20: 889 – 893.

[33] Mishra, A. and Adhikary, S. P. (1988). Effect of different neem preparations on growth protein content, Nitrogen fixation and extracellular amino-acids of Anabaena variables. UU 147.

[34] Moore, K. G. (1965). Senescence of leaves of Acer pseudoplantanus L and Parthenocissus aricuspidalis plarch 1, changes in some leaf constituents during maturity and senescence. *Ann. Bot.,* 29: 433 – 444.

[35] Moreland, D. E. (1980). Mechanism of action of herbicides. *Ann. Rev. Plant. Physiol.,* 31: 597.

[36] Murty, K. S., Nayhak, S. K. and Sahu, G. (1973). Photosynthetic efficiency in rice varieties. *ISNA. News Letter*, 2: 5 – 6.

[37] Norberg-King, T. J., Durhan, E. J. and Ankley, G. T. (1991). Application of toxieity identifieation evaluation proeedures to the ambient waters of the Colusa Basin Drain, California. *Environ. Toxicol. Chem.,* 10: 891-900.

[38] Onsa, G. H., Saari, N. B., Selamat, J., Bakar, J. (2000). Latent polyphenol oxidase from Sago Log (Metroxylon sagu): Partial purification, activation, and some properties. *J. Agric. Food Chem.,* 48: 5041–5045.

[39] Onsa, G. H., Saari, N. B., Selamat, J., Bakar, J. (2004). Purification and characterization of membrane-bound peroxidases from Metroxylon sagu. *Food Chem.,* 85: 365–376.

[40] Padhy, B., Khan, P. A., Acharya, B. and BuxiPatro. N. P. (1992). Allelopathic effects of Eucalyptus leaves on seed germination and seedling growth of finger millet. In: Proc. of First National Symposiums on Allelopathy in Agroecosystems, (Eds. P. Tauro and S.S. Norwal), Indian Society of Allelopathy. Haryana Agriculture University, Hisar, India, Pp. 102-104.

[41] Panda, P. (1994). Allelopathic studies on crop plants, M.Phil. Dissertation, Berhampur University, Berhampur, Odisha, India.

[42] Peter, H. F., John, R. L. W. (1996). Inhibition of diphenol oxidases: A comparative study. *J. Food Biochem.,* 20:15–30.

[43] Ramanand, K., Sharmila, M., Singh, N., Sethunathan, N. (1991). Metabolism o f carbamate insecticides by resting cells and cell-free preparations of a soil bacterium, *Arthrobacter* sp. Buli. *Environ. Contam. Toxicol*., 46: 380- 386.

[44] Rath, B. B. and Adhikary, S. P. (2018). Assessment of influence and efficacy of some biocides on rice plant. *Int. J. Current Microbology and Applied Science,* Vol. 7(10): 1326-1347.

[45] Sahoo, A., Sahu, S. K., Sharmila, M. and Sethunathan, N. (1990). Persistenee of carbamate inseetieides, earbosulfan and carbofuran in soils as influenced by temperature and mierobial aetivity. *Buli. Environ. Contam. Toxicol.,* 44: 948- 954.

[46] Schmutterer, H. (1990). Properties and potential of natural pesticides from the neem tree, Azadirachta indica. *Annu. Rev. Entomol*., 35: 271--297.

[47] Seiber, J. N., Catahan, M. P., Barrill, C. R. (1978). Loss of carbofuran from rice paddy water: ehemieal and p hysieal faetors. *J. Environ. Sei. Health 13(B)*: 131- 148.

[48] Sethi, A., McAuslane, H.J., Rathinasabapathi, B., Nuessly, G. S., Nagata, R. T. (2009). Enzyme induction as a possible mechanism for latex-mediated insect resistance in romaine lettuce. *J Chem Ecol.,* 35: 190–200.

[49] Stout, M. J., Riggio, M. R., Yang, Y. M. J. (2009). Direct induced resistance in Oryza sativa to Spodoptera frugiperda. *Environ Entomol*., 38:1174–1181.

[50] Sugai, A. Y. and Tadini, C. C. (2006). Thermal inactivations of mango (Mangifera indica variety Palmer) puree peroxidase. Proceedings of 2006 CIGR Section VI International Symposium on Future of Food Engineering; Warsaw, Poland.

[51] Tejada, A. W. and Magallona, E. D. (1985). Fate earbosulfan in a rice paddy environmental. Philipp. *Entomol.,* 6: 255- 273.

[52] Trevizan, L. R. P., Mattos, M. L., Martins, J. F. S., Baptista, G. C., Mello, M. and Dias, R. A. (2002). Carbofuran and 3-hydroxy-earbofuran residues in irrigation water of a rice ecosystem in Southern Brazil. In: l0th International Congress on the Chemistry of Crop Protection, 10, 2002.

[53] Usha Rani, P. and Jyothsna, Y. (2010). Biochemical and enzymatic changes in rice as a mechanism of defense. *Acta Physiol Plant*. 32: 695–701.

[54] Vergara, B. S. (1966). Crop reponse to light variations. Relationship between growth duration and grain yield of rice plants. *Soil Sci. and Plant. Nutr.,* 12: 137-153.

[55] War, A. R., Paulraj, M. G., War, M. Y., Ignacimuthu, S. (2011). Herbivore- and elicitor-induced resistance in groundnut to Asian armyworm, *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae). *Plant Signal Behav.,* 6: 1769–77.

[56] War, A. R., Paulraj, M. G., War, M. Y. (2011). Ignacimuthu S. Jasmonic acid- mediated induced resistance in groundnut (*Arachis hypogaea* L.) against *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae). *J. Plant Growth Regul.,* 30: 512–23.

[57] Yoshida, S. and Ahn, S. B. (1968). The accumulation process of carbohydrate in rice verities in relation to their response to nitrogen in the tropics. Int.rice Ros. Inst. Loss Banes, Phillipines. 38-41.

[58] Zhang, S. Z., Hau, B. Z., Zhang, F. (2008). Induction of the activities of antioxidative enzymes and the levels of malondialdehyde in cucumber seedlings as a consequence of *Bemisia tabaci* (Hemiptera: Aleyrodidae) infestation. *Arthropod-Plant Interact*, 2: 209–213.

[59] Zhao, L. Y., Chen, J. L., Cheng, D. F., Sun, J. R., Liu, Y., Tian, Z. (2009). Biochemical and molecular characterizations of *Sitobion avenae*– induced wheat defense responses. *Crop Prot.,* 28: 435–42.

[60] Zhu-Salzman, K., Luthe, D. S., Felton, G. W. (2008). Arthropod-inducible proteins: broad spectrum defenses against multiple herbivores. *Plant Physiol.,* 146: 852–858.

Table – 1 Effect of Neem oil on chlorophyll content and enzyme activities of Oryza sativa var.IR- 36 on fourteen day old seedling (values are in mean ± S.D. of ten samples). Correlation coefficient (r) values are calculated between different concentration of Neem oil on fourteen days and different parameters studied. Significance levels are shown (p)value in parentheses with d.f.20

Treatment (ml/lit.) / Parameters		Control	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	Correlation coefficient with level of significance (p value)
Chlorophyll Content mg/g	Chl. a	3.04± 0.06	2.68± 0.06	2.45± 0.05	2.27± 0.05	2.06± 0.05	1.85± 0.04	1.68± 0.04	1.57± 0.04	1.48± 0.03	1.07± 0.03	0.89± 0.02	r=-0.978 (p≤0.001)
	Chl. b	0.89± 0.02	0.83± 0.02	0.78± 0.02	0.72± 0.02	0.68± 0.02	0.63± 0.02	0.57± 0.02	0.45± 0.02	0.47± 0.02	0.43± 0.02	0.37± 0.01	r=-0.967 (p≤0.001)
	Total ch1	3.93± 0.09	3.51± 0.08	3.23± 0.08	2.99± 0.07	2.74± 0.06	2.48± 0.06	2.25± 0.05	2.11± 0.05	1.95± 0.04	1.50± 0.04	1.26± 0.03	r=-0.976 (p≤0.001)
Peroxidase content Mg/g	Leaves	1.19± 0.32	1.06± 0.27	1.01± 0.21	0.97± 0.19	0.93± 0.16	0.86± 0.19	0.81± 0.17	0.75± 0.15	0.71± 0.12	0.68± 0.09	0.62± 0.06	r=-0.961 (p≤0.001)
	Roots	1.26± 0.41	1.19± 0.39	1.15± 0.32	1.11± 0.31	1.07± 0.29	1.03± 0.29	0.97± 0.26	0.92± 0.23	0.86± 0.17	0.82± 0.13	0.76± 0.09	r=-0.968 (p≤0.001)
Poly phenol oxidase content mg/g	Leaves	1.65± 0.56	1.57± 0.53	1.52± 0.49	1.47± 0.46	1.42± 0.41	1.35± 0.32	1.31± 0.38	1.24± 0.34	1.17± 0.27	1.13± 0.23	1.08± 0.18	r=-0.972 (p≤0.001)
	Roots	1.43± 0.48	1.32± 0.46	1.26± 0.39	1.21± 0.36	1.16± 0.33	1.12± **0**.38	1.06± 0.29	1.01± 0.24	0.95± 0.19	0.87± 0.16	0.81± 0.14	r=-0.976 (p≤0.001)

Table – 2 Effect of Carbosulfan 25% E.C. (Marshal) on chlorophyll content and enzyme activities of Oryza sativa var.IR- 36 on fourteen day old seedling (values are in mean ± S.D. of ten samples). Correlation coefficient (r) values are calculated between different concentration of Carbosulfan 25% E.C. (Marshal) and different parameters studied. Significance levels are shown (p)value in parentheses with d.f.20

Treatment (ml/lit.) / Parameters		Control	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	Correlation coefficient with level of significance (p value)
Chlorophyll Content mg/g	Chl. a	3.04± 0.06	2.46± 0.05	2.32± 0.05	2.12± 0.05	1.91± 0.04	1.72± 0.04	1.52± 0.04	1.42± 0.03	1.31± 0.03	0.92± 0.02	0.76± 0.02	r=-0.953 (p≤0.001)
	Chl. b	0.89± 0.02	0.68± 0.02	0.64± 0.02	0.62± 0.02	0.58± 0.02	0.54± 0.02	0.49± 0.02	0.43± 0.02	0.38± 0.01	0.34± 0.01	0.27± 0.01	r=-0.974 (p≤0.001)
	Total ch1	3.93± 0.09	3.14± 0.07	2.96± 0.07	2.74± 0.06	2.49± 0.06	2.26± 0.05	2.01± 0.05	1.85± 0.04	1.69± 0.04	1.26± 0.03	1.03± 0.02	r=-0.958 (p≤0.001)
Peroxidase content Mg/g	Leaves	1.19± 0.32	0.92± 0.25	0.86± 0.21	0.82± 0.21	0.78± 0.18	0.73± 0.15	0.68± 0.12	0.64± 0.12	0.58± 0.09	0.51± 0.08	0.45± 0.06	r=-0.972 (p≤0.001)
	Roots	1.26± 0.41	1.07± 0.36	1.03± 0.32	0.97± 0.29	0.92± 0.25	0.87± 0.21	0.83± 0.24	0.76± 0.21	0.71± 0.19	0.64± 0.16	0.57± 0.13	r=-0.968 (p≤0.001)
Poly phenol oxidase content mg/g	Leaves	1.65± 0.56	1.42± 0.45	1.37± 0.42	1.31± 0.37	1.25± 0.36	1.19± 0.32	1.16± 0.29	1.08± 0.24	1.02± 0.21	0.94± 0.16	0.86± 0.12	r=-0.964 (p≤0.001)
	Roots	1.43± 0.48	1.17± 0.29	1.12± 0.26	1.06± 0.28	1.02± 0.25	0.95± **0**.19	0.89± 0.16	0.83± 0.18	0.75± 0.14	0.69± 0.07	0.63± 0.08	r=-0.961 (p≤0.001)

Table – 3 Effect of Topstar (Oxadiargyl 80% W.P.) on chlorophyll content and enzyme activities of Oryza sativa var.IR- 36 on fourteen day old seedling (values are in mean ± S.D. of ten samples). Correlation coefficient (r) values are calculated between different concentration of Topstar (Oxadiargyl 80% W.P.) and different parameters studied. Significance levels are shown (p)value in parentheses with d.f.20

Treatment (ml/lit.) / Parameters		Control	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	Correlation coefficient with level of significance (p value)
Chlorophyll Content mg/g	Chl. a	03.04± 0.06	2.39± 0.05	2.27± 0.05	2.08± 0.05	1.87± 0.04	1.68± 0.04	1.47± 0.03	1.38± 0.03	1.24± 0.03	0.87± 0.02	0.68± 0.02	r=-0.961 (p≤0.001)
	Chl. b	0.89± 0.02	0.66± 0.02	0.63± 0.02	0.61± 0.02	0.56± 0.02	0.52± 0.02	0.46± 0.02	0.41± 0.02	0.36± 0.01	0.31± 0.01	0.23± 0.01	r=-0.952 (p≤0.001)
	Total ch1	3.93± 0.09	3.05± 0.07	2.90± 0.07	2.69± 0.06	2.43± 0.06	2.20± 0.05	1.93± 0.04	1.79± 0.04	1.60± 0.04	1.18± 0.02	1.91± 0.02	r=-0.969 (p≤0.001)
Peroxidase content Mg/g	Leaves	1.19± 0.32	0.88± 0.23	0.83± 0.21	0.79± 0.19	0.75± 0.17	0.69± 0.16	0.65± 0.18	0.65± 0.15	0.54± 0.11	0.48± 0.09	0.41± 0.07	r=-0.967 (p≤0.001)
	Roots	1.26± 0.41	1.04± 0.34	0.99± 0.29	0.93± 0.24	0.89± 0.28	0.85± 0.25	0.78± 0.12	0.76± 0.24	0.68± 0.19	0.59± 0.16	0.53± 0.13	r=-0.959 (p≤0.001)
Poly phenol oxidase content mg/g	Leaves	1.65± 0.56	1.38± 0.43	1.34± 0.39	1.27± 0.31	1.21± 0.28	1.17± 0.23	1.11± 0.25	1.04± 0.21	0.96± 0.16	0.89± 0.19	0.79± 0.14	r=-0.958 (p≤0.001)
	Roots	1.43± 0.48	1.14± 0.27	1.08± 0.32	1.03± 0.28	0.97± 0.23	0.92± **0**.25	0.85± 0.21	0.78± 0.18	0.72± 0.15	0.65± 0.12	0.57± 0.09	r=-0.956 (p≤0.001)