RESEARCH ARTICLE

Phytotoxicity of Profenofos 50% EC (Curacron 50 EC) to *Vigna radiata*, L. seedlings: III. Studies on Secondary metabolites and enzymes

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ABSTRACT

Profenofos 50% EC (Curacron 50 EC) is a hazardous pesticide commonly used in agriculture is also an important contaminant of environment. Its presence in biological system has gained importance due to bioaccumulation in food chain. The phyto-toxic effect of profenofos was assessed based on the secondary metabolites like Phenolic Compounds, Flavinoids) and Enzymes of the test species, *Vigna radiata, L*. The concentrations of pesticide chosen were based on EC50 and are in the range of 0.02, 0.05, 0.08, 0.1 and 0.2 % of profenofos. The changes in phenol and flavinoids enzymes (peroxidase and polyphenol oxidase) are not dose dependant but the catalase enzyme activity was profenophos concentration dependant.

Keywords: Profenofos, organophosphate, seedlings, morphology, pigments

INTRODUCTION

Pesticides are playing a pivotal role in meeting the food, cotton fibre and tobacco demand of escalating population and control of vector-borne diseases. However, most of the applied pesticides get dispersed in the environment and affects the health of un-protected agricultural and industrial workers. The three major routes of entry for pesticides include contamination of the skin, lungs and the gut. Exposure to pesticides is one of the most important occupational risks among farmers in developing countries (Wesseling *et al*., 2001; Konradsen *et al*., 2003 and Coronado *et al.* 2004). Occupational exposure to pesticides is of great interest in order to identify the hazards of pesticide use and the establishment of safe methods of pesticide handling. This is

because pesticide misuse in various sectors of the agriculture often has been associated with health problems and environmental contamination worldwide (Soares *et al*., 2003; Mancini *et al*., 2005; Remor *et al*., 2009). Misuse of highly toxic pesticides, coupled with a weak or a totally absent legislative framework in the use of pesticides, is one of the major reasons for the high incidence of pesticide poisoning in developing countries (Konradsen *et al*., 2003).

Profenofos is the International Organization for Standardization (ISO) approved name for(*RS*)-*O*-4-bromo-2-chlorophenyl *O*-ethyl S-propyl phosphorothioate (IUPAC). The Chemical Abstracts Service (CAS) chemical name for profenofosis *O*-(4-bromo-2-chlorophenyl) *O*-ethyl S-propyl phosphorothioate (CAS No.41198-08-7). It is a broad-spectrum organo-phosphorus insecticide that is used to control insect pests in cotton, maize, sugar beet, soya bean, potato, vegetables and other crops.

Application of organophosphate insecticides inhibits the seed germination seedling growth of *Peniesetum americanum* L. (Siddiqui *et al*., 1999). Like wise use of systemic fungicides produced chlorosis irregular depression at the central marginal portions of saffron leaf (Reyes, 1975), induced sharp decrease in cell division (Coman *et al*., 1990) inhibited seedling growth of pea (John *et al*., 1976). Despite the facts, use of systemic agrochemicals is the need of present time.

MATERIALS AND METHODS

Selection of profenofos concentration

The concentrations of pesticide, Profenofos chosen were, 0.02, 0.05, 0.08.0.1 and 0.2%. A control set was maintained with distilled water only for comparison purpose.

Selection of test seed

The prime pulse seed Vigna *radiata*, var. PDM 139 Samart popularly called as mung commonly used in eastern state of India, particularly Odisha State has been chosen for study. Healthy seeds of *Vigna radiata*, were obtained from OUAT extension Ratnapur, Ganjam for the experimentation. A standard filter paper method was used. Mung seeds (20 per replication) were placed in Petridishes (6'') on filter paper moistened with 10 ml of test solution.

The mung seeds surfaces sterilized with sodium hypochlorite for 10 minutes and were incubated in the dark at 25±2 °C for 7 days in Seed Germinator (REMI- 6C). After seven days (168hours) the following assessments were made on germination (%), Phyto pigments (Chlorophyll a, Chlorophyll b, Total Chlorophyll, Caretonoids and Phaeophytin) following the methods of Arnon (1949), length of seedling roots and shoots (cm) and fresh and dry weight (g) of roots and shoots. The experiment was set in three replicates. Estimation of total phenolic content and total flavonoid content (Malik and Singh, 1980) was done in shoot of the 7 days old seedlings.

The activity of catalase was assayed after the modified method of Kar and Mishra (1976). The shoot/ root sample weighing about 200 mg were homogenized with 10 ml of phosphate buffer pH 7.2 (0.1 M) and was centrifuged at 2^0C for 15 minute at 17000 g in a refrigerated centrifuge. The clear supernatant was taken as the enzyme source. The plant material shoot/ root of 200 mg weight was ground with 0.1 M phosphate buffer PH 7.2 in a pre-chilled mortar and pestle, and the homogenate was centrifuged at 15000 g at 4^0C for 30 min and the aliquot was used as the source of the enzymes.

The enzyme activity of peroxidase was assayed by modified method of Kar and Mishra (1976).The plant material was ground with 0.1 M phosphate buffer PH 7.2 in a pre-chilled mortar and pestle, and the homogenate was centrifuged at 15 000 g at 4^oC for 30 min and the aliquot was used as the source of the enzymes. The activity of polyphenol oxidase was assayed by the method of Kar and Mishra (1976) with slight modification.

The data are expressed as mean values of (n=5) and were analyzed employing Correlation Analysis to determine whether the values were significantly different from control at 0.05P with 4 d.f.

RESULTS

Effect of Profenofos on the secondary metabolite activity of *Vigna radiata* L. seedlings were studied after treatment for seven days at different concentration of profenofos. The secondary metabolite activity of root and shoot of the seedlings were studied and recorded in separate tables and graphs (Table-1 and Fig. 4-5).There is a significant increase in Phenol content of the root and shoots, of *Vigna radiata* L. seedlings treated with different concentrations of Profenofos 50 % EC solution. Phenol content in the controlled roots was 0.396 (A) of g^{-1} fresh wt. It was increased up to 0.472 (A) at 0.01 % of Profenofos 50 % EC then declined to 0.426 (A) at 0.02 % of Profenofos.

The trend of Phenol content in shoot was similar to that of the root of *Vigna radiata* L. Seedlings when treated with different concentrations of Profenofos 50 % EC solution. Phenol content in the control shoots was 0.049 (A) of g^{-1} fresh wt. And it increased up to 0.180 (A) with 0.01 % of Profenofos (an increase of 267.34 %) and then it declined to 0.113 (A) at 0.02 % of Profenofos treatment . However the effect of Profenofos 50 % EC on increasing the phenol content was much more in the roots at all the concentrations with comparision to shoots.

Regression analysis and ANOVA indicates the statistical insignificance and the change in phenol content in root was not profenofos treatment dependant. (Fig. 5 and Table. 1)

Table 1: Correlation Analysis of different parameters observed after Treatment of Profenofos to 07 days old seedlings of *Vigna radiata.* L.

PARAMETER	Correlation Coefficient (r- Value)	d.f	P level	Statistical Significance
Concentration of Profenofos Vs Catalase activity (shoot)	-0.971	5	0.01	Statistically Significant
Concentration of Profenofos Vs Catalase activity (root)	-0.974	5	0.001	Highly Significant
Concentration of Profenofos Vs peroxidase activity (shoot)	-0.208	5	NS	Statistically not Significant
Concentration of Profenofos Vs peroxidase activity (root)	-0.250	5	NS	Statistically not Significant
Concentration of Profenofos Vs polyphenoloxidase activity (shoot)	-0.044	5	NS	Statistically not Significant
Concentration of Profenofos Vs polyphenoloxidase activity (root)	-0.142	5	NS	Statistically not Significant
Concentration of Profenofos Vs phenol activity (shoot)	-0.378	5	NS	Statistically not Significant
Concentration of Profenofos Vs phenol activity (root)	-0.312	5	NS	Statistically not Significant
Concentration of Profenofos Vs flavanoid activity (shoot)	-0.040	5	NS	Statistically not Significant
Concentration of Profenofos Vs flavanoid activity (root)	-0.237	5	NS	Statistically not Significant

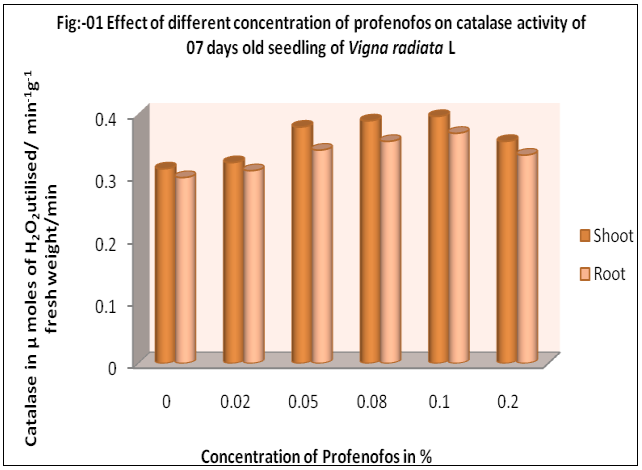

Fig. 1: Effect of different concentration of profenofos on catalase activity pf 07 days old seedling of *Vigna radiate* L.

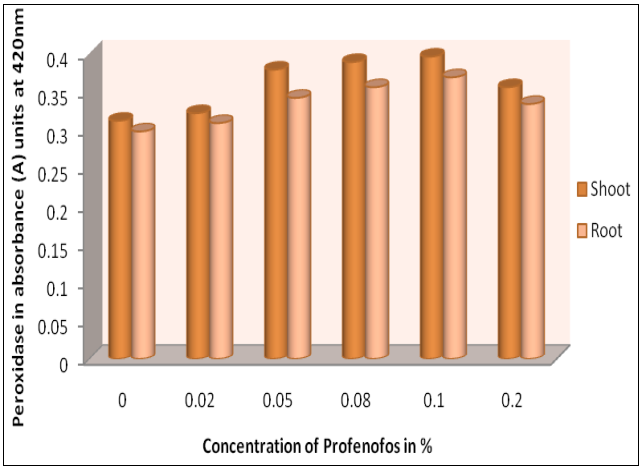

Fig. 2: Effect of different concentration of profenofos on Peroxidase activity of 07 days old seedling of *Vigna radiate* L.

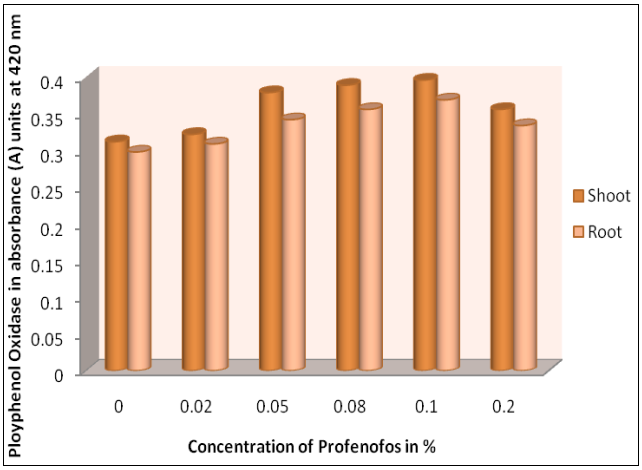

Fig. 3: Effect of different concentration of profenofos on polyphenol oxidase activity of 07 days old seedling of *Vigna radiate* L.

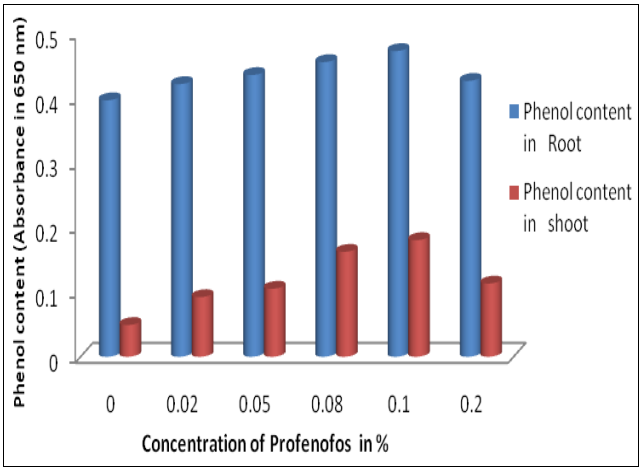

Fig. 4: Effect of different concentration of profenofos on Phenol Content of 07 days old seedling of *Vigna radiate* L.

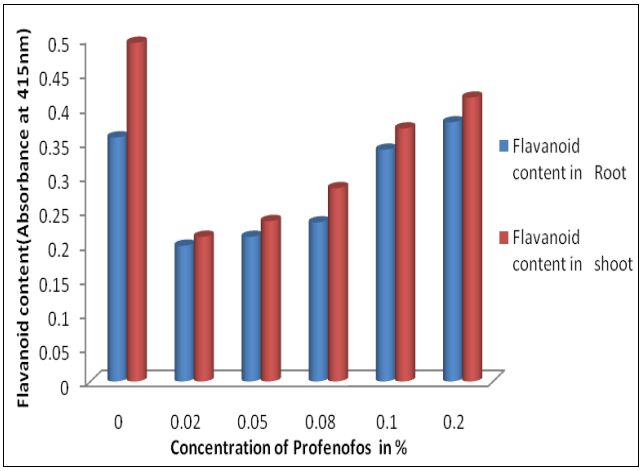

Fig. 5: Effect of different concentration of profenofos on Flavanoid content of 07 days old seedling of *Vigna radiate* L.

There is a significant decrease in Flavanoid content of the root and shoots, of *Vigna radiata* L. Seedlings treated in different concentrations of Profenofos 50 % EC solution. Flavanoid content in the controlled roots were measured in absorbance units was 0.356 (A) of fr wt. And a drastic decline of 0.198 (A) at 0.02 % of Profenofos 50 % EC that is a decrease of 44.39 % over the control value but then the decline was lessened to 0.338 (A) at 0.1 % of Profenofos that is a decrease of 5.06 % over the control value but at 0.02 % of treatment the Flavanoid content increased to 0.378 (A) that is an increase of 6.17 % over the control value.

The trend of Flavanoid content in shoot was nearly similar to that of the root of *Vigna radiata* L. Seedlings when treated in different concentrations of Profenofos 50 % EC solution. Flavanoid content in the controlled shoots were measured in absorbance units was 0.494 (A) of fr wt. and decreased up to 0.211 (A) at 0.01 % of Profenofos 50 % EC that is an decrease of 57.29 % over the control value but then there was a significant recovery up to 0.02 % with 0.414 (A) and an increase of 16.20 % over the control value. Regression analysis and ANOVA indicates the statistical insignificance and the change in flavanoid content in root was not profenofos treatment dependant.

Enzyme Activity:

The Profenofos 50 % EC application to the seedlings of *Vigna radiata* L. increased in the catalase activity of root with the increase of the test chemical from 0.02 % to 0.2 % . There was very significant increase by 210 catalase in µ moles of H_2O_2 utilized / min $^{-1}g^{-1}$ fresh root weight/ min in the enzyme activity and an increase of 840 % at 0.2 % of Profenofos over the control values (Fig,-1).

A similar observation was also noticed with shoot with the application of Profenofos 50 % EC to the seedlings of *Vigna radiata* L. increased in the catalase activity of shoot with the increase of the test chemical from 0.02 % to 0.2 % . There was very significant increase by 270 catalase in µ moles of H_2O_2 utilized / min $^{-1}g^{-1}$ fresh root weight/ min in the enzyme activity and an increase of 771.4 % at 0.2 % of Profenofos over the control values (Fig.1).

Increased activity of peroxidase was observed in root and shoot with increase in Profenofos 50 % EC concentrations (0.02,0.05,0.08,0.1 &0.2 %) At 0.1 % the percent increased was 23.82 in roots and 26.92 in shoot over the control values, but at 0.2 % there was a percent decrease with respect to the treatment at 0.1 % by 12.08 in root and 14.1 in shoot over the control value. (Fig.2).

Polyphenol oxidase which oxidises phenol in the absence of hydrogen peroxide, shows a increase in the activity with application of Profenofos 50 % EC concentrations (0.02,0.05,0.08,0.1 &0.2 %) At 0.1 % the percent increased was 19.19 in roots and 15.31 in shoot over the control values, but at 0.2 % there was a percent decrease with respect to the treatment at 0.1 % by 7.57 in root and 3.15 in shoot over the control value. (Fig. 3)

DISCUSSION

Increase in total phenols at higher concentrations of systemic fungicide provides further insight to the reduction in growth parameters discussed above. Production of phenols in the plants subjected to fungicidal spray is a response of the plants that not only helps them to cope with the resulting chemical stress but at the same time act as protective compound to check the growth of invaded pathogens (Reid *et al*., 1992). It is presumed that increase in total phenols may act as prophylactic measure against pathogens before invasion. On the other hand phytotoxin in the form of phenols have been found to have an adverse affect on germination and growth parameters (Hafeez *et al*., 1988; Macias *et al*., 1992; Berger and Cwick, 1990; Ahmed and Siddiqui, 1995; Siddiqui *et al*., 1997).

Einhellig (1995) proposed that a primary effect of phenolic acid is on the plasma membrane, and this perturbation contributes to a number of physiological effects causing growth reduction. It seems that cultivation of soybean plants in a soil treated with higher concentration of pesticide initiate some kind of abiotic stress (chemical stress) in plants triggering formation of phenolic compounds like iso-flavones (Genistein, diadzein), phenolic acid (elagic, tannic and vanilic acid) and hydroxycinnamic acid derivatives (ferulic acid, *ρ*- hydroxy benzoic acids and *ρ*-caumeric acid). These compounds are potential inhibitors of germination and plant growth (Einhellig *et al*., 1985; Macias *et al*., 1992; Mersie and Singh 1993). Few reports have elucidated the physiological mechanism of phenols induced inhibition on plant growth. Einhellig *et al*., (1985) who proposed that Ferulic and ρ-coumaric acids reduce leaf water potential and stomatal diffusive conductance in sorghum and soybean. Another study by Einhellig (1995) found that a primary effect of phenolic acids is on the plasma membrane, and this perturbation contributes to a number of physiological effects causing growth reduction. High level of ρ-coumaric, Ferulic, Cinnamic and Vanillic acids and Coumarins severely suppressed the photosynthesis of soybean and *Lemna minor* L., (Patterson, 1981; Einhellig, 1986). Three phenolic acids, ρ-Coumaric acid, Ferulic and Vanillic acids, were also reported to severely inhibit photosynthesis and protein synthesis of isolated leaf cells of velvet leaf *Abutilon theophrasti* (Mersie and Singh, 1993). Friend (1977) is of the opinion that these very compounds may act as protective compounds against pest as well.

Increase in total phenolic content and flavanoids in the test species usually indicates some kind of chemical stress produced by the application of insecticide . It has been suggested that plant treated with the chemical pesticide suffer from the chemical stress and phenolic compound produced as a result of the stress may act as a protective compound against pest and disease (Friend, 1977; Siddiqui *et al*., 1997). Stress condition cause abnormal changes in metabolic pathway resulting in production of toxic phenolic compound (Reid *et al.1992*). Phytotoxin in the form of phenolic compound and flavanoids are responsible for limiting cell division, nodulation, respiration, photosynthesis, disruption of cell membrane and reduction in total prtein and carbohydrate content of various plant species (Wilson , 1970; Bernestein and Ogata, 1966; Hafeez *et al.*1988; Siddiqui and Ahmed, 1996, Siddiqui *et al*.,1997). Consequently , the synthesis of carbohydrate, DNA and RNA may also be affected by the application of insecticide specially at higher concentration.

In the present study the shoot and root of green gram seedling showed a significant increase in phenol and flavanoid content with a gradual increase in profenofos concentrations, and our observations are similar to all of the above reports.

Enzyme activity indicates the phyto-toxicity of the stress material. The effect of pesticides on the enzyme activity in plants has been reviewed (Van Assche and Clijsters, 1990).

The activity of catalase increased in the shoot and root of green gram seedling with the increase of concentration of profenofos significant increase of catalase activity to 771.4% and 840.0% in shoot and root of green gram seedlings at 0.2 % profenofos treatment was recorded in present investigation. A highly significant positive correlation ($r=0.974$, $p \leq 0.01$, $d.f=4$) was found between the concentration profenofos and the catalase activity of shoot and root of pigeon pea seedlings.

The stress induced decline in catalase activity has also been reported by Somasekaraiah *et al*., (1992) and Galeogo *et al*., (1996). Accumulation of hydrogen peroxide in higher quantities may be cytotoxic and therefore the hydrogen peroxide formed due to the activity of the superoxide dismutase may be degraded by catalase and peroxidase resulting in the formulation of water

and oxygen (Scott *et al*.,1987). Therefore concurrent increase in catalase a predominant utiliser of hydrogen peroxide may become necessary. The hydrogen peroxide accumulation may result from the increased activities of superoxide dismutase and reduced activities of catalase. The end product, hydrogen perioxide will in turn may be toxic to the green gram seedlings.

Conflicting reports exist on the activities of catalase in plant tissues exposed to stress; Subhadra *et al*., (1991) reported an increase in catalase activity in roots of *Lemna minor* L and *Allium Cepa* L. Whereas Cakmak and Horst (1991) and Luna *et al*., (1994) reported a decrease in catalase activity in roots and leaves respectively. Stress seems to affect the pathway of synthesis of the enzyme and its activity in leaves at seedling stage was drastically inhibited by the metal; catalase being photosensitive needs constant fresh synthesis (Feierbend *et al*., 1992).

Peroxidases are antioxidant enzymes which play a crucial role in plant growth and development, and activities of these enzymes are changed under both abiotic and biotic stress conditions (Doganlar and Atmaca, 2011). Sandalo *et al*., (2001) noticed a decrease in peroxidase activity in pea under the influence of stress. Pesticides causes oxidative stress, probably through an interaction with the anti oxidative defense, disruption of the electron transport chain, or induction of lipid peroxidation. Stimulation of anti oxidant enzyme activity at low stress levels could play a significant role in protecting cells against stress -induced oxidative stress (Scebba *et al*., 2006). Profenofos is a highly toxic, non essential mutable and biodegradable pesticide that undergoes many changes during transfer through different levels of food chain. Pesticides treated seedlings of *phaselous vulgaris* showed reduction or inhibition of the growth of the main axis of the root with a consequent reduction in root length. There was an inverse correlation between cell wall peroxidase and growth . Zinc induced rise in peroxidase was reported in the leaves of *phaseolus vulgaris* (Van Assche *et al*.,1988). The author found a significant correlation between peroxidase induction and the presence of metals like zinc, cadmium and copper in plant tissues. Reddy and Prasad (1992) observed an increased peroxidase activity in *Oryza sativa* treated with different concentration of cadmium. Patro *et al*., (2001) reported that all concentration of the effluents found to have strong effect on the activity of peroxidase in the leaves of *oryza sativa* L. Very low cadmium levels in vitro have shown to stimulate the activities of certain enzymes like peroxidases, acid phosphatases etc. (Ernst 1980, Shah and Dubey 1997).

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