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RESEARCH ARTICLE

BIOCHEMISTRY

EFFECT OF CHLOROPYRIFOS AND MALATHION ON ANTIOXIDANT ENZYMES IN TOMATO AND BRINJAL

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ABSTRACT

Modern agriculture associated with monoculturing has favoured noxious insects and pests. This has promoted a heavy reliance on synthetic pesticides to control and limit their spread. Amongst vegetables tomato and brinjal are very commonly used throughout India and especially in the state of Maharashtra. Because of this they have occupied maximum cultivable land as compared to other vegetables. However both the vegetables are highly susceptible to insect attack. To enhance the yield and net economic return from these vegetables, growers apply heavy doses of chloropyrifos and malathion, which generate xenobiotic / pollution stress on these crops leading to creation of reactive oxygen species (ROS) in them which culminate the plants into death through cellular damages. Scavenging of ROS through stimulation of antioxidant enzymes such as SOD, POD and PPO is the most adaptive mechanism for the tolerance of pollution. The results of the present investigation revealed that both the pesticides have caused highly significant stimulation in the activities of SOD, POD and PPO, and it increased with the increasing dose of chloropyrifos and malathion.

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KEY WORDS

Antioxidant enzymes, Brinjal, Chloropyrifos, Malathion, Pesticides, Tomato.

INTRODUCTION

Use of pesticides to protect the vegetables has become indispensible and their effectiveness depends upon their properties. Organophosphate pesticides are more soluble and degrade quite readily in soil. Hence organophosphate pesticides play a major role in controlling insect pests in agriculture and their judicious use is improving the vegetable production. But their indiscriminate use had caused irreparable damages to the environment, especially soil, water and air. The impact of heavy applications of chloropyrifos and malathion to the vegetables like tomato and brinjal has not yet been studied in depth. Hence the biochemical and enzymological changes pertaining to the applications of both the pesticides has been attempted in present study.

MATERIALS AND METHODS

Assay of antioxidant enzymes

Randomly sampled, one gram fresh tissues of third leaf, from the top of treated and control plants at 50% flowering stage, were homogenized in 5.0 ml of 0.1 M phosphate buffer (pH 7.0). The extract was centrifuged in refrigerated centrifuge at 15,000 rpm for 20 minutes and the supernatant was used as enzyme source for the assay of superoxide dismutase, peroxidase and polyphenol oxidase

Superoxide dismutase (EC 1.15.1.1)

The activity of SOD was determined according to the standard method[1]. 3.0 ml reaction mixture contained 13 mM methionine, 25 mM nitroblue tetrazolium chloride (NBT), 0.1 mM EDTA, 50 mM phosphate buffer (pH 7.8), 50 mM sodium biocarbonate and 0.1 ml enzyme extract. The reaction was started by adding 2.0 µl of riboflavin and placing the tubes below 15 W fluorescent lamp for 15 minutes. The reaction was stopped by switching off the light and covering the tubes with black cloth. Tubes without enzyme extract developed maximum colour. A non-irradiated complete reaction mixture that did not develop colour was used as a blank. The absorbance was recorded at 560 nm and one unit of enzyme activity was taken as that quantity of enzyme, which reduced the absorbance reading to 50% in comparison with the tubes lacking enzyme. The enzyme assay was carried out at 27 ± 2 ºC by recording the absorbance at 560 nm on UV-visible spectrophotometer (Shimadzu - 1601).

Peroxidase (EC. 1.11.1.7.)

The assay[2] mixture of 3.0 ml contained 1.8 ml of 0.1 M phosphate buffer (pH 7.0), 1.0 ml freshly prepared 10 mM guaicol, 0.1 ml enzyme extract and 0.1 ml of 12.3 mM H_2O_2. Initial optical density was read at 430 nm and then increase in optical density was noted at an interval of 30 seconds on UV-visible spectrophotometer (Shimadzu-1601). The amount of protein from 0.5 ml of enzyme extract was precipitated with eight volumes of acetone (containing 14 µl β- mercaptoethanol $l^{-1}$of acetone) at 0 ºC for five hours and then centrifuged at 10,000 rpm for 20 minutes. The supernatant was discarded and pellet was dissolved in 2.5 ml of sodium hydroxide (1 N) and used for protein estimation. The enzyme activity was expressed as ΔOD min^{-1} mg^{-1}protein recording the absorbance at 595 nm in UV-visible spectrophotometer (Shimadzu - 1601).

Polyphenol oxidase (EC 1.14.18.1)

Activity of the enzyme was assayed as per the prescribed method[2]. The oxidation of catechol was measured from the reaction mixture containing 2.0 ml of phosphate buffer (pH 6.5), 0.5 ml of enzyme extract and 1.0 ml of 0.01 M catechol. Initial absorbance was

recorded at 495 nm and then the absorbance was measured at the interval of every 30 seconds in UV- visible spectrophotometer (Shimadzu-1601). The enzyme activity was expressed as ΔOD $min^{-1}mg^{-1}$ protein.

Statistical analysis

The data were analyzed statistically using ANOVA test.

Superoxide dismutase (SOD)

The results recorded in Figs. 1(a) and (b) on the changes in activity of SOD in tomato and brinjal revealed that the activity was at par with control with the application of lower concentrations of chloropyrifos and malathion, but it was stimulated with increase in their concentrations. This clearly indicated the increased xenobiotic stress level in tomato and brinjal.

RESULTS

Figure 1(a)

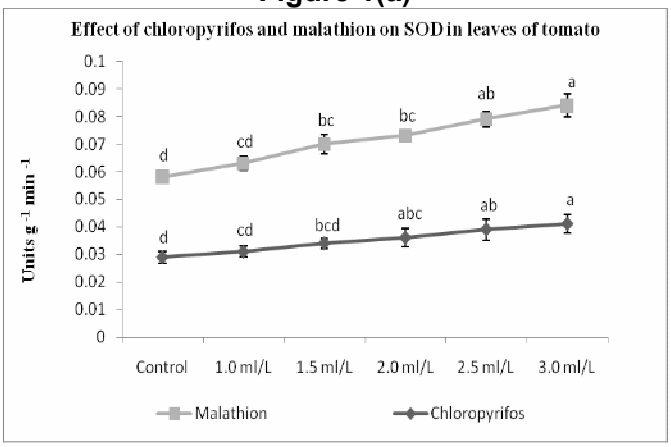
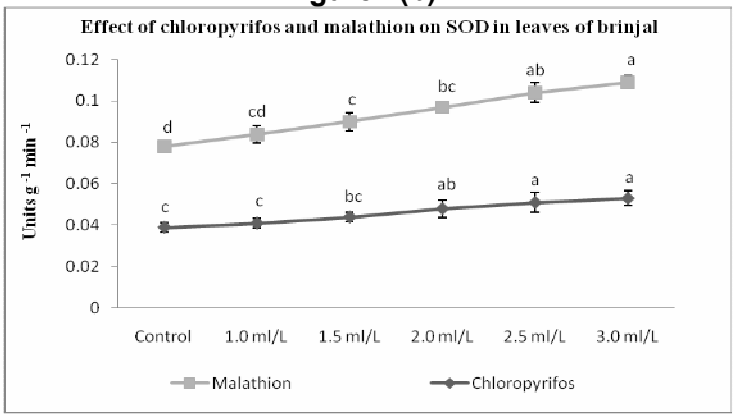


Figure 1(b)



Peroxidase (POD)

Changes in the activity of peroxidase enzyme under the influence of chloropyrifos and malathion in tomato and brinjal is explained in Figs. 2 (a) and (b). The results indicated that the lower concentration treatments (1.0 ml/L) caused very less stimulation in peroxidase activity and it was almost at par with control, while towards higher concentrations (2.5 and 3.0 ml/L), the activity was highly stimulated as compared to control.

Figure 2(a)

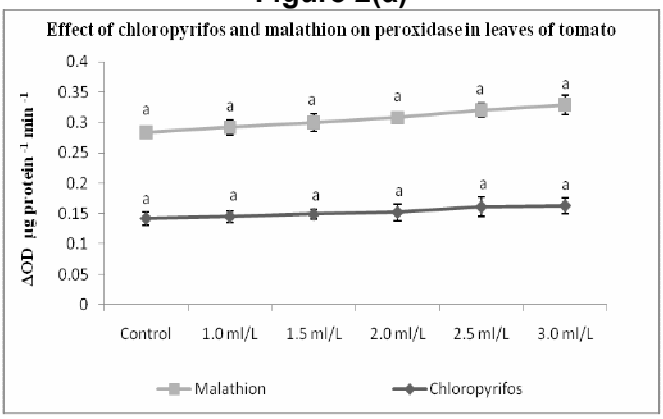
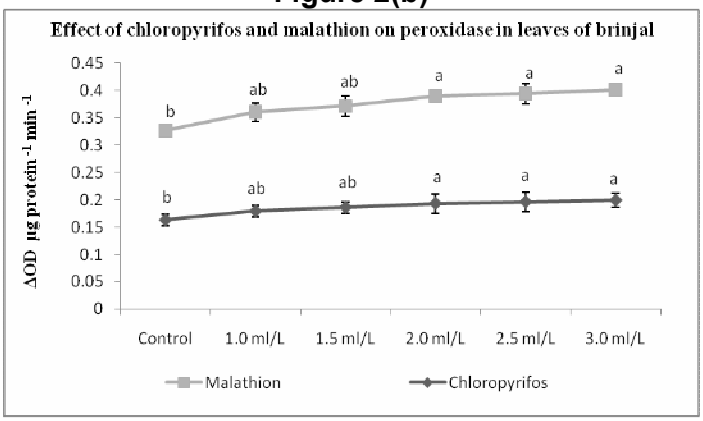


Figure 2(b)



Polyphenol oxidase (PPO)

The result presented in Figs. 3 (a) and (b) indicated that the activity of polyphenol oxidase was progressively stimulated over control in tomato and brinjal with all the concentrations of chloropyrifos and malathion. The highest stimulation in PPO activity was recorded at highest concentration of both the pesticides (3.0 ml/L).

Figure 3(a)

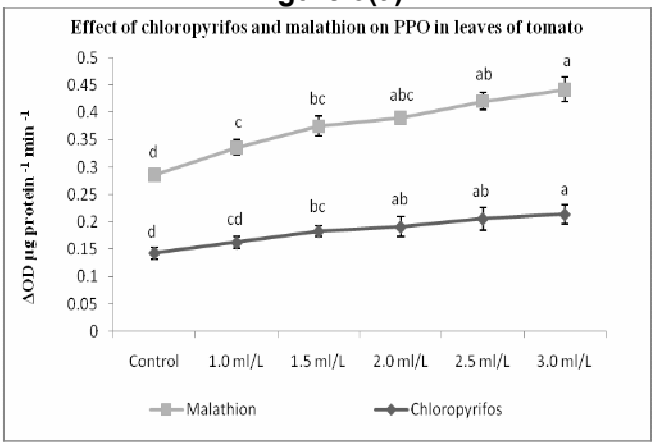
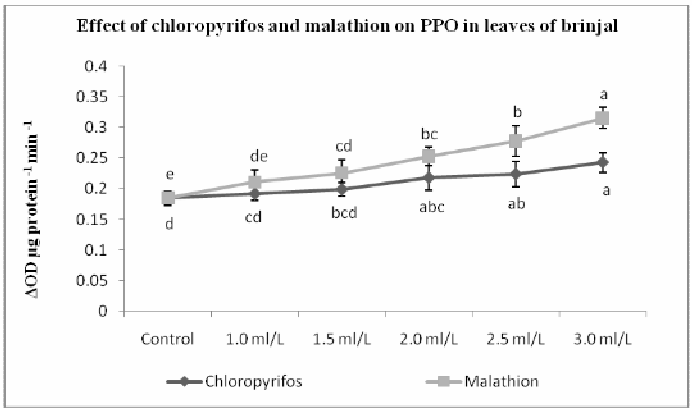


Figure 3(b)



DISCUSSIONS

Superoxide dismutase (SOD)

The higher concentration treatments of both the pesticides have imposed xenobiotic stress on treated plants, generating free radicals reactive oxygen species (ROS). The activities of antioxidant enzymes are usually stimulated on exposure to oxidative stress[3]. Once the free radicals such as superoxide anions are produced in the treated plants, due to higher concentrations, the plant cells get damaged; hence for self defense the plants stimulate the activities of free radical scavenging enzymes like SOD. Antioxidative enzymes, such as superoxide dismutase (SOD), peroxidases (POD) and polyphenol oxidase (PPO) are the most important components in the ROS scavenging system[4, 5, 6, 7]. The disparity in SOD and PPO activities is directly correlated with lipid peroxidation[8]. SOD dismutates O_2^- to H_2O_2, POD and PPO subsequently scavenge the H_2O_2.

The enzymatic status of SOD indicates the level of generations of free radicals or reactive oxygen species (ROS) in any plant, exposed to biotic and abiotic stress conditions[9]. They further explained that the scavenging of ROS is associated with antioxidative processes of the cell and the osmoprotectants. The harmful influence of ROS on macromolecules in cell is alleviated by the activities of SOD. Some research workers[10, 11] have claimed that the activities of SOD and POD were correlated with membrane lipid peroxidation which in involved

in destruction of membrane systems in plants under xenobiotic stress. Similar findings were also reported[8, 12].

Peroxidation of plasmalemma leads to the leakage of cellular contents, rapid desiccation and cell death. Intracellular membrane damage can affect respiratory activity in mitochondria, causing pigments to break down and leading to the loss of the carbon fixing ability in chloroplasts[13]. The stimulation in the activity of SOD in tomato and brinjal under high concentration treatments of chloropyrifos and malathion may be in response to protect them from such damages and injuries. The enhanced activity of SOD had protected the treated plants from the adverse impact of pesticides. The stimulation or inhibition in the activities of antioxidant enzymes like SOD may help for predicting the tolerance of test plants towards the application of high dose of pesticides. It has been explained[4, 14] that SOD is acting as a pro-oxidant in presence of hydrogen peroxide. The activities of antioxidant enzymes in tomato were assessed[15] and the stimulation in SOD due to the application of pesticides were reported. Similar stimulation in SOD activity due to application of melittin in tomato was reported[16]. The stimulation in SOD in test crops can be attributed to xenobiotic stress imposed by chloropyrifos and malathion.

Peroxidase (POD)

The enzyme peroxidase is an important antioxidant enzyme[17] which plays a pivotal role in plant growth and development. A close correlation exists between the enhanced activity of peroxidase (POD) and the concentration of phenolic substances[18]. The increase in POD has been linked with resistance to stress and self defense.

Under stress conditions the rate of respiration increases with a stimulation in peroxidase activity[19, 20, 21, 22]. In the present study the activation of peroxidase was correlated with the increased rate of respiration in treated plants due to pesticidal treatments. An elevation in the activity of peroxidase in tomato leaves, treated with bavistin and calixin was also observed[23]. The stimulation in peroxidase activity indicates the stress tolerance and intensity of stress to which the treated plants are exposed.

The effect of fungicide folpet on duckweed (*Lemna minor*) has been studied[24] and stimulated activities of peroxidase under fungicidal stress were recorded. They proposed the involvement of peroxidase in the tolerance of fungicide. While working on antioxidant response of *Cucumis sativus* to fungicides like carbendazim, it was concluded that[25] increased application of pesticides to the plants could improve their tolerance. The stimulation in peroxidase activity in tomato due to the application of melittin[16] was also reported.

Polyphenol oxidase (PPO)

The phenolic compounds under stress conditions are accumulated in crop plants, the oxidation and degradation of these toxic substances occur through the activity of polyphenol oxidase. The increased PPO activity might have caused oxidation of phenolics and thereby reduction in their content. Few research workers[26] observed that polyphenol oxidase catalyses the oxidation of phenolics to quinones, which confers resistance towards the insects and pathogens. Manipulation of PPO activity could provide resistance simultaneously to both diseases and insect pests, and therefore might be used as a component of effective integrated pest management. The increase in polyphenol oxidase activities in tomato due to melittin applications were noted[16].

PPO and POD scavenge the free radicals formed during oxidative stress and protect the plants from harmful effects of stress conditions tolerance. Phenolic compounds are believed to be important in offering resistance to diseases and insects attacking the plants and polyphenol oxidase (Catecholase and Cresolase) enzyme has been reported to be responsible for in *vitro* synthesis and accumulation of these compounds[27]. A close correlation has been found between the enhanced activity of polyphenol oxidase and peroxidase and the concentration of phenolic substances[18].

CONCLUSION

The results on the changes in the activities of antioxidant enzymes under the influence of pesticide treatments very clearly indicated the significant stimulation of all the enzymes like SOD, POD and PPO. The stimulation was increased with increasing concentrations of pesticide treatments. Maximum stimulation in the activities of antioxidant enzymes is a common phenomenon, with increasing xenobiotic stress, which is an adaption for stress tolerance. The enhanced activities of antioxidant enzymes scavenge the ROS, produced under xenobiotic stress and protect the plants from many cellular injuries and damages. In absence of enzymatic and non enzymatic antioxidants the plants are unable to survive under any type of stress, they became either susceptible or sensitive to stress and culminate into death. Because the ROS cause lipid peroxidation of the membranes, destabilize it and affect its permeability.

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