RESEARCH PAPER | BIOTECHNOLOGY | Volume : 5 | Issue : 1 | Jan 2015 | ISSN - 2249-555X

Role of Zinc on Pesticides-Induced Oxidative Damage and Seed Germination on Brassica Species in-Vitro

KEYWORDS: Chlorpyrifos, zinc, rye seeds, germination, oxidative stress

Dr. Ashima Pathak	Gursimran Bedi
Department of Biotechnology, GGDSD College, Sector 32, Chandigarh.	Department of Biotechnology, GGDSD College, Sector 32, Chandigarh

ABSTRACT *Use of pesticides in agriculture is important to ensure crop viability but when used indiscriminately, these can lead to destruction of crops. The present research focuses on the risk of chlorpyrifos on germination and oxidant status of rye seeds and effect of zinc under such conditions. Seeds in control group were normal water treated. 2 µm zinc (ZnSO4.6H2O) was given to the seeds and 1% chlorphyrifos was given. The results showed that zinc and chlorpyrifos are not exerting toxicity as indicated by the seed germination. Chlorpyrifos caused an increase in phenolic content but significant increase in glutathione levels and catalase activity was found to be increased in rye seeds. However when zinc was administered alongwith chlorpyrifos, zinc exerts a toxic effect, thus producing free radicals which led to oxidative stress. It can be concluded that risk to the seeds may occur through indirect damage by oxidative stress.*

Introduction

Pesticides are widely used for the protection of economic crops from a variety of noxious pests [1]. Their repeated and indiscriminate uses and extreme stability have led to their accumulation in plants, animals, soils and sediments, thus causing widespread contamination to the environment [2]. Some of the pesticides are reported to be beneficial for plant growth if used at lower concentration but become phytotoxic at higher dose [3, 4]. Chlorpyriphos is one of the most commonly applied insecticide (Su et al. 1998). It has been demonstrated that exposure to chlorpyriphos can differentially modify endogenous antioxidants which can lead to the development of oxidative stress in some tissues [6].

Heavy metals have also become a subject of great interest in recent years [7]. Some of the heavy metals are necessary for the growth of higher plants. However, they act toxically at high concentrations [8]. Among heavy metals, zinc is one of the essential micronutrients required for both plants and animals [9]. Zinc, when present in low concentrations, is an important micronutrient, while at high concentrations; it has become toxic to plants. Increasing the concentration of micronutrients, especially zinc, in food crop plants is a growing global challenge, which potentially has great implications for both crop production and human health. The antioxidative effect of zinc has been well documented [3]. It is also a part of several enzymes such as Superoxide dismutase and catalase, which prevent oxidative stress in plant cells. Studies on the toxicity of zinc on seed survival, seed germination and antioxidative property is still ongoing but the present endeavor aimed at evaluating the ameliorative, effect of zinc, an antioxidative trace element, on antioxidative status and lipoperoxidative changes induced in rye seeds by chlorpyriphos exposure.

Materials and Methods

For in-vitro germination studies, healthy & uniform size black rye seeds were procured from Durga Nursery, Mani Majra and were surface sterilized by treating them with 2% Bavistan for 6-7 minutes. These were washed three times with autoclaved water before inoculation. The seeds were divided into four groups. Group 1 – Control group- Seeds or oup were normal water treate $ZnSO_4$ tr Zinc treated seeds were given $ZnSO_4.6H_2H_2$o at dose of 2µm mixed in distilled water. Group 3– Chlorpyrifos treated- The seeds belonging to this group were given 1% chlorpyrifos. Group 4 – Zinc + Chlorpyrifos treated. After one hour of different treatments, all seeds were washed and established on filter paper in petri plates. During their germination period, number of roots, shoots, primary root length, secondary root length, shoots length, and numbers of leaves that appeared were measured.

The plant extracts of in vitro raised plantlets were prepared in water. For preparing the aqueous extract, after 7 days the plant parts were dried for 7-10 days at room temperature and then ground. Dried material was stirred in 250 ml distilled water for 15 minutes at 95 °C. Then these extracts were filtered and resulting filtrate was freeze-dried and the powder was used to study various biochemical parameters. Total phenolic content of extract of the plantlets was determined spectrophotometrically by using Folin –Ciocalteau method. Total flavinoids were determined using 2% AlCl3 ethanol solution. Reduced glutathione was determined using the modified method of Einhellig [10]. Catalase activity in the plant extract was estimated by the method of Ruch et al. [11].

Results & Discussion

Increase in the rate of seed germination, shoot length, number of roots, shoots and leaves of rye plant may suggest a minimum, threshold limit of chlorpyrifos that can be tolerated by the plants. At lower concentration, chlorpyrifos seemed to elevate the growth parameters. In case of zinc treated seeds, the overall growth of seeds was found to be increased, thus indicating that zinc is not toxic to these seeds. The root length was found to be decreased in zinc treated seeds, thus suggesting that the metal has somewhat accumulated in the roots and has exerted toxic effects on the roots [12]. These results suggest that rye seeds have great capacity to absorb zinc without any disturbance in the metabolism (Figure 1, 2).

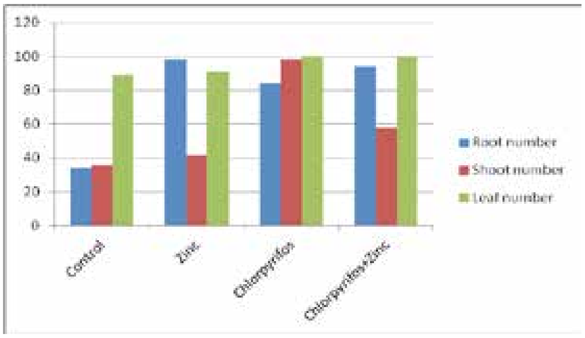

Figure 1: Effect of zinc on root, shoot and leaf number of chlorpyrifos treated seeds

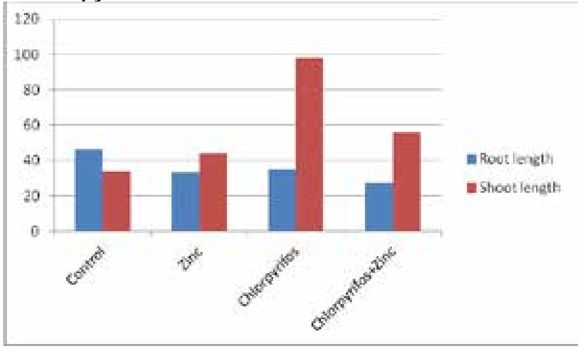

Figure 2:Effect of zinc on root and shoot length of chlorpyrifos treated seeds

It is well known that polyphenolic compounds are widely distributed in plant kingdom and they have shown to possess strong antioxidant properties [13]. A decrease in total phenols was observed in all the treatment groups and it was found to be further decreased when combined zinc and chlorpyrifos treated seeds was compared to the zinc treated as well as chlorpyrifos treated seeds. This may be due to the cumulative effect of both zinc and chlorpyrifos (Table 1). This suggests that zinc is not playing any role in this case and all changes are due to the pesticide alone. The decrease in the level of phenolics may be due to their consumption to fight the free radicals generated by chemical stress which occurs during the exposure to pesticides. It has been reported that pesticides initiate some kind of chemical stress in plants triggering formation of phenolic compounds. These compounds are found to be potential inhibitors of germination and plant growth, as observed in the present study. Einhellig [10] found that a primary effect of phenolic compounds is on plasma membrane and this perturbation contributes to a number of physiological effects causing growth reduction. Also it has been suggested that only reduced forms of phenolic compounds act on antioxidants. It is possible that in rye, these phenolic compounds occur in oxidised form (phenoxy radicals) which are ionic and lead to increase in the lifetime of free radicals. Similar explanation may hold good for zinc exposure which also produce the ionic effects by increasing the life time of phenoxy radicals. Flavonoids are large class of benzo-pyron derivatives exhibiting antioxidant activity. Similar trend was noticed in flavonoid contents with decrease noticed in all the treatment groups (Table 2). However, these levels were found to be raised when zinc was administered to the chlorpyrifos treated seeds (Table 1).

Treatments	Total Phenol (mg/g tannic acid equivalent)	Total flavonoids (mg/g quercetin equivalent)	Catalase activity (% inhibition)	Glutathione level (mg/g)
Control	0.021 ± 0.001	0.0047 ± 0.0001	0.644 ± 0.010	279.1 ± 15.0
Zinc	0.018 ± 0.001	0.0031 ± 0.0002	0.650 ± 0.009	238.8 ± 6.3
Chlorpyrifos	0.017 ± 0.001	0.0028 ± 0.0001	0.905 ± 0.029	479.1 ± 8.4
Chlorpyrifos +Zinc	0.012 ± 0.004	0.0039 ± 0.0001	0.836 ± 0.002	120.7 ± 7.2

Values are expressed as mean ± ± S.D.

Table 1: Effect of zinc on phenolic, flavanoid and glutathione contents and catalase activity of seeds treated with chlorpyrifos

Catalase forms an integral component of the cellular antioxidant defence mechanism. The activity was found to be raised in all the treatment groups. Elevation in catalase activity suggests that catalase is most sensitive to oxidative stress and hence results in increased neutralizing of free radicals which were formed during pesticide treatment. Significant increase in catalase activity during zinc treatment suggests the antioxidative potential of zinc. Decrease in the enzymatic activity in the combined treatment group confirms the antioxidative potential of zinc in controlling the generation of free radicals and hence oxidative stress. The phenolic and flavonoids contents were found to be decreased after zinc treatment as well as after exposure to pesticides but catalase activity were found to be enhanced. This suggests that in the present set up, catalase activity is playing a predominating role with regard to combating the deleterious effect of free radicals. Glutathione is a chemical containing tri peptide (glutamyl cysteinyl glycine) which in its reduced state participates in scavenging of toxic free radicals. The glutathione levels were also found to be decreased in all the treatment groups except in case of chlorpyrifos treated seeds where the levels were found to be increased significantly. Zinc treatment to the seeds treated with pesticides caused a further decrease in the levels of glutathione. This might be due to the dominant effect of zinc over chlorpyrifos which led to a reduction in the levels of glutathione (Table 1).

The aim of the present study was to achieve a better understanding of the effects of zinc on oxidative stress and on the possible induction of a defense mechanism in rye seeds, and to see the influence of pesticide, chlorpyrifos, in the defense strategies of the seed. In the present study, it was found that plants can tolerate and cut off range of systemic pesticide concentration that show normal or enhanced growth due to the suppression of pathogen, but once cut off range is exceeded, the phytotoxic so formed to combat the pathogen attack, also begin to retard the plant's growth parameters. Only source of scavenging of radicals is the increased activity of catalase. The polyphenols were found to be decreased after chlorpyrifos treatment, thus suggesting that pesticide treatment leads to chemical stress, due to which more and more free radicals are being formed. However, the activity of catalase was increased thus suggesting that only antioxidative enzymes i.e. catalase is playing some positive role. Here also zinc is playing an antioxidative role in decreasing the elevated levels of phenols and flavonoids, thus confirming that more and more of these are used up in combating the destructive effects of free radicals.

Acknowledgments:
We are highly grateful to The Principal, GGDSD College for providing the space and chemicals for pursuing this research. It is declared that there is no commercial or financial conflict of interest among the authors.

REFERENCE [1] Wasim A, Sengupta D, Chowdhury A (2009) Impact of pesticides use in agriculture: their benefits and hazards. Interdiscip Toxicol 2(1): 1–12. | [2] Isman MB (2006) Botanical insecticides, deterrents and repellents in modern agriculture and an increasingly regulated world. Annu Rev Entomol 51: 45–66. | [3] Praasterink FM (2000) FAO Inter-Country Programme for the Development and Application of Integrated Pest Management in Vegetable Growing in South and South-East Asia. Cabbage Integrated Pest Management: An Ecological Guide. Vientiane, Lao PDR. 2000. | [4] Schmutterer H (2002) The Neem Tree (Azadirachta indica A. Juss) and other Meliaceous Plants: Sources of unique natural products for integrated pest management, Medicine, Industry and other Purposes. 1st edition. Mumbai: Neem Foundation. | [5] Su T, Mulla MS (1998) Antifeedancy of neem products containing Azadirachtin against Culex tarsalis and Culex quinquefasciatus (Diptera: Culicidae). J Vector Ecol 23: 114–122 | [6] Mordue (Luntz) AJ, Nisbet AJ (2000) Azadirachtin from the neem tree Azadirachta indica: its actions against insects. Ann Entomol Soc Brasil 29: 615–632. | [7] Singh N, Mishra AK, Saxena A (1996) Use of neem cream as a mosquito repellent in tribal areas of central India. Indian J Malariol 33: 99–102. | [8] Sharma VP, Ansari MA, Razdan RK (1993) Mosquito repellent action of neem (Azadirachta indica) oil. J Am Mosq Control Assoc 9, 359–360. | [9] Champtiaux N, Changeux JP (2004) Knockout and knockin mice to investigate the role of nicotinic receptors in the central nervous system. Prog Brain Res 145: 235–251. | [10] Einhellig FA (1995) Mechanism of Action of Allelochemicals in Allelopathy pp. 96-116. In:Allelopathy: Organisms, Processes, and Applications, Inderjit, K.M.M. Dakshini, 321 and F.A. Einhellig (eds.), ACS symposium Series NO.582, Am. Chem. Soc., Washington, D.C. | [11] Ruch RJ, Cheng SJ, Klavning JE (1989) Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis 10: 1003- 1008. | [12] Mazhoudi S, Chaoui A, Ghorbal MH, El Ferjani E (1997) Response of antioxidant enzymes to excess copper in tomato (Lycopersicon esculentum,Mill.). Plant Science 127: 129–137. | [13] Aliyu AB, Musa AM, Ibrahim MA, Ibrahim H, Oyewale AO (2009) Preliminary phytochemical screening and antioxidant activity of leave extract of Albizia chevalieri harms (Leguminoseae-mimosoideae). Bayero J Pure and Appl. Sciences 2(1): 149 – 153. |