A Review of Pesticide toxicity on the Morphological and physiological response of agriculture crops

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Abstract: This review aims to highlight the effect of pesticides on plants; Pesticides, which are used to increase the yield and protect the plants from pests, insects, fungus etc. But it can also have a toxic effect on the plants. Therefore, this paper reviews the changes in germination, biochemical, physiological and various enzyme activity.

Key words: Pesticides, Fungicides, Herbicides, Antioxidant enzymes

1. Introduction:

Agriculture plays a vital role in India's economy. Agriculture is the principal means of majority of the Indians (58%) of their livelihood. Disease caused by various pathogens is one of the major biotic stresses which reduce the crop yield; thereby the entire economy is affected.

To reduce the disease incidence and thereby to improve the crop yield synthetic chemicals are used in agriculture. Pesticides are defined as "chemical substances used to prevent, destroy, repel or mitigate any pest ranging from insects, rodents, weeds, to microorganisms i.e., algicides, fungicides or bactericides" (Donaldson D et al, 1998-99, Repetto R, Baliga S 1996). This review focuses on the impacts of herbicides, insecticides, and fungicides on the biochemical changes in agricultural crops.

2. Effect on germination and Biochemical parameters:

2.1. Germination

Pesticides will have an effect on variety of physiological processes which includes changes in chlorophyll, root growth, shoot growth, cell division, etc (William et al., 1995).

Shive and Hugh (1976) showed that a fungicide triarimol not only prevented the gibberellins synthesis in *Phaseolus vulgaris* and also checked the dry weight increase over fresh weight at higher concentrations. Treatment with fungicides severely affected the germination and seedling vigour in cotton, rice and beans have been reported by Marcos and Perri (1977).

Seed germination reduced not only by the individual concentration of fungicides and insecticides, when these two are applied in combination then the effect will be severe on both germination and early growth of crop plants have been reported by Karup and Kivera (1982) and Gupta *et al*., (1983). Ahmed *et al.,* (1983) have reported the effects of systemic fungicide benomyl on sunflower, cotton and cowpea plants which showed a reduction of mean rates of transpiration and the photosynthetic pigments. There was a reduction in the photosynthetic activity of sunflower plant and significant enhancement in cotton and cowpea plants.

Fletcher and Nath (1984) have studied several pronounced side effects in plants including the development of shorter and more compact shoot in wheat plants treated with triadimefon fungicide. Izumi *et al.,* (1984) have studied the effect of S-3307 in rice plants, which retarded the plant height. Fletcher and Arnold (1986) evaluated the effect of triazole treatment in cucumber which was found to increase root growth with increased levels of cytokines. Swietlik and Fucik (1988) have reported that PBZ treatment (fungicide) was found to reduce the leaf size and number of leaves in citrus. Morphological effects of azoles molecules on plants include reduced root elongation and trichome length, increased epicuticular wax and larger chloroplasts were reported by Fletcher and Hofstra (1988), Gao *et al*., (1988).

Patil and Shirashyad (1989) observed the negative response in seed germination under the treatment of methyl parathion and phosphoamidon. Marc and Bugbee (l996) studied phytotoxic effects of benzimidazole fungicide on bedding plants. They reported that at higher concentrations of fungicide, net photosynthesis and carbon gain

was reduced. The fungicide application caused chlorosis in the plants, and delayed flowering which further suggested some of the benzimidazole fungicides can cause growth reduction. Further, Siddiqui *et al.,* (1997) have reported that usage of a low dosage of methylthiophenate (fungicide) treatment in *Sesbania sesban* has been found to cause an increase in fresh and dry weight, where higher dosages have been found to be deleterious. Though most of the systemic fungicides are used as seed dressing, yet here are several reports showing phytotoxicity on various plants.

Siddiqui *et al.,* (1999) reported the effect of systemic fungicide (topsin-M) and insecticide (dimecron) in *Pennisetum americanum* L. Dimecron showed a negative effect on seed germination and seedling growth in *Pennisetum americanum* compared to the topsin-M. At higher concentrations root growth was reduced than shoot growth but the combination of both the insecticide and fungicide reduced phytotoxicity and increased the phenolic content. Further Siddiqui and Soaliha (1999) have reported the phytotoxic effect of dipterex on the carbohydrate, RNA and DNA contents in *Vigna radiate* and *Vigna mungo*.

Mohammad Ashraf *et al.,* (2000) evaluated the effect of priming with water and sodium chloride and the changes in electrical conductance of leachates in wheat seeds. Pretreatment with water showed enhanced germination whereas sodium chloride treated seeds showed reduced germination. Tajbakhsh (2000), has also studied the relationship between electrical conductivity of imbibed seed leachates and subsequent seedling growth, viability and vigour in omid wheat and reported a reduced vigour index with solute leakage from the imbibed seeds.

Kwang Soo Roh *et al.,* (2001) studied the effect of benomyl fungicide on photo synthetic capacity in soybean leaves and reported a negative effect of benomyl fungicide on pigments and enzymes of photosynthesis. A significant decrease in the activity of rubisco activase was observed. Siddiqui and Sadaf Khan (2001) have studied the effect of systemic fungicide (topsin-M) and insecticide (dimecron) on *Vigna radiata* (L.) Wilczek and reported an enhanced chlorophyll and phenolic contents.

Siddiqui et al., (2002) have studied the effect of two systemic fungicides on benlate and calixin on a susceptible and resistant variety of wheat and have reported a reduction in total protein, increased amino acid and phenolic content. Pablo *et al.,* (2002) have evaluated the effect of carbendazim treatment on healthy tobacco plants and reported that high concentration caused the significant decrease in dry weight and all the leaf pigments, as well as all the nutrient minerals like sodium, phosphorus, potassium, calcium and magnesium and treatment lower than the recommended dosage increased the above parameters.

The effect of baytan, vitavax, benlate and captan fungicides in wheat was studied by Khalil *et al.,* (2002) and reported a significant increase in the seedling emergence number, number of grains per spike, grain weight and grain yield with all the fungicides. Mahesh and Hosmani (2002) studied the effect of ethyl bromide on the nutrient uptake by four cultivars of rice and noticed a reduction in root and shoot length and nutrient uptake. Mahesh and Hosmani (2003) have reported a drastic reduction in root and shoot length and nutrient uptake of sodium, phosphorus, potassium, calcium and magnesium in Blitox treated rice cultivars.

Ruske *et al.,* (2003) have studied the effect of triazole and strobilurin fungicide on nitrogen uptake in wheat cultivars and found that all fungicide treatments have significantly increased the amount of nitrogen. Tort and Turkyilmaz (2003) studied the physiological effects of captan fungicide on Pepper *(Capsicum annuum.*L*.)* plant and showed a higher amount of chlorophyll a, a/b ratio and carotenoid contents with recommended dosage whereas the reduction in the the chlorophyll b and total chlorophyll contents were observed in all treatments. Saladin *et al.,* (2003) have reported the flumioxazin herbicide treatment on photosynthesis in *Vitis vinifera* L.and have shown a reduction in chlorophyll and carotenoids contents.

Mahesh and Hosmani (2004) have studied the effect of Bavistin treatments in rice plants and reported the phytotoxic effect of the fungicide on morphological and nutritional parameters. Zamin Siddiqui and Arif-uz-zaman (2004) analysed the effects of fungicide benlate in *Zea mays* L. and have showed increased germination and phenolic contents.

Siddiqui and Soaliha (2006) studied the combined effects of pesticides, viz., Topsin M, Benlate (benomyl), Demacron (phosphomedon), and Chlorosuphuron, Cypermethrin and Cypermethrin dimethride (Lazer) on growth and nutritive composition of soybean plants. A lower concentration of the pesticides had a positive effect on the growth stages but high concentrations have significantly affected the growth.

Radha Bisht *et al.,* (2007) evaluated the effect of triazoles on net photosynthetic rate, transpiration rate and stomatal resistance in *Setaria italica.* Transpiration rate and stomatal resistance increased with increased concentrations of triazoles. Kishorekumar *et al.,* (2007) evaluated the comparative effects of different triazole compounds on growth, photosynthetic pigments and carbohydrate metabolism of *Solenostemon rotundifolius* and reported that triazole compounds increased the chlorophyll contents. Whereas reduction in the fresh and dry weight of shoot and leaf area was reported. Ozlem *et al.,* (2007) have studied the effect of herbicide, fusilade on lentil seeds. The result showed a reduction in seed germination, mitotic frequency.

Andreas *et al.,* (2008) studied the effect of the triazole compounds on barley and they reported that seed treatment with triadimenol significantly reduced the length of sub crown internodes and shoot length while flutriafol triazole partially inhibited shoot elongation, but did not affect dry mass accumulation and root growth. Seed dressing with strobilurin had no marked plant growth activities on seedling emergence, shoot length and sub crown inter node, but slightly stimulated the root growth.

Catherine S and Palle P (2009) studied the effect of fungicides (tebuconazole and pyraclostrobin) on soya bean growth and yield and concluded that environmental conditions and assessment of disease levels should be used as a guide for foliar fungicide application on soya bean. Meena Rajalekshmi *et al.,* (2009) have evaluated the effect of triazole growth regulators on growth and pigment contents in *Plectranthus sps*. and found that triazole treatments increased fresh and dry weight and pigment contents.

Vidya Sagar *et al.,* (2009) reported the phytotoxic effect of endosulfan in mediating stress response in sorghum and found that all the concentrations of endosulfan inhibited the seed germination, biomass, chlorophyll- a and total chlorophyll contents.

Moore and Kroger (2010) screened the effect of three insecticides and two herbicides on germination and growth of rice *(Oryza saliva)* seedlings. The root-shoot system showed sensitivity to the pesticides. Coleoptile growth was lowered in metolachlor/atrazine mixture, diazinon, and lambda-cyhalothrin while radicles of fipronil-exposed seeds were significantly higher than untreated seedlings. Sitansu *et al.,* (2010) studied the effect of propineb, prochloraz, difenoconazole and flusilazole fungicides on mung bean, mustard and rice and reported the inhibitory effect of fungicides on seed germination, plumule length and viguor index.

Sammaiah *et al.,* (2011) studied the pesticide induced alterations in *Solanum melongena* L. The results showed a beneficial effect in physiological parameters with regards to seed germination and seedling growth at lower doses of endosulfan and in comibination with kitazin but they proved to be phytotoxic at higher concentrations.

Kavina *et al.,* (2011) evaluated the comparative effect of plant growth regulators (gibberellic acid, abscisic acid) and difenoconazole fungicides on growth and biochemical contents in *Mentha piperita* Linn., difenoconazole and abscisic acid treatment increased the fresh weight, dry weight, root growth, total chlorophyll and reduced shoot length.

Rajashekar *et al.,* (2012) showed the effect of pendimethalin on the seed germination and physiological behaviour of maize seedlings. Increased concentration of pesticide showed a negative effect on germination percentage, length of radical and plumule, reduced shoot-root axis was observed.

Wondimeneh *et al.,* (2012) has investigated the effect seed treatment with ridomil, mancozeb and metalaxyl on seed germination, emergence and seedling vigor in maize. In the lab trial the fungicides affected the emergence rate, fresh root weight, shoot weight and shoot dry weight but had no significant effect on seedling height and average leaf number. Whereas in the field experiment, the fungicides improved the seedling height, average leaf number, fresh and dry weight of maize seedling as compared to control. Among the fungicides metalaxyl was more efficient followed by mancozeb.

2.2. Biochemical changes/Metabolic enzymes

Pesticides in plants can have an influence on vital physiological and metabolic pathways, such as carbohydrate metabolism, amino acid metabolism, and fatty acid metabolism thus leading to an altered level of these biomolecules and the hydrolytic enzymes.

Sahoo and Das (1994) have reported a drastic reduction in carbohydrate and protein content in rice seedlings treated with carbofuran. Hence, they made a suggestion that application of this pesticide is not beneficial.

Soaliha and Siddiqui (1999) studied the positive effect of topsin fungicides on chlorophyll, protein and phenolic content of *Hibiscus esculentus* and *Capsicum annuum*. Topsin fungicide increased all the parameters studied with a difference in response of both the species.

Siddiqui *et al.,* (1999) reported the effect of systemic fungicide (topsin-M) and insecticide (dimecron) in *Pennisetum americanum* L. Dimecron showed a negative effect on seed germination and seedling growth in *Pennisetum americanum* compared to the topsin-M. At higher concentrations root growth was reduced than shoot growth but the combination of both the insecticide and fungicide reduced phytotoxicity and increased the phenolic content. Further Siddiqui and Soaliha (1999) have reported the phytotoxic effect of dipterex on the carbohydrate, RNA and DNA contents in *Vigna radiate* and *Vigna mungo*.

The effect of carbofuran, butachlor and carbendazim treatments on chlorophyll and carbohydrate contents in rice cultivars was studied by Bhattacharya *et al.,* (2001) and reported that carbendazim treatment at panicle emergence stage significantly reduces the carbohydrate level. Prabhjot *et al.,* (2001) have investigated the changes in growth, soluble sugars and water relations of sorghum seedlings grown in light and dark conditions. Reduction in the relative water content, water potential and fresh weight of different parts of the seedlings under the imposed stress was observed whereas a significant increase in dry weight and sugar level was reported.

Ahmed Sohail *et al.,* (2003) determined the comparative efficacy of cypermethrin, regent and carbofuran in maize and have noticed an increase in total soluble protein, total free amino acids, carbohydrates and crude fibres at various time points of growth, following the application of insecticides. Enhanced level of protein, proline and abscisic contents in the leaf were observed in recommended dosage and at higher concentration on treatment with Captan fungicide in Pepper plant (Tort and Turkyilmaz 2003). An accumulation of sugars and starch were reported in *Vitis vinifera* L on flumioxazin herbicide treatment (Saladin *et al.,* 2003)

Anwarul Islam *et al.,* (2003) has evaluated the effect of NPK fertilizers, cow dung, foliar spray of urea and chemicals on the changes in the enzyme contents of matured jute leaves. It was found that the activities of amylases, protease, cellulase, peroxidase, invertase and ascorbic acid oxidase increased significantly over control whereas the highest activity was that of protease followed by cellulase and invertase.

Jha and Dubey (2005) have studied the effect of arsenic on the enzymes of carbohydrate metabolism in germinating rice seedlings and found that arsenic toxicity caused a marked decline in the activities of α-amylase and β- amylase in endosperms as well as embryo axis of germinating rice seed.

High concentration of all the pesticides Topsin M, Benlate (benomyl), Demacron (phosphomedon), and Chlorosuphuron, Cypermethrin and Cypermethrin dimethride (Lazer have affected the protein and lipid content but total phenolic compounds were found to be increased (Siddiqui and Soaliha 2006)

α- amylase activity increased with increased concentrations of the herbicide fusilade on lentil seeds (Ozlem *et al.,*2007).

Inhibition of Protease activity and significant increase in the level of hydrolytic enzyme amylase by Endosulfan is reported by Vidya Sagar (2009).

Chibi Fatiha and Sayah Fouad (2011) reported the endosulfan induced alterations in *Lycopersicum esculenlum* L. during germination and the results showed an over expression of the protein and increased proline levels during germination. Treatment even with low concentrations showed six folds increase in the esterase activity and three folds increase in the protease activity while inhibition of α-amylase activity was observed.

The activity of amylase decreased in lower doses of endosulfan and in comibination with kitazin in *solanum melangena* but protease activity increased in individual treatment of endosulfan whereas protein content in both treatments showed inhibitory effect (Sammaiah *et al,* 2011)**.** Increased protein and amino acid content in *Mentha piperita* Linn., difenoconazole and abscisic acid treatment (Kavina *et al* 2011)

The maize seedlings showed the increased content of total protein, total carbohydrate, starch and reducing sugar in endosperm by pendimethalin seed treatment (Rajashekar *et al.,*2012). Avinash and Hosmani (2012a) emphasized the effect of carbendazim on morphological and biochemical parameters of *Sorghum bicolor.* The study showed a strong correlation between vigor index and non-reducing sugar, total carbohydrates in the samples treated with the fungicides.

Moumit Roy *et al.,* (2013) investigated the carbaryl mediated biochemical alterations in egg plant. The result showed a phytotoxic effect on germination. Soluble sugar, free amino acid and amylase activity were found to be reduced whereas the total protein and insoluble sugar content increased significantly.

Gabreil *et al.,* (2013) reported the effect of triazole fungicide on biochemical and antioxidant enzymes activity in okra *(Abelmoschus esciilentus* L.) plant under drought stress. Both stress conditions increased amino acid, proline, glycine and betaine content was observed.

2.3. Antioxidant system: Enzymatic and non-enzymatic

Overproduction of reactive oxygen species is observed under Abiotic stress which can affect the plant productivity by altering many physiological processes (Apel and Hirt, 2004). Plants are developed a variety of enzymatic and non-enzymatic antioxidants system to scavenge excess ROS production. Manor Antioxidant enzymes in plants include superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione peroxidase (GPX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), glutathione reductase (GR), and glutathione S-transferase (GST) (Mittler et al., 2011).

Enhanced activities of SOD, CAT and GR were found to be related with natural resistance shown by perennial ryegrass (Harper and Harvey, 1978). Casano et al., (1994) have experimented on paraquat concentration-dependent effects in barley leaves and observed an induction in all the antioxidant enzymes except SOD.

CATs are proved to be involved in the herbicide tolerance with trichloroacetate as reported from the studies of Radetski et al., (2000). SOD and CAT were found to be increased in the presence of herbicide mefenacet in rice seedlings (Zhang *et al.* 2001). Treatment with fungicide azoxystrobin and epoxiconazole significantly increased the activity of SOD and CAT in spring Barley (Wu et al. 2002).

CATs were found to increase in the herbicide norflurazon treated maize (Jung et al.,2006). CAT activity was increased by glyphosate application in maize plants (Sergiev *et al*., 2006). An herbicide 2-benzoxazolinone, in V. radiata plants was found to increase the activity of ROS scavenging enzymes like CAT and SOD in the root and leaf tissues (Batish et al., 2006).Wang and Zhou, (2006) has demonstrated the upregulation of POD upon exposure to the herbicide chlorimuron- ethyl has been demonstrated in wheat (Triticum aestivum).

Zhang et al., (2007) have reported a change in the activity of the enzymes SOD, CAT, and GPX enzymes in the roots, stem, and leaves of cucumber on treatment with carbendazim.

Youbi Mustapha *et al.,* (2009) have reported the effects of artea, a systemic fungicide on the antioxidant system and the respiratory activity of durum wheat *(Triticum durum* L.) The results showed that treatment with artea enhanced the respiratory activity and antioxidative enzymatic levels in roots and showed increased activities of catalase, ascorbate-peroxidase and guiacol-peroxidase with increased concentrations of fungicide. Vidya Sagar *et al.,* (2009) reported effect of endosulfan in sorghum which inhibited the activity of the enzyme catalase and but there was a significant increase in the level of the defense enzymes peroxidase, polyphenol oxidase, superoxide dismutase were reported.

Somnath Roy *et al.,* (2010) have evaluated the combined formulation of carbendazim and mancozeb, sole application of carbendazim, mancozeb and methyl jasmonate in post infectional defense responses in chilli seedlings against *Sclerotium rolfsii* and reported the over expression of peroxidase isoforms and esterase. Thirumal *et al.,* (2010) have shown alterations of antioxidative metabolism induced by triazoles in sweet potato. Triazole compounds increased the contents of antioxidant compounds like ascorbic acid, tocopherol, riboflavin, anthocyanin, xanthophyll and activities of ascorbate peroxidase, superoxide dismutase, and catalase.

Mahnaz *et al.,* (2011) studied the effect of chloropyrifos and malathion on antioxidant enzymes in tomato and brinjal and reported that increased concentrations of pesticides increased the activities of defense enzymes like superoxide dismutase, peroxidases and polyphenol oxidase.

Peroxidase activity increased in the leaf tissues whereas no significant difference observed with catalase on treating with carbaryl in egg plant (Moumit Roy et al., 2013). The increase in the antioxidant system was observed in okra under triazole treatment which could cause a partial recovery of the damaging effect of stress induced by drought (Gabreil *et al.,* 2013).

3. Conclusion:

Number of studies are available to understand the pesticide induced toxicity, and its effect on crop physiology, and metabolic processes. But the effect cannot be generalized due to the complexity of various classes of pesticides and their interactions with biomolecules in different crop species. Hence future studies need to be more species specific in order to elucidate the exact mechanism.

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