

Research Article

Effect of Chloropyrifos and Malathion on Stress and Osmolyte Parameters in Tomato and Brinjal

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Abstract: With the ever-increasing population and escalating demand for food, scientists are toiling hard to enhance the production level through the introduction of new high yielding cultivars, effective cultural practices and adopting advanced technologies. Pesticides are one of the most essential inputs in modern agriculture for insuring food security particularly in the developing countries where population growth exceeds the agricultural growth. Among the various strategies adopted to combat pest of tomato, insecticides from the first line of defense. Most of the insecticides used on agriculture crop are based on quite limited number of chemically different classes and most important organic insecticides that are used against the pest on tomato and brinjal is belong to organophosphates. But the indiscriminate use of pesticides, in modern agriculture has led to various negative impacts on the environment. Some of the pesticides remain persistent (Recalcitrant) and move into the environment. The Tomato, *Lycopersicon esculentum* (Miller) and *Solanum melongena* are an important vegetables crop grown throughout the year in the India. However these vegetables crop suffers heavily from ravages of various insect pests and disease, which reduce not only the yield but also the quality of the fruits. The pesticidal / xenobiotic stress at high concentration of pesticides adversely affect the Chlorophyll Stability Index (CSI), Membrane Stability Index (MSI) and Relative Water Content (RWC).

Keywords: Chloropyrifos, Malathion, Phenols, Glycine betaine, Proline, CSI, MSI, RWC..

INTRODUCTION

Amongst the different factors responsible for yield reduction, pesticide attack on the crops is major one, especially for vegetables, the most sensitive horticultural crops. The crop losses by pests are common all over the world including India, where the losses ranged from 40% under normal situation.

Pesticides play an important role in plant protection, but their excess and continue use has harmful effects on growth, development, yield and quality in crop plants.

Among pesticides of synthetic origin, organophosphate pesticides play an important role in controlling insect pests in agriculture but the farmers usually go for excess application of chloropyrifos and malathion to protect the susceptible vegetables like tomato and brinjal from different pests in shortest possible time. As a result of this, the stress parameters have contributed to develop stress tolerance and adversely affect the Chlorophyll Stability Index (CSI), Membrane Stability Index (MSI) and Relative Water Content (RWC).

MATERIALS AND METHODS

Analysis of osmolites and stress parameters

Phenols

Total phenols in the composite leaf samples were estimated as per the method of Farkas and Kiraly [1]. One gram composite fresh leaf samples were homogenized in 10 ml of 80 % ethanol, centrifuged at 15000 rpm for 15 minutes and the supernatant was condensed on hot water bath to approximately 1.0 ml. The final volume was made up to 50 ml with distilled water. From this, 0.5 ml was taken and adjusted to 3.0 ml with distilled water. The reaction mixture was prepared with Folin-Ciocalteu reagent and 20% Na₂CO₃. The absorbance of the blue colour developed was recorded at 650 nm in UV-visible spectrophotometer (Shimadzu-1601). Tannic acid at the concentration of 100 µg ml⁻¹ was used to prepare the standard curve.

Proline

The proline content in composite leaf samples was determined by following the method of Bates *et al.* [2]. The samples (500 mg) were homogenized

separately in 10 ml of 3% aqueous sulphosalicylic acid and the homogenate was filtered through Whatman No.1 filter paper. The filtrate (2.0 ml) was taken in a test tube and was mixed with an equal volume of glacial acetic acid and acid ninhydrin. The reaction mixture was kept in boiling water bath for one hour. The reaction was terminated by placing the test tube in an ice bath, to this 4.0 ml of toluene was added and it was vigorously shaken for 20-30 seconds. The reaction was allowed to stand for few minutes to separate upper toluene layer at room temperature. The chromophore containing toluene was separated and its absorbance was recorded at 520 nm in UV-visible spectrophotometer (Shimadzu-1601). A series of standards with different concentrations of proline was run in a similar way to obtain the standard curve.

Glycine betaine

It was estimated as per the method of Ishitani *et al.* [3] by using 100 mg oven dried leaf samples, which were incubated in 20 ml of 1 N H₂SO₄ for 18 hrs. at 25°C. The suspension was centrifuged at 2000 rpm for 10 minutes and the supernatant (0.25 ml) was used in the reaction mixture with 0.75 ml H₂SO₄ (1 N) and 2.0 ml of cold I₂KI reagent. The mixture was mixed well and cooled to 0 °C for two hrs in ice bath with stirring of the reaction mixture at frequent intervals. The tubes were centrifuged again, to which 10 ml of ethylene dichloride was added to precipitate in each test tube. The absorbance of red colour developed in the reaction mixture was recorded at 365 nm in UV-visible spectrophotometer (Shimadzu-1601). Glycine betaine concentration was calculated from the calibration curve of 100 µg ml⁻¹ betaine (Sigma, USA).

Relative water content (RWC)

The leaf samples were cut in to small discs of uniform size using leaf punch. Twenty-five such discs were weighed accurately to obtain fresh weight. These discs were then suspended in distilled water for four hours. These discs were surface blotted gently to remove water and the turgid weight was recorded. These discs were placed in an oven at 72 °C for about 24 hours to get their dry weight. The relative water content was determined by using the formula given by Barrs and Weatherley [4].

Membrane stability index (MSI)

The membrane stability index (MSI) was determined according to the method of Deshmukh *et al.* [5]. Leaf discs (0.2 g) of control and treated plants were thoroughly washed in running tap water and double distilled water, and they were placed in 20 ml of doubled distilled water at 40 °C for 30 minutes, after that electrical conductivity (EC) was recorded by conductivity bridge (C₁). Subsequently, the same

samples were placed in boiling water bath (100°C) for 10 minutes and the electrical conductivity was recorded (C₂). The membrane stability index was calculated by using the formula:

$$MSI = [1 - C_1 / C_2] \times 100$$

Chlorophyll stability index

The chlorophyll pigments are thermo sensitive and their degradation occurs when subjected to higher temperature. This method is based on pigment change induced by heating. The chlorophyll destruction commences rapidly at critical temperature of 55 to 56°C. Thus, chlorophyll stability is function of temperature.

Two clean glass tubes were taken and 5.0 g of representative leaf sample was placed in them with 50 ml of distilled water. One tube was then subjected to heat in water bath at 56°C ± 1°C for exactly 30 minutes. Other tube was kept as control. The leaves are taken out and ground in mortar for 5 minutes with 100 ml 80% acetone. The extract was filtered through Whatman filter No.1. The filtrate was taken for recording the absorbance at 645 and 663 nm on UV-visible spectrophotometer (Shimadzu-1601). The chlorophyll stability index was calculated by using the formula:

$$CSI = \frac{\text{Chlorophyll content of boiled sample}}{\text{Chlorophyll content of normal sample}}$$

Statistical analysis

The treatments of chloropyrifos and malathion were laid out in a completely randomized design with three replicates. Data were expressed as mean value of three replicates. One way ANOVA was used to compare the mean values. Duncan's Multiple Range Test (DMRT) was applied as post hoc test at p = 0.05 to compare the mean difference and determine the significance. All the calculations were made by using a Statistical Package for Social Science (SPSS) for windows version 14.0 and Microsoft Excel 2007 to analyze the data.

RESULTS

Proline

The results presented in (Fig.1) indicated that proline was significantly accumulated with increasing concentrations of chloropyrifos and malathion in tomato and brinjal plants as compared to control. Maximum increase in proline was noted at the highest concentration (3.0 ml/L) of both the pesticides.

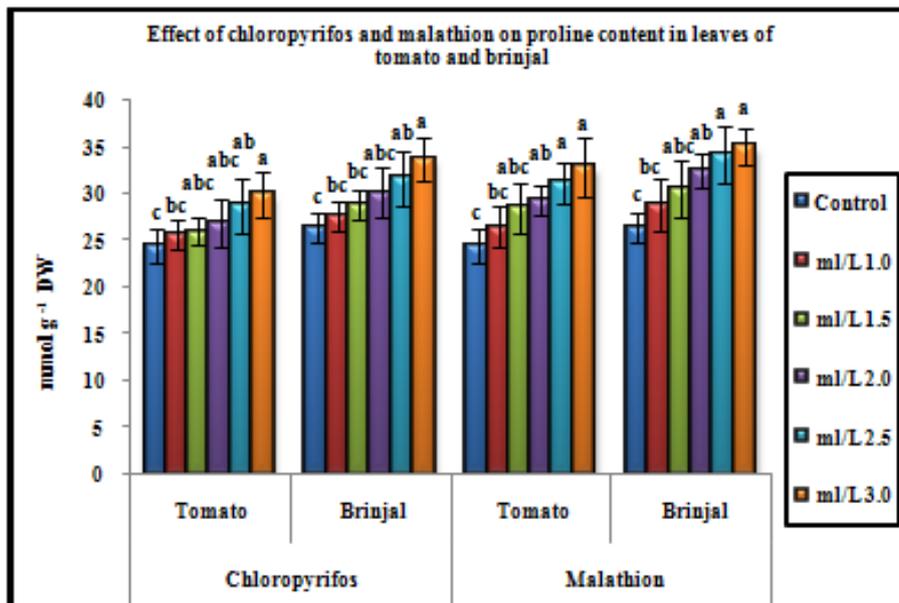


Fig 1: Effect of chloropyrifos and malathion on proline content in leaves of tomato and brinjal

Glycine betaine

The result of present investigation on glycine betaine (Fig. 2) had shown maximum accumulation in both the vegetable crops at higher concentrations of chloropyrifos and malathion. Glycine betaine contents increased with increasing concentrations of pesticides.

The lower as well as higher concentration treatments caused increase in GB contents. GB accumulation was maximum at highest concentration (3.0 ml/L) of chloropyrifos and malathion. In the beginning the increase in GB was very less but it slowly reached to maximum at higher concentration.

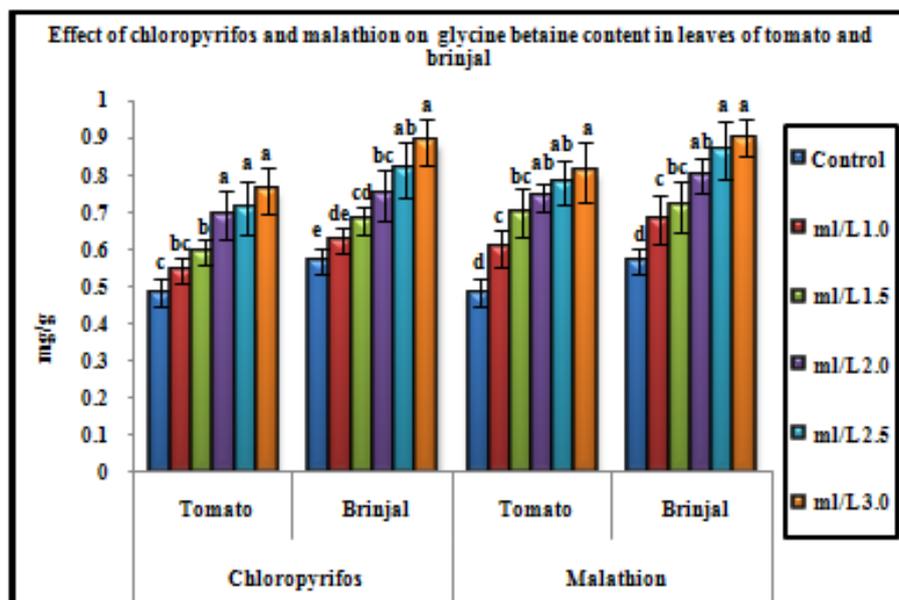


Fig. 2: Effect of chloropyrifos and malathion on glycine betaine content in leaves of tomato and brinjal

Phenols

The results on phenolic contents showed in (Fig. 3) revealed significant increase, due to lower as well as higher concentration treatments of chloropyrifos and malathion in both the vegetable crops. With increasing

concentrations there was progressive increase in phenolics and caused highest accumulation at 3.0 ml/L concentration of both the pesticides.

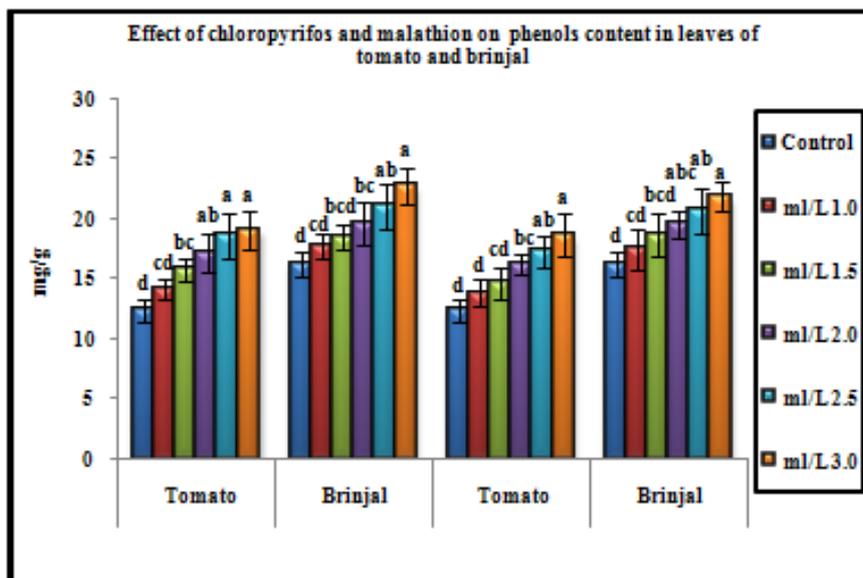


Fig 3: Effect of chloropyrifos and malathion on phenol content in leaves of tomato and brinjal

Relative water content (RWC)

The results on relative water content (RWC) presented in Table 1 indicated considerable reduction with increasing concentrations of chloropyrifos and malathion in tomato and brinjal plants. The results on RWC indicated that malathion was more effective to influence relative water contents in both the vegetables. The decrease in RWC is noted with lower as well as higher concentrations of both the pesticides.

Membrane stability index (MSI)

The results on MSI shown in Table 1 revealed that with increasing concentrations of pesticides the membrane stability in both the test crops was significantly decreased. The membrane stability index was highly reduced with the increasing concentrations

of both the pesticides indicating loss of structure and functioning of membranes under the influence of pesticides.

Chlorophyll stability index (CSI)

Results presented in Table 1 indicated highly significant decline in chlorophyll stability index with increasing concentrations of both the pesticides in both the vegetable crops under investigation. The CSI values successively decreased with the increasing concentrations of chloropyrifos and malathion indicating the adverse impact of xenobiotic stress on chlorophyll stability, structure and functioning. The lower concentrations of pesticides were less inhibitory as compare to higher concentration, which have very high negative influence on CSI.

Table 1: Effect of chloropyrifos and malathion on osmolytes intomato and brinjal

Treatments	Chloropyrifos		Malathion	
	Tomato	Brinjal	Tomato	Brinjal
Relative water content (RWC) (%)				
Control	67.71±5.08 a	76.73±4.80 a	67.71±5.08 a	76.73±4.80 a
1.0 ml/L	65.28±4.24 ab	74.21±4.33 ab	65.19±5.35 ab	71.39±6.85 ab
1.5 ml/L	63.43±3.49 ab	72.93±4.08 ab	62.61±5.89 abc	68.62±6.72 abc
2.0 ml/L	60.94±5.79 ab	68.62±6.18 abc	60.22±3.25 abc	64.73±3.75 bc
2.5 ml/L	58.32±5.83 ab	65.56±6.06 bc	57.36±4.24 bc	61.45±5.62 bc
3.0 ml/L	57.64±4.61 b	62.27±4.17 c	55.41±5.43 c	60.35±3.35 c
SEM	1.31	1.58	1.41	1.74
Sig. (p-value)	0.158	0.031	0.069	0.019

Membrane stability index (MSI) (%)				
Control	12.28±0.92 b	13.18±0.82 a	12.28±0.92 a	13.18±0.82 a
1.0 ml/L	12.04±1.49 a	12.23±0.71 ab	11.52±0.94 ab	12.1±1.16 ab
1.5 ml/L	11.31±0.62 bc	11.65±0.65 bc	10.84±1.02 abc	11.13±1.09 b
2.0 ml/L	10.24±0.97 c	10.28±0.93 cd	10.12±0.55 bc	9.63±0.56 c
2.5 ml/L	10.01±1.00 c	9.45±0.87 d	9.65±0.71 cd	8.54±0.78 c
3.0 ml/L	9.46±0.76 c	8.86±0.59 d	8.32±0.82 d	8.12±0.45 c
SEM	1.15	0.40	0.35	0.48
Sig. (p-value)	<0.001	<0.001	<0.001	<0.001
Chlorophyll stability index (CSI) (%)				
Control	1.57±0.118 a	0.94±0.059 a	1.57±0.118 a	0.94±0.059 a
1.0 ml/L	1.41±0.092 ab	0.87±0.051 a	1.38±0.113 b	0.85±0.082 ab
1.5 ml/L	1.34±0.074 bc	0.84±0.047 ab	1.35±0.127 bc	0.82±0.080 b
2.0 ml/L	1.29±0.123 bc	0.76±0.068 bc	1.27±0.069 bc	0.77±0.045 bc
2.5 ml/L	1.23±0.123 bc	0.7±0.065 cd	1.21±0.090 bc	0.67±0.061 cd
3.0 ml/L	1.17±0.094 c	0.64±0.043 d	1.16±0.114 c	0.62±0.034 d
SEM	0.04	0.03	0.04	0.03
Sig. (p-value)	0.007	<0.001	0.006	<0.001

Data are the means of three replicates ± standard deviation. 'p-value' denoted the significance of differences between the means by one way ANOVA statistics. ^a The values followed by different letters differ significantly by Duncan's multiple range test at p=0.05.

DISCUSSION

One of the most common responses in plants to abiotic stresses is overproduction of different types of compatible organic solutes, which protect the plants from stress injuries by cellular osmotic adjustment, detoxification of ROS, protection of membrane integrity and stabilization of enzymes/ proteins [6]. The antioxidants also protect cellular components from dehydration injury. These solutes include proline, sucrose, polyols, trehalose and quaternary ammonium compounds (QACs) such as glycine-betaine, alanine-betaine, proline-betaine, choline *O*-sulfate, hydroxyproline-betaine and pipercolate-betaine [7].

Amongst the many quaternary ammonium compounds known to accumulate in plants, glycine betaine occurs most abundantly in response to dehydration stress [8, 9]. GB is abundant mainly in chloroplast where it plays a vital role in adjustment and protection of thylakoid membrane, thereby maintaining photosynthetic efficiency [10, 11].

Proline is a five carbon cyclic amino acid, which belongs to glutamate family and accumulates in leaves in large quantities under stress conditions. It has been suggested that free proline synthesized from glutamate serves as an energy donor during

environmental stress [12]. It acts as an osmolyte and a reservoir of carbon and nitrogen. It elevates consistently in numerous plant species under varied range of environmental conditions. The abiotic stress conditions generate superoxide anion and other reactive oxygen species, which induce lipid peroxidation of cell membranes [13-15], causing cellular damages and culminate into death of plant [16]. These ROS are scavenged through the mechanism of non-enzymatic (proline, glycine betaine) and enzymatic (SOD, POD and PPO) antioxidant mechanisms.

Among these antioxidants proline, which is acting as compatible solute or osmoprotectants [17, 18] has very important role in abiotic stress tolerance. It also serves as free radical scavenger [19, 20] and redox potential buffer [21]. Nanjo *et al.* [22] had indicated that proline is an important component of cell wall protein. Bohnert and Jensen [6] indicated very high accumulation of proline (up to 80%) under stress conditions. The biosynthesis and accumulation of proline is important in the determination of growth response of plants towards the imposed stress [23]. The increased or decreased level of proline usually correlates with the magnitude of stress, which governs the physiological status, growth and yield of the plants [16].

The enhanced accumulation of proline may be helping in the above manner to the tomato and brinjal plants exposed to high concentration of chlorpyrifos and malathion. The accumulation of proline has helped the test crops under xenobiotic stress, which might be helping to the test crop to maintain membrane stability, water relations, nitrogen and energy metabolism. It might have also helped to maintain the growth and yield of the pesticide treated plants.

Proline is one of the cyclic amino acids, normally accumulates in higher plants in response to various environmental stresses [24]. Stewart and Hanson [25] are of the opinion that, accumulation of proline in plants is linked with water relations, nitrogen and energy metabolism. An accumulation of proline in a wide variety of plant species under varied kinds of stresses and possible role in adaptive mechanism have been reviewed by Aspinall and Paleg [26]. The accumulation of proline in the cytoplasm is accompanied by a reduction in the concentration of less compactable solutes and an increase in cytosolic water volume [27]. According to Yoshida *et al.* [28] in many plants proline is widely distributed as osmolyte which does not interfere with normal biochemical reactions and acts as osmoprotectant under stress conditions. The proline can be considered as a strong compound supplying reductants, reduced nitrogen and carbon skeleton for post stress recovery [29]. Pratibha and Gupta [30] recorded the effects of insecticide endosulfan on the accumulation of free proline content.

The considerable accumulation of proline in tomato and brinjal treated with chlorpyrifos and malathion is attributed to the xenobiotic stress imposed on treated plants by the pesticides.

The free radicals are constantly generated under stress conditions, which are quenched by an efficient antioxidant network in the plant body. The complex network of such adaptive mechanisms at physiological and molecular levels cause changes in the synthesis and accumulation of various osmolytes, antioxidants and antioxidant enzymes, which provide stress tolerance to the plants [31, 32]. Very few reports are available as this aspect influenced by pesticide applications.

Glycine betaine accumulates in various plants under different stress conditions and serves as an osmolyte by lowering the osmotic potential of the cell and prevents the movement of water from the cell to outside. Thus it helps to prevent the denaturation of macromolecules like proteins and many enzymes. Robinson and Jones [10], Yang *et al.* [33], recorded similar trend regarding accumulation of GB under various abiotic stress conditions. According to Chander *et al.* [15] the increased level of GB protects the cellular proteins, enzymes, cell organelles and membranes

against stress injury. The enhanced GB in tomato and brinjal plants might be helping to protect the cellular proteins, enzymes, cell organelles and membranes against pesticide injury imposed by chlorpyrifos and malathion in tomato and brinjal. GB along with proline may be highly useful to the stressed plants to protect them from cell injuries and metabolic dysfunctioning. The increasing level of GB clearly indicated the increasing stress level with higher concentration treatments of pesticides. These adaptive physiological mechanisms in the plants of tomato and brinjal must be very helpful to them under adverse xenobiotic stress. The denaturation of various macromolecules like protein and many enzymes is avoided by enhanced GB and proline and the plants of tomato and brinjal could survive under the pesticidal stress.

Phenolic compounds play a vital role in the biochemistry of plants. They serve as secondary metabolites and are responsible for the control of oxidative stress. These secondary metabolites indicate intensity of stress and act as defense compounds in plants during biotic and abiotic stresses. They have also a very profound effect on seed germination, growth, development and metabolic functioning of plants.

Treatment of pesticides activated the biosynthesis of phenolic compounds in several plants. Many workers have studied the effect of different pesticides on polyphenol accumulation and biosynthesis in different plants. Karadge and Karne [34] have reported an increase in polyphenols in the leaves of tomato treated with bavistin and calixin. Very scanty information is available about the effect of insecticides on polyphenols. Thirumaran and Xavier [35] reported that 0.01% methyl parathion caused increase in phenol contents of black gram. Kulkarni *et al.* [36] also observed that sprays of methyl parathion and phosphomidon stimulated polyphenol synthesis in tomato, okra and guar. Wang [37] recording stimulation in polyphenol contents due to application of insecticides. The treatments of monocrotophos also exhibited similar results showing pronounced increase in the polyphenol content after spraying and the amount increased with the concentration of monocrotophos.

The researchers like Abbas *et al.* [38] claimed that phenolics inhibit CO₂ dependant O₂ evolution in intact chloroplast. Singh *et al.* [39] reported that phenolic compounds inhibit photosynthesis in intact plants, which results in reduced growth and yield. The decrease in chlorophylls and photosynthetic rate may affect the plant growth. The reduction in growth and yield in tomato and brinjal when treated with high concentration of pesticide can be attributed to enhanced level of phenolics in these plants.

Phenolics interfere with growth and other energy-dependent activities by uncoupling the oxidative phosphorylation. The formations of highly reactive

quinones due to oxidation of phenols inhibit enzymes by complexing with metal ions and reacting with sulphhydryl group of proteins. The phenolic compounds also affect fundamental processes such as photosynthesis, chlorophyll biosynthesis, plant water relations [40], protein synthesis [41] and membrane permeability [42]. The pesticide treated tomato and brinjal show very high accumulation of phenolics, which had influenced negatively the process of photosynthesis, chlorophylls biosynthesis, many enzymes, plant water relations and protein synthesis. These metabolic irregularities created by phenols finally caused the reduction in growth and yield in tomato and brinjal.

Water potential and relative water content (RWC) are two fundamental concepts that characterize water relations of plants and are widely used as indicators for plant water status. RWC indicates the actual content of water in the plant based on maximal water content it can hold at full turgidity. The crop plants are always exposed to different types of biotic and abiotic stress conditions like drought, salinity, temperature and pesticidal pollution (Xenobiotic), leading to the adverse impact on its physiological, biochemical and enzymological processes. These alterations affect its growth, development, flowering, fruiting and yield. The plant water status under any stress condition has predominant role in its survival and growth. The ability of plant to maintain the turgor and related physiological processes even under xenobiotic or other stress conditions has correlation with stress tolerance in terms of osmoregulatory activities [4].

RWC has been identified as a reliable trait for screening drought tolerance in winter wheat (*Triticum aestivum*) [43, 44]. Several studies also indicated positive correlations between grain yield in cereals and RWC [44-46]. RWC was found to be a better tool for evaluating genotypic differences in drought tolerance in soybean compared to water potential [47]. Schonfeld *et al.* [43] found greater genetic variations in RWC. The lower as well as higher concentration treatments of chloropyrifos and malathion had influenced the values of RWC. With increasing concentrations there was decrease in RWC, indicating adverse impact on plant water status, which resulted in to reduced growth, development, flowering, fruiting and yield of tomato and brinjal. Generally, the phenomenon of growth is accompanied by changing water relations and osmotic adjustments leading to build up the turgor pressure, which is involved in extension of cellwall and growth [48]. This may be due to membrane damage causing leakage of water, as a result of changed permeability.

The membrane proteins participate in signal reception and in transport of specific solutes. Hence membrane stability plays an important role in survival of plants under the influence of abiotic stress like pesticides, water, salt etc. Electrical conductivity of cell, which depends on various solutes with different electrical

charges oozing out of the tissue as a result of membrane injury, is used as the basis for studying membrane stability in terms of percent membrane injury or MSI.

A major impact of environmental stress like pesticides is on cellular membrane modification, which may result in an impaired function or total dysfunction in plants. However, the cellular membrane dysfunction due to stress is expressed as increased permeability and leakage of ions, which can be readily measured by the efflux of electrolytes. Hence, the estimation of membrane stability under stress by measuring cellular electrolyte leakage from affected plant tissues into an aqueous medium has been widely used as a screening tool for stress acclimation and tolerance by the plants. Stuart [49] recommended expressing electrolyte leakage as an index percentage of total electrolytes in the tested tissues. Based on Stuart's method Flint *et al.* [50] developed an index of measuring electrolyte leakage for stress injury.

Cellular mechanisms of stress tolerance like MSI, RWC and CSI are important to understand the level of stress and the tolerance capacity of any plant towards the stress to which it is exposed. Any abiotic stress like drought, salt, temperature and pesticides affects various physiological processes both at whole plant and cellular level [51]. Stress affects the membrane integrity [52]. The free radicals produced under stress condition cause lipid peroxidation, inhibit protein synthesis by hydrolysis of m-RNA [53]. The membrane stability is loss due to lipid peroxidation.

Although numerous metabolic processes show change during stress, the membranes are among the first, affected by environmental stresses, and membrane changes may constitute the initial response of a plant to stressful conditions [54]. MSI and RWC are important physiological parameters offering additional mechanisms for abiotic stress tolerance in plants.

MSI is closely associated with abiotic stress tolerance of the plants. The first impact of any abiotic stress is at cell membrane level, disturbing its stability, integrity and normal functioning. The changes in cell membrane permeability are indicated by the degree of stress injury caused by pesticides or the xenobiotic stress to cell membrane of treated plants is indicated by the MSI. The results on MSI corroborate with concentrations of chloropyrifos and malathion used. The physiological injuries to cell membrane result into dysfunctioning of all metabolic processes, growth, development, flowering and yield. MSI is linked with the complex network of reactions in the plant body and once is disturbed almost all the events and steps in pesticide treated plants of tomato and brinjal will be disturbed leading to either death or complete loss in yield.

With increasing concentrations of chloropyrifos and malathion there was decrease in MSI indicating loss in structure and function of the membrane due to

xenobiotic stress generated by pesticides. The results on MSI and RWC had confirmed the negative and toxic impact of pesticides of cell membrane. The destabilization of membrane structure is the basic reason for all metabolic alterations in pesticide treated plants.

MSI decides the extent of membrane perturbations in structure and dysfunctioning in the cellular activities during the stress conditions [55]. The membrane stability index (MSI) is very important parameter that gives idea about the stress tolerance ability of invasive and native weeds. The results recorded in Table 4.8 on MSI of weeds under investigations agree with this. Increase in allelochemicals in these weeds might also be helping the weeds to get more stress tolerance. Membranes are barriers isolating aqueous compartments of the cells and the membrane proteins participate in signal reception and in transport of specific solutes giving them stability and thereby afford stress tolerance to the plants [56]. The higher values of MSI in both the invasive weeds recorded in the present investigation may be having similar role as mentioned above, because of which these weeds are tolerating extreme environmental conditions, survive comfortably and invade successfully in the new habitats. On the contrary the native weeds are not able to tolerate the stress conditions and hence make the place for highly tolerant invasive weeds. This results in to loss of native phytodiversity in that particular ecosystem.

The photosynthetic pigments are the important biochemicals for harvesting the solar energy and converting them into various photoassimilates, which determines the yield and productivity of the crop plants. The abiotic stress induces several types of damages and injuries to chlorophyll molecules and reduces the rate of photo synthesis. The stability of chlorophylls and degradation caused by stress conditions like xenobiotic is highly important to understand the tolerance of plants. The CSI values determine the tolerance or resistance of plants to the stress conditions, as well as it can also predict the degree of loss in yield of the particular crop plant.

As stated by Ali *et al.* [57] the chlorophyll stability index is inversely related to the degree of stress conditions imposed on the plants. The higher treatments of chloropyrifos and malathion in tomato and brinjal were found to be harmful inducing higher degradation of chlorophylls and reducing the values of CSI. Scanty work

is done on this aspect under the influence of pesticide stress or xenobiotic stress. But there are reports available on the impact of drought or salinity stress on CSI.

CONCLUSION

The osmolites or compatible solutes and antioxidants have major role in biotic and abiotic stress tolerance, including xenobiotic stress, created by high concentration of pesticides. The reactive oxygen species (ROS) or active oxygen species (AOS) generated in the plants under stress conditions, cause cellular damages, which finally culminate into their death. But most of the plants tolerate the impact of abiotic stress conditions, through the special type of adaptive mechanisms of osmolites and antioxidants like proline, glycine betaine, phenols, reducing sugars etc. These compounds protect the cell membrane structure, its integrity and functioning. The metabolic processes and biosynthetic pathways in cells are precisely regulated by the accumulation of these osmolytes. Not only this but also the toxic reactive oxygen species are detoxified by these antioxidants. This may be the reason for the significant accumulation of proline, GB and phenols in tomato and brinjal under the high as well as low concentration treatments of chloropyrifos and malathion. The level of accumulation of osmolites and antioxidants was positively correlated with the concentrations of pesticides applied, confirming the positive correlation between the degree of stress and the level of accumulation of such compounds. These adoptive mechanisms helped the plants to survive even under stressful environmental conditions.

The other parameters like RWC, MSI and chlorophyll stability index (CSI) also play the major role in stress tolerance. The RWC decreased with increasing concentrations of pesticides. While MSI and CSI increased with increasing concentrations of pesticides and contribute in tolerance of xenobiotic stress (pesticide stress). For any plant water content in protoplasm, membrane stability and chlorophyll stability are of immense importance for its survival and growth.

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