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# ANTIOXIDANT POTENTIAL OF SOYBEAN (GLYCINE MAX) GROWN UNDER WATERLOGGING STRESS

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## Abstract

Analysis of antioxidant potential was carried out from soybean to determine the effect of herbal extracts (UltraSil and UltraK) and PGRs like GABA (Gamma amino butyric acid), Putrescine, and Biotonic under waterlogged conditions. Foliar sprays of above herbal extracts and PGRs were given at 20 DAP and 45 DAP of soybean seedlings. The foliar application of 0.3% UltraSil, 0.2% UltraK, 10 ppm GABA, Putrescine and 100 ppm Biotonic were applied to both normal and waterlogged plants. It was noticed that $H_2O_2$, $OH^-$ and NOx, radical activities were induced due to waterlogging stress as compared to control. This elevation in $H_2O_2$, $OH^-$ and NOx, radical scavenging activities were due to application of UltraSil, UltraK, GABA, Putrescine and Biotonic. This will helps to improve waterlogging tolerance of sensitive soybean.

**Key words:** $H_2O_2$, $OH^-$ and NOx, Putrescine and GABA.

## Introduction

In many abiotic stresses ROS are the main cause of serious damages to the membrane systems of various cell organelles in plants. According to Shewfelt and Purvis, (1995) during waterlogging ROS causes enzyme inactivation, lipid peroxidation and oxidative damages to DNA. By scavenging of the free radicals it prevents the oxidation of biomolecules. (Shenoy and shirwaikar, 2002). Antioxidative enzymes and low molecular weight metabolites like ascorbic acid, glutathione, proline and glycine betaine are also take part in detoxifying various ROS.

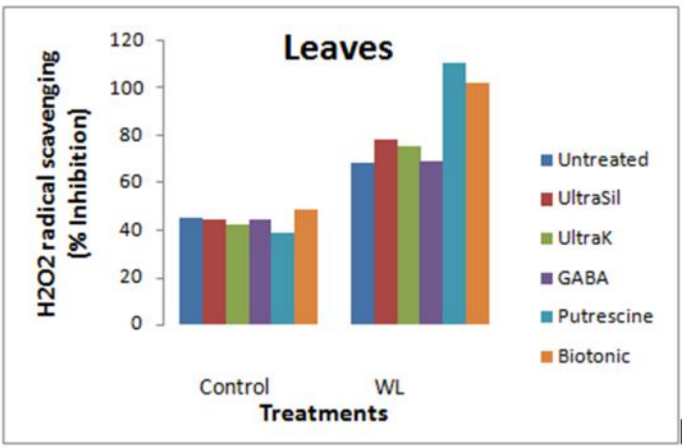
Soybean is an important, protein rich, oil yielding crop. Soybean fulfils the major requirement of protein and minerals in the nutrition of human diet. PGRs have ant oxidative properties that can reduces the effects of stress. Hence an attempt has been made to study the effect of herbal extracts and PGRs on antioxidant potential under waterlogging stress conditions of important oil yielding crop soybean.

## Material and Methods

The present study was conducted in the field of Botany Department at Shivaji University, Kolhapur. Seeds of soybean (Variety JS-335) were sown in the twelve pots with two replications (first 6 with normal irrigation and other 6 with water logging stress). The seeds were allowed to grow for 20 days with equal irrigation of tap water in both the replications. First spray was given at 21st day with 0.3% Ultrasil (Adhatoda vasica, Cardiospermum halicacabum, Embelia ribes and Aqua solvent), UltraK 0.2% (Adhatoda vasica, Catharanthus tinctorius, Embelia ribes and Aqua solvent) and PGR such as 10 ppm GABA, Putrescine and 100 ppm Biotonic formulation (Cystein, Methionine, Lysine, Valine, ABA, vitamins Nicotinic acid and Riboflavin, Saccharides (Myoinositol), Cytokinine (6 BA) and BSA) respectively to the each pot of normal & waterlogged stress. The same sprays were repeated on 35th days flowering stage. After 2nd spray waterlogging stress was applied for 6 days to the second replication (6 spots). The influence of foliar application of0.3% UltraSil, 0.2% UltraK, 10 ppm GABA, Putrescine and 100 ppm Biotonic on radical scavenging potential of soybean were studied separately for normal irrigated and stress pots.The methods given by Anwar et al., (2006) and Sultana et al., (2008) were followed for the preparation of methanolic extracts. The analysis were carried out to find antioxidant potential by using assays like in $H_2O_2$, $OH^-$and NOxradical scavenging activity. $H_2O_2$ radical scavenging activity was determined according to method given by Govindrajan et al., (2003). The method described by Halliwell et al., (1987) was followed to find out $OH_2^-$ radical scavenging activity, and Singh et al., (2009) described the method for estimation of NO scavenging activity was applied.

**Results and discussion:**

The $H_2O_2$ radical scavenging activity was increased in leaf and root tissue of soybean under waterlogging stress. Further foliar applications of herbal extracts and PGRs were also effective to increase in all waterlogged plants of soybean. (Fig. 1)



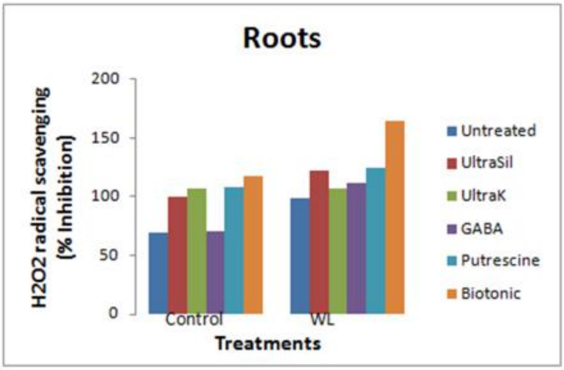
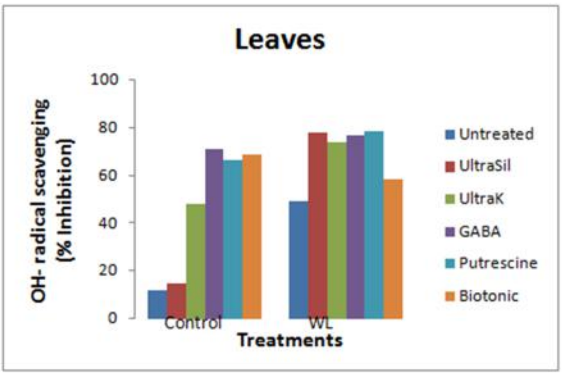



Figure:1. Effect of foliar sprays of UltraSil, UltraK, GABA, Putrescine and Biotonic on $H_2O_2$ radical scavenging activity of the leaves and roots of soybean under unstress and waterlogging condition**.**

Gill and Tuteja, (2010) noticed that at low concentration $H_2O_2$ act as signalling molecule for triggering tolerance to various biotic and abiotic stresses.Waterlogging increases rate of superoxide radicals and their products, $H_2O_2$, can directly attack membrane lipids and inactivate SH-containing enzymes in mungbean and citrus (Ahmed et al., 2002)El-Enany, et al., (2013) noticed that $H_2O_2$ was increased significantly in shoots and roots of cow pea plants due to waterlogging as compared to control. Exogenous application of polyamines reduced the formation and accumulation $H_2O_2$ in Salvinia natans (Mandal et al., 2013)

During waterlogging, the $OH^-$ radical scavenging activity was increased significantly in leaf tissue and decreased slightly in root tissue of soybean. The foliar applications of herbal extracts and growth regulators increases percentage of $OH^-$ radicals scavenging under waterlogging conditions. (Fig. 2)



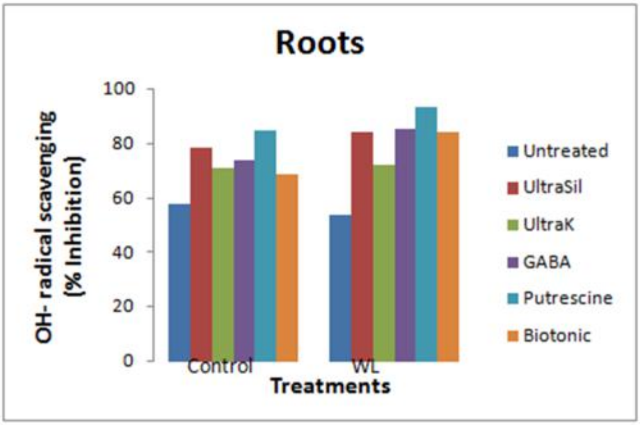
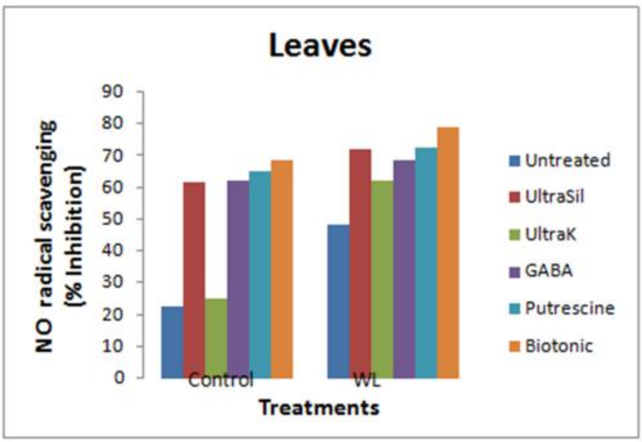



Figure:2. Effect of foliar sprays of UltraSil, UltraK, GABA, Putrescine and Biotonic on OH- radical scavenging activity of the leaves and roots of soybean under unstress and waterlogging condition.

According to Smirnoff and Cumbes, (1989) hydroxyl radicals generated in Fenton type reactions. Hydroxyl radicals causes damages to DNA, proteins and lipids and get neutralize if provided with H atom (Spencer et al., 1994). Ha et al., (1998) reported that polyamines act as scavengers of hydroxyl radicals. GABA application effective in scavenging $OH^-$ radicals (Smirnoff and Cumbes, 1989).

During waterlogging, the NOx radical scavenging activity was increased significantly in leaf tissue and root tissue of soybean. The foliar applications of herbal extracts and growth regulators increase NOx radicals scavenging activity in unstressed and waterlogging conditions. (Fig. 3)



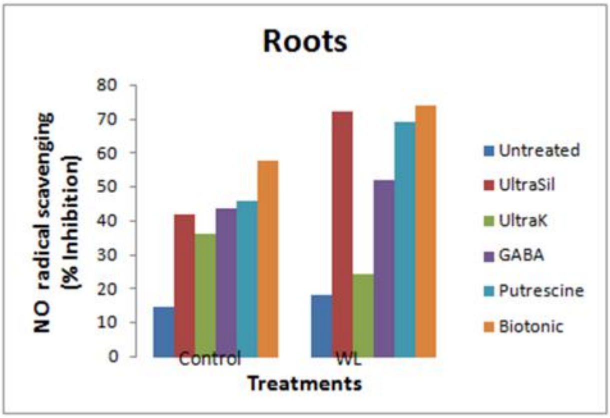



Figure:3. Effect of foliar sprays of UltraSil, UltraK, GABA, Putrescine and Biotonic on NO radical scavenging activity of the leaves and roots of soybean under unstress and waterlogging condition.

Nitric oxide is smaller, unstable and highly diffusible free radical. According to Beligni and Lamattina, (1999) NOx able to destruct ROS and involved in cytotoxic processes mediated by plant tissue. During hypoxia nitrate reduced to Nitric oxide by nitrate reductase and subsequent oxidation through oxyhaemoglobin are important mechanism to maintain plant cell energetics (Igamberdiev and Hill, 2004). Waterlogging induces NOx production in soybean nodules and that NO is generated from bacteriodal denitrification during hypoxia (Meakin et al., 2007)

The foliar application of herbal extracts and growth regulators considerably increases hydrogen peroxide radical scavenging percentage. The higher potential of hydrogen peroxide radical scavenging in root and leaf tissue might be helpful for reduction of $H_2O_2$ production. Thus the overall scavenging of $H_2O_2$ was reduced under waterlogging conditions which might be helpful for reducing its toxic effects on membrane and cell organelles of waterlogged soybean.

The foliar application of herbal extracts and growth regulators shows increase in percentage of OH- radicals scavenging under waterlogging conditions. The higher percentage of OH- radicals scavenging was noticed in waterlogged soybean leaf tissue. The OH- radicals are toxic to the lipid molecules in membranes and damage the integrity membrane structure. Thus the scavenging of OH- radicals under waterlogged conditions might be helpful for the improvement of waterlogging tolerance of soybean.

The foliar application of UltraSil, UltraK, GABA, Putrescine and Biotonic formulation results in an increase in the NO scavenging activity in unstressed and waterlogging leaves and roots of soybean.The elevation in NO scavenging percentage which might be found beneficial for the protection of various biomolecules from its adverse effects.

Increase in $H_2O_2$, $OH_2^{-}$ and NOx radical scavenging activity in waterlogged stressed as well as foliar sprayed waterlogged and unstressed soybean plants. It will found beneficial for the improvement of waterlogging tolerance of soybean and protection of various biomolecules from adverse effects.

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