EFFECT OF PAEONOL ON GROWTH AND METABOLISM IN ORYZA SATIVA L. UNDER ARSENIC STRESS

Chaya Rani[1*], Deepak Kumar Gautam[2], Nancy[2], Gaurav Kumar[2], Adesh Kumar[3], Yeshowardhana[1]

Abstract:
The effect of arsenate with or without paeonol on the growth and metabolism in rice seedlings of Basmati rice was studied. In the test, cv. arsenic was more toxic for root growth, than for shoot growth, where root hairs were less and short, roots were characteristically stubby, brittle and root tips gradually turned brown. Arsenic caused damage to the root epidermal cells and the aerenchymatous cortex. The levels of total chlorophyll, chlorophyll-a, chlorophyll-b and fluorescence intensity were decreased in arsenic treated rice seedlings. Arsenic toxicity affected the activities of different antioxidant scavenging enzymes in the test seedlings. Activities of superoxide dismutase and ascorbic acid oxidase were increased, whereas catalase and catechol peroxidase activities were decreased by arsenic application. In these seedlings, oxidative stress has been observed with arsenic treatments, and the levels of proline and H_2O_2 have increased. The combined application of paeonol and arsenic showed significant alterations on all parameters tested under the purview of arsenic treatment alone, leading to better growth and metabolism in rice seedlings. Thus, the use of paeonol in arsenic contaminated soil may improve the production of healthy rice plants.

Keywords: arsenic; growth and metabolism; amelioration; paeonol; rice

[1*]Department of Chemistry, D.N. College, Meerut (Uttar Pradesh)
[2]Department of Chemistry, Meerut College, Meerut (Uttar Pradesh)
[3]Department of Botany, Keral Verma Subharti College of Science, Swami Vivekanand Subharti University, Meerut (Uttar Pradesh)

***Corresponding Author:** Chaya Rani
*Department of Chemistry, D.N. College Meerut (Uttar Pradesh)

DOI: 10.48047/ecb/2023.12.si5a.0540

Introduction

In recent years, toxic metals have received considerable attention as a consequence of the increased environmental pollution from industrial, agricultural, energetic, and municipal sources. Arsenic contamination is wide- spread due to geologic and anthropogenic activities such as melting operation, fossil fuel combustion (Nriagu and Pacyna 1988) and arsenic based agro- chemicals, fertilizer and disposal of municipal and industrial wastes (Requejo and Tena 2006). It is one of the world's most serious ecological toxicants entering the cell cycle through topographical and anthropogenic activities (Chowdhury *et al*., 2018) with an elevated concentration in drinking water (McCarty *et al*., 2011) particularly in South East Asia, causing carcinogenicity (Chandrakar*et al*., 2016; Mehmood, *et al*., 2017) and other chronic diseases to millions of human beings and animals *via* plants (Zhu *et al*., 2019).

Rice is one of the main crops in India particularly in West Bengal and arsenic contamination of rice is a newly uncovered disaster on a massive scale (Meharg 2004), where millions of humans are at risk of arsenic poisoning due to drinking of arsenic-contaminated water (Pearce 2003). Further, irrigation of soils with arsenic-contaminated water significantly elevates the arsenic levels in the soil. Its everely affects the growth and development of plants, and causes toxicity resulting in various biochemical and physio-logical disorders (Li et al. 2006). Constant arsenic toxicity is related to a few sorts of chronic infections including skin, bladder, lung, liver, kidney malignant growth (Waalkes*et al*., 2004) in people, while in plants arsenic up-take adversely affects the plant metabolism and lead to bring about different physiological and biochemical variations from the norm (Li *et al*., 2006) and hinders the development of plants in various manners (Singh *et al*., 2015), for example, restraintof seed germination, lessened development in the root and shoot (Tang and Miler, 1991), Low yield of foods grown from the ground (Marin *et al*., 1992). Arsenic is among the toxic metals concentrated to the generation of ROS such as superoxide radical (O2-), hydroxyl radical (OH) and hydrogen peroxide (H2O2) in different cell frameworks (Shahid *et al*., 2017) and causes oxidative stress (Shi *et al*., 2004), which bringing about cell damage by enacting oxidative flagging pathways. These reactive oxygen species can be constrained by increasing the production of various enzymes such as SOD, APX, GPX, CAT, glutathione reductase and externally supplied antioxidants especially polyphenolic compounds.

Paeonol is a major bioactive ingredient of cortex moutane (Paeonia suffruticosa) which is utilized as a homegrown cure in treating different infections. It has an assortment of corrective impacts including its antibacterial (Rehman and Mairaj, 2013), antifungal (Mairaj*et al*., 2016), mitigating,cancer prevention agent, (Sun *et al*.,2004), invulnerable framework strengthing, antimutagenic activities mutagenic activities (Hsieh *et al* 2006), antidiabetic (Valensi*et al*., 2005). It improved liver capacity in hepatoxic rodents (Bendong*et al*., 2012), upgraded blood flow through its inhibitory consequences for both platelet conglomeration and blood coagulation (Abraham *et al*.,2003), brought down histopatho-logical scores and lessened myelope-roxidase-receptivecells of polymorphonuclear neutrophils accumulation.

Inthecurrent examination,we study the role of pretreatment of rice seeds with paeonol extracted from the root of paeony suffruticosa plant weinvestigatedthearsenic induced toxicity in rice (*Oryza sativa* L.) by studying the morphological and anatomical changes, the degree of oxidative damages, changes in the level of chloroplasticpigments and oxidative enzymes.

Materials and methods

Chemical

Chemicals used for the analysis were of analytical grade and purchased from Sigma-Aldrich/ Merck. Paeonol was extracted from the root of *Paeonia suffruticosa* plant.

Isolation of paeonol (2-hydroxy-4-methoxy acetophenone)

The root of the *Paeonia suffruticosa* plant is sterilized with 70% ethanol followed by 0.1% mercuric chloride ($HgCl_2$), shade dried and powdered. Then 50 g of the dried powdered root was transferred into a conical flask containing a sufficient amount of ethanol and macerated for about seven days. The dried plant extract was analyzed for the qualitative analysis of its secondary metabolites by standard protocols.The active phenolic component (Paeonol) was isolated from other secondary metabolites of dried root extract by steam distillation (Chen *et al*., 2017). The distillate is collected set at 4ºC overnight. The crystal was dried in a silica gel desiccator (m.p. 50ºC) and characterized by different parameters and spectral analysis.

Plant material and treatments

Rice (*Oryza sativa* L.) seeds Basmati obtained from the Indian Agriculture Research Institute (IARI) New Delhi, were surface sterilized with $HgCl_2$ (0.1%, w/v) for about 30 seconds, after that seeds were washed 2-3 times by double distilled water and devided into two group. One group of seeds that will later be treated with only arsenic were soaked in double distilled water while another group of seeds was soaked in paeonol concentration range 10, 50 and 100 μM for pretreatment for one day. These seeds were then transferred to Petri-dishes and kept in the culture room at 27ºC for 5 days in dark condition for proper germination. The seedlings were grown in a medium (Dave *et al*., 2013) for 10 days before treatment and then exposed to AsIII concentration range 0, 10, 50 and 100 μM for a weak at 27ºC in a humidified culture chamber. All treatments were in triplicate. After a weak all plants were harvested, rinsed by double-distilled water and used for different parameters.

Morphological and anatomical studies

The root and shoot lengths of arsenic treated andand joint application with paeonol rice seedlings were measured followed by anatomical studies. The sections were stained with 0.25% safranin (w/v) for tissue differentiation and mounted in 10% glycerine (v/v) and observed under light microscope and photographed with a digital camera fitted to a microscope.

Chlorophyll and carotenoid contents

Total chlorophyll, chlorophyll-a and chlorophyll -b contents were measured from the rice leaves according to Arnon (1949). For this, 1 g of fresh leaves were extracted with 25 ml 80% acetone (v/v) and chlorophyll contents were estimated spectrophotometrically at 645 nm and 663 nm. The chlorophyll contents were expressed in terms of mg chlorophyll present g^{-1}

Antioxidant enzymes assay and oxidative stress markers assay

Treated and untreated rice seedling samples were homogenized in a chilled mortal with 3 ml 0.1 M Na3PO4 buffer at seven pH having 1.0% polyvinyl polypyrrolidone, 1.0 mM disodium-EDTA and 0.5 M sodium chloride.The amalgamate were centrifuged at 10,000 rpm about 15 minute at 4□C andthe supernatant was utilized to determine APX, SOD, CAT, GPX activities. The ascorbate peroxidase was estimated according to Nakano and Asada (1987) The superoxide dismutase was measured as per Beauchamp-Fridovich (1971) *via* assessing the inhibition of the reduction of NBT-dye by superoxide dismutase anion. The GPX-activity was estimated as per Hammer Schmidt *et al*., 1982. The catalase was assessed according to Aebi (1984) The malondialdehyde content was assessed according to theimproved method of Hodges (1999).

Statistical analysis

All investigations were conducted triplicate in structure. Analysis of variance (ANOVA) was done with all the data to confirm the variability of data and validity of results and Duncan's multiple range test (DMRT) was performed to determine the significant difference between treatments at ($p<0.05$).

RESULTS AND DISCUSSION

The level of pigment concentration and antioxidants [APX, CAT, SOD, GPX] of seven days oldpaeonol pre-treated rice seedling, exposed to applied concentrations (100 μM, 50 μM and 10 μM) of arsenite, paeonol and their dose treatment of paeonol with As exposure were shown in Fig 1 and 2.

Effect of arsenic on seedlings growth and root anatomy Arsenic exposure to the growth of rice seedlings caused a significant reduction in root and shoot lengths (Figure 1). This inhibitory effect was more prominent on root than on shoot length. The root length was decreased significantly in 50 mM and 100 mMarsenic treatments which was more than 47% reduction, on average. In contrast, the reduction in shoot length was comparatively lower amounting to 32% reduction, on an average.When rice seedlings were treated with arsenic along with paenol, rootas well as shoot lengths were increased as compared to only arsenic treatments (Figure 1) and those were 12% and 5% increments on an average in root and shoot growth, respectively.

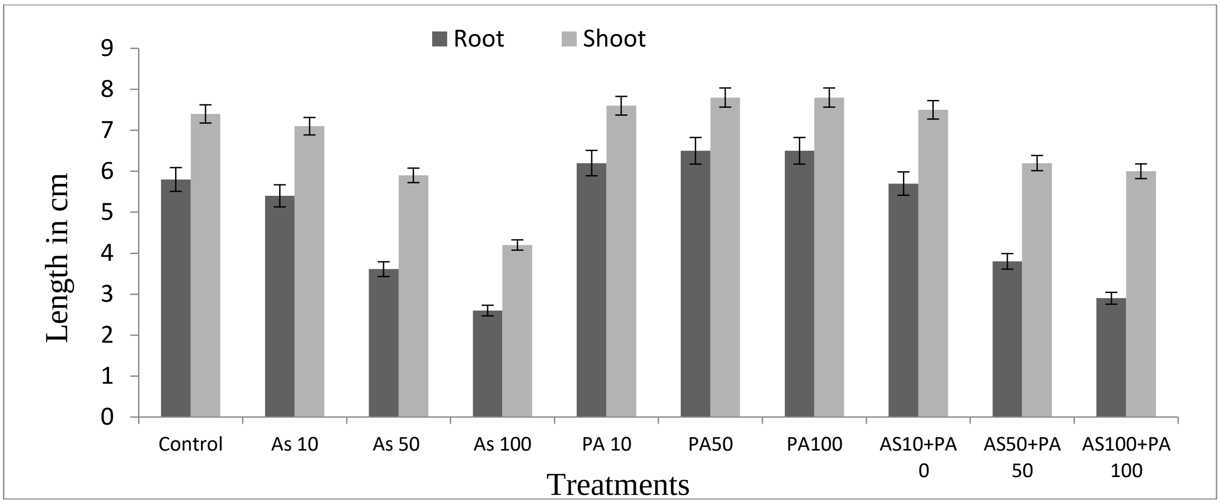


Figure1. Effect of arsenate and paeonol on root and shootlength in rice (cv. MTU 1010) seedlings.

Effect on pigment contents

There was a linear decrease in the levels of total chlorophyll, chlorophyll-a, chlorophyll-b, under increasing concentrations of arsenic treatments. Compared to water control, the pigment contents decreased, on an average,by 32% fortotalchlorophyll,30%forchlorophyll-a,24%for chlorophyll-b (Table 1). But when treated with paenol it was observed that increase a significant level in total chlorophyll, chlorophyll a and chlorophyll b content increased as compared to water control.

It was further observed that in case of joint application of paeonol with arsenic, total chlorophyll, chlorophyll a and chlorophyll b content were increasedinall(arsenicpaeonol)treatedsamplesas compared to samples treated with arsenic alone (Table 1),

Table 1. Effect of arsenate ($Na_2HAsO_4.7H_2O$) and paeonol ($C_9H_{10}O_3$) applied either singly or in combination on the chlorophyll contents in rice (cv. MTU 1010) seedlings. The data were recorded from seven-day-old rice seedlings.

Treatments	Total Chl (mg g^{-1}f w)	Chl-a(mg g^{-1}f w)	Chl-b (mg g^{-1}f w)
Control	0.2290	0.1190	0.0490
Arsenate 10 (µM)	0.1990	0.1090	0.0480
Arsenate 50 (µM)	0.1390	0.0720	0.0390
Arsenate 100 (µM)	0.1290	0.0690	0.0250
Paeonol10 (µM)	0.2310	0.1290	0.0690
Paeonol 50 (µM)	0.2330	0.1290	0.0690
Paeonol 100 (µM)	0.2330	0.1290	0.0690
Arsenate 10 (µM) + Paeonol50 (µM)	0.2160	0.1270	0.0490
Arsenate 50 (µM) + Paeonol 50 (µM)	0.1690	0.1050	0.0470
Arsenate 100 (µM) +Paeonol 50 (µM)	0.1490	0.0800	0.0310

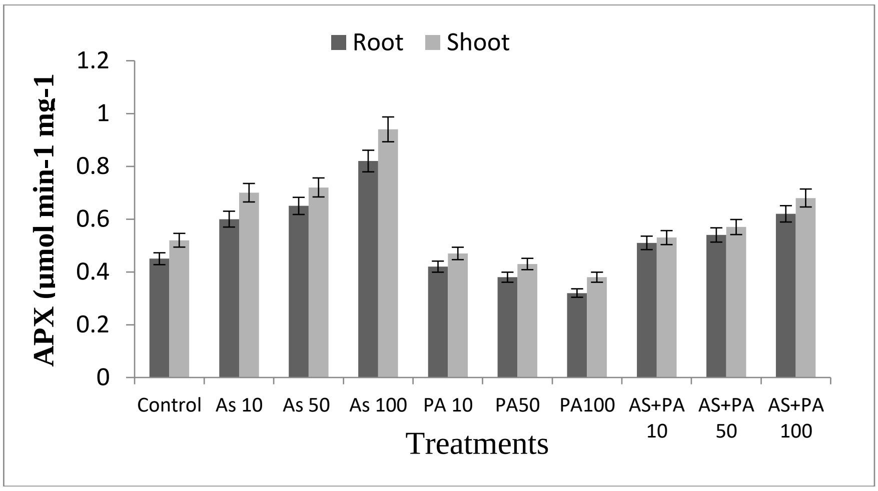
Effect of antioxidant

The APX increased by 33%, 44% and 82% in roots while in same pattern increase in shoot with treated 10 µM 50 µM and 100 µM,respectively as compared to control with As accumulation. Upon paeonol pretreatment under the same day and same concentration as As treatment, the APX decreased in root and shoot as compared to control and positively correlated with As accumulation. Upon dose treatment of paeonol along with As the increased level of APX in root and shoot was reduced as compared to only As treated rice seedling under the same concentration only and was positively correlated with As accumulation was shown (Fig 2a).

The CAT increased very high in root and shoot at 7 d pre-treatment with 100 µM, 50 µM,10 µM As respectively and was positively correlated with As accumulation. Upon paeonol pretreatment under the same condition, the CAT decreasedin roots and shoots as compared to control.Upon those treatment of paeonol along with As the increased level ofCAT in root and shoot was reduced as compared to As treated rice seedling under the same concentration(Fig 2b)..

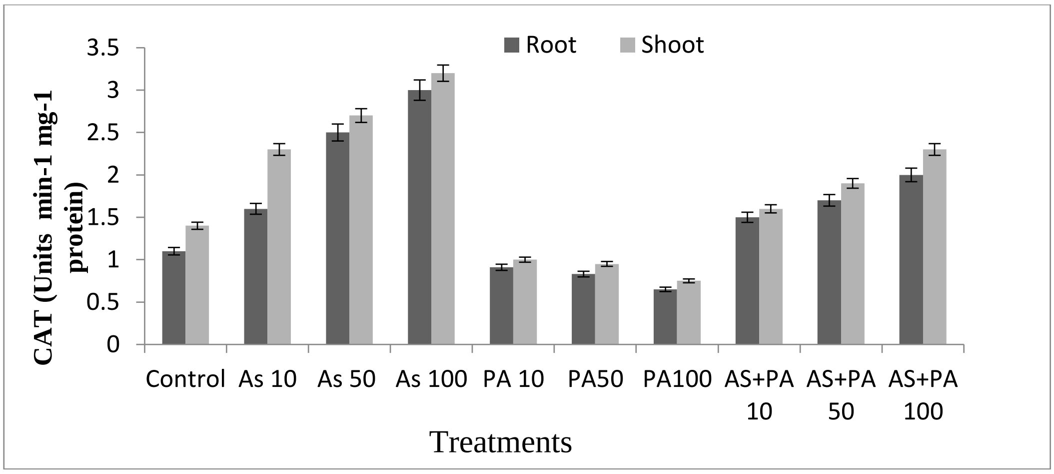
The GPX increased very high in root and shoot at 7 d pre-treatment with 100 µM, 50 µM,10 µM As respectively and was positively correlated with As accumulation. Upon paeonol pretreatment under the same condition, the GPX decreased in roots and shoots as compared to control.Upon those treatment of paeonol along with As the increased level of GPX in root and shoot was reduced as compared to As treated rice seedling under the same concentration (Fig 2c).

The SOD increased very high in root and shoot at 7 d pre-treatment with 100 µM, 50 µM,10 µM Asrespectively and was positively correlated with As accumulation. Upon paeonol pretreatment under the same condition, the SOD decreased in roots and shoots as compared to control.Upon those treatment of paeonol along with As the increased level of SOD in root and shoot was reduced as compared to As treated rice seedling under the same concentration (Fig 2d)..



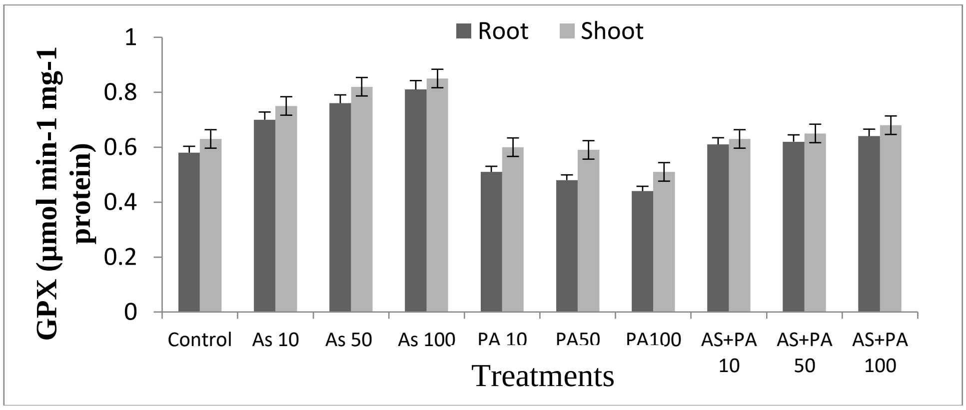
(a) APX

Figure2 (a). Effect of arsenate and paeonol APX contents in rice (cv. MTU 1010) seedlings.



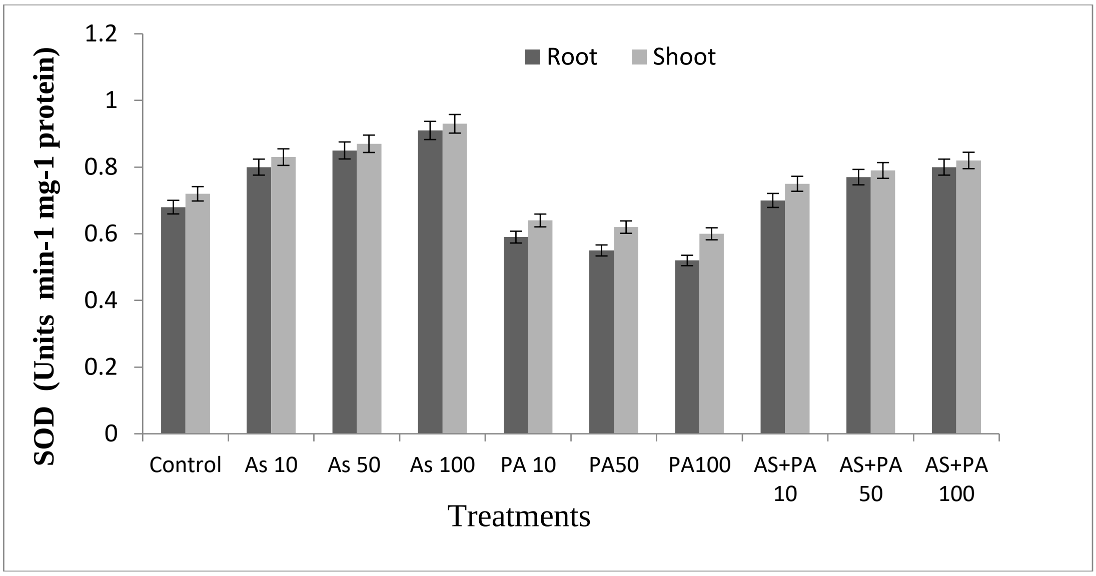
(b) CAT

Figure2(b). Effect of arsenate and paeonol CAT contents in rice (cv. MTU 1010) seedlings.



(c) GPX

Figure2(c). Effect of arsenate and paeonol GPX contents in rice (cv. MTU 1010) seedlings.



(d) (SOD)

Figure2(d). Effect of arsenate and paeonol SOD contents in rice (cv. MTU 1010) seedlings.

CONCLUSION

In view of the outcomes, it inferred that the assimilation of As by rice plant roots and shoots was fundamentally decreased within the sight of paeonol, prompting an expansion in plant growth and biomass, which may clarify on the formation of a non-poisonous complex with paeonol. Pigment content (total chlorophyll, chlorophyll a and chlorophyll b) decreased with supplement by arsenic.The oxidative stress caused by arsenic may increase the activities of antioxidative enzymes like ascorbateperoxidase (APX), glutathione peroxidase (GPX) catalase (CAT) and superoxide dismutase (SOD). Paeonol supplementation with arsenite causes a significant decrease in antioxidentlevels, which shows the mitigating impact of paeonol on oxidative stress achieved by arsenic. Therefore paeonol plays a protective role against the oxidative stress brought about by arsenic. It concluded that toxic arsenic, liable for oxidative stress may be converted to the non-lethal complex by the interactionpaeonol and play an important role in plant growth under arsenic stress.

Acknowledgment: This work is a part of the PhD. Research program of Chaya Rani. We thankfull to the Management of D.N. College, Meerut for the facilities they provided for conducting the research**.**

Reference

1. Aebi, H. (1984). Catalase *in vitro*. Methods in Enzymology. 105: 121-126. https://doi.org/10.1016/S0076-6879(84)05016-3.
2. Chandrakar, V., Parkhey, S., Dubey, A., Keshavkant, S. (2017b). Modulation in arsenic-induced lipid catabolism in G*lycine max* L. using proline, 24-epibrassinolide and diphenylene iodonium. Biologia. 72: 292-299.
3. Chandrakar, V., Naithani, S.C., Keshavkant, S. (2016). Arsenicinduced metabolic disturbances and their mitigation mechanisms in crop plants: A review. Biologia. 71: 367-377.
4. Chowdhury, M.T.A., Deacon, C.M., Steel, E., Imamul Huq, S.M., Paton, G.I., Price, A.H., Williams, P.N., Meharg, A.A., Norton, G.J. (2018). Physiographical variability in arsenic
5. dynamics in Bangladeshi soils. Sci. Total Environ. 612: 1365-1372.
6. Hodges, D.M., DeLong, J.M., Forney, C.F., Prange, R.K. (1999). Improving the thiobarbituric acid-reactive-substances assay as for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. Planta. 207: 604-611.
7. Li WX, Chen TB, Huang ZC, Lei M, Liao XY. 2006. Effect of arsenic on chloroplast ultrastructure and calcium distribution in arsenic hyperaccumulator *Pteris vittate* L. Chemosphere. 62:803−809.
8. Gautam, A., Pandey, K.A., Dubey, S.R. (2019). Azadirachta indica and *Ocimum sanctum* leaf extracts alleviate arsenic toxicity by reducing arsenic uptake and improving the antioxidant
9. system in rice seedlings. Physiol. Mol. Biol. Plants.
10. McCarty, K.M., Hanh, H.T., Kim, K.W. (2011). Arsenic geochemistry and human health in South East Asia. Rev. Environ.

Health. 26: 71-78.

11. Nriagu JO, Pacyna JM. 1988. Quantitative assessment of worldwide contamination of air, water and soils by trace metals. Nature. 333:134−139.
12. Requejo R, Tena M. 2006. Maize response to arsenic toxicity as revealed by proteome analysis of plant shoots. Proteomics. 6:156−162.
13. Mehmood, T., Bibi, I. Shahid, M., Niazi, N.K., Murtaza, B., Wang, H., Ok, Y.S., Sarkar, B., Javed, M.T., Murtaza, G. (2017). Effect of compost addition on arsenic uptake, morphological and physiological attributes of maize plants grown in contrasting soils. J. Geochem. Explore. 178: 83-91.
14. Zhu, N., Qiao, J., Yan, T. (2019). Arsenic immobilization through regulated ferrolysis in paddy field amendment with bismuth impregnated biochar. Sci. Total Environ. 648: 993-1001.
15. Meharg AA. 2004. Arsenic in rice-understanding a new disaster for South-East Asia. Trends Plant Sci. 9: 415−417.
16. Pearce F. 2003. Arsenic's fatal legacy grows. New Sci. 179:4−5.Raghothama KG. 1999. Phosphate acquisition. Annu Rev Plant Physiol Mol Biol. 50:665−693.
17. Marin AR, Masscheleyn PH, Patrick WH. 1992. The influence of chemical form and concentration of arsenic on rice growth and tissue arsenic concentration. Plant Soil. 139:175−183.
18. Requejo, R., Tena, M. (2006). Maize response to arsenic toxicity as revealed by proteome analysis of plant shoots. Proteomics. 6: S156-162.
doi: 10.1002/pmic.200500381.
19. Shahid, M., Shamshad, S., Rafiq, M., Khalid, S., Bibi, I., Niazi, N.K., Dumat, C., Rashid, M.I. (2017). Chromium speciation, bioavail ability, uptake, toxicity and detoxification in the
20. soil-plant system: A review. Chemosphere. 178: 513-533. [CrossRef] [PubMed].
21. Singh, A.P., Dixit, G., Mishra, S., Dwivedi, S., Tiwari, M., Mallick, S., Tripathi, R.D. (2015). Salicylic acid modulates arsenic toxicity by reducing its root to shoot translocation in rice
22. (*Oryza sativa* L.). Front. Plant Sci. 6: 340.
23. Shi, H., Shi, X., Liu, K.J. (2004). Oxidative mechanism of arsenic toxicity and carcinogenesis. Mol. Cell Biochem. 255: 67-78.
24. Srivastava, S., Sinha, P., Sharma, Y.K. (2017). Status of photosynthetic pigments, lipid peroxidation and antioxidative enzymes in *Vigna mungo* in presence of arsenic. J. Plant Nutr. 40: 298-306.
25. Tiwari, S., Sarangi, B.K. (2017). Comparative analysis of the antioxidant response by *Pteris vittata*and *Vetiveriazi zanioides* towards arsenic stress. Ecol. Eng. 100: 211-218.
26. Tang T, Miller DM. 1991. Growth and tissue composition of rice grown in soil treated with inorganic copper, nickel, and arsenic. Commun Soil Sci Plant Anal. 22:2037−2045.
27. Waalkes, M.P., Liu J., Ward, J.M., Diwan, B, A. (2004). Mechanisms underlying arsenic carcinogenesis: Hypersensitivity of mice exposed to inorganic arsenic during gestation.
28. Toxicology. 198: 31-38.
29. Rehman, F. and Mairaj, S. (2013). Antimicrobial studies of allicin with paeonol and its oxime. International Journal of Pharma and Bio-sciences. 4(1): 240-249.
30. Mairaj, S, Rehman, F., Jetley, U.K. (2016). Comparative Antifungal activities of specified and modified secondary metabolites of *Paeonia suffruticosa* Root extract against aflatoxigenic fungus-*Aspergillus flavus* under different condition. World Journal of Pharmaceutical Research. 5(11): 1401-1417.
31. Sun, Y.C., Shen, Y.X., Sun, G.P. (2004). Advances in the studies of major pharmacological activity of paeonol. Chin. Tradit. Pat. Med. 26: 579-582.
32. Hsieh, C.L., Cheng, C.Y., Tsai, T.H., *et al*. (2006). Paeonol (Extracted from *Paeonia suffruticosa* Root) pretreatment lends antioxidant protection against arsenite stress in rice (*Oryza sativa* L.) seedlings. Journal of Ethnopharmacology. 106 (2): 208-215.
33. Hsieh, C.L., Cheng, C.Y., Tsai, T.H., *et al*. (2006). Paeonol (Extracted from *Paeonia suffruticosa* Root) pretreatment lends antioxidant protection against arsenite stress in rice (*Oryza sativa* L.) seedlings. Journal of Ethnopharmacology. 106(2): 208-215.
34. Bendong, C., Mingliang, N. and Guangshhun, Y. (2012). Effect of paeonol on antioxidant and immune regulatory activity in *Hepatocellular carcinoma* rats. Molecules. 17: 4672-4683.
35. Abraham, E. (2003). Neutrophils and acute lung injury. Critical Care Medicine. 31(4): S195-9. DOI: 10.1097/01.CCM. 0000057843. 47705.E8.
36. Chen, Y., Sun, S.K., Tang, Z., Liu, G., Moore,

K.L., Maathuis, F.J.M., *et al*. (2017). The nodulin 26-like intrinsic membrane protein OsNIP3; 2 is involved in arsenite uptake by lateral roots in rice. J. Exp. Bot. 68.11.3007-3016. 12: e017368. 101093/jxb/erx165.

37. Dave, R., Tripathi, R.D., Dwivedi, S., Tripathi, P., Dixit, G., Sharma, Y.K., Trivedi, P.K., Corpas, F.J., Barroso, J.B., Chakrabarty, D. (2013). Arsenate and arsenite exposure modulate antioxidants and amino acids in contrasting arsenic accumulating rice (*Oryza sativa* L.) genotypes. J. Hazard. Mater. 262: 1123-1131.
38. Arnon DI. 1949. Copper enzyme in isolated chloroplast. Plant Physiol. 24:1−15.
39. Nakano and Asada, K. (1987). Purification of ascorbate peroxidase in spinach chloroplast as inactivation in ascorbate depleted medium and reactivation by monodehydroascorbate radical. Plant and Cell Physiology. 28: 131-140.
40. Beauchamp, C., Fridovich, I. (1971). Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Anal. Biochem. 44: 276-287.
41. Hammerschmidt, R., Nucles, E.M. and Kuc, J. (1982). Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. Physiological Plant Pathology. 20: 73-82.