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Effect of Imidacloprid on Germination and Biochemical Nature of Cowpea *(Vigna unguiculata* L. Walp) and Sunflower *(Helianthus annuus* L.)

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ABSTRACT

Indiscriminate use of pesticides in agricultural fields affect the soil quality. One of the effective remedies to reduce excessive pesticides in soil is use of plants that can degrade pesticides. Present work was performed to study the effect of imidacloprid on seed germination and Biochemical parameters of Cowpea (*Vigna unguiculata* L. Walp) and Sunflower (*Helianthus annuus* L.). Proteins, Carbohydrates, Stress indicators and Phenolics of plants increased with increase in imidacloprid concentration. Photosynthetic pigments reduced slightly in treated plants. The results strongly suggest that Cowpea and Sunflower can thrive well in high imidacloprid concentrations with meager changes in their biochemical compositions. Thus, these plants can be potential members of the phyto-restoration system which can be used to reduce imidacloprid in soils. Farmers can undertake cultivation of these plants on an imidacloprid contaminated soil to restore its quality.

Key words : *Imidacloprid, Cowpea, Sunflower, Phyto-restoration*

Introduction

Introduction of modern agricultural practices have resulted in contamination of agricultural soils with pesticides.They remain in soil and affect its quality and can be toxic to animals and non-target plants (Aktar *et al.,* 2009). The ill effects of pesticide application can be seen in the form of stunted growth, chlorosis, necrosis of plant and formation of Reactive Oxygen Species (Shakir *et al.* 2016; Sharma *et al.* 2018, 2020).

Imidacloprid (a neonicotinoid) is a systemic, moderately hazardous pesticide that is used to protect plants from insect manifestations (Stoner and Eitzar, 2013). Some control measures should be taken to prevent the excessive spread of this pesticide in soil. In order to select a plant which can remediate soil efficiently, properties like root depth, rhizosphere microflora, climate and the nature of contaminant are very crucial (Chirakkara *et al.* 2016). Screening of plants at seed germination stage is important as they are more susceptible to pesticide exposures at this stage (Tian *et al.* 2014).

The present research was done to study the effect of imidacloprid on seed germination and biochemical parameters of Cowpea (*Vigna unguiculata* L. Walp) and Sunflower (*Helianthus annuus* L.). This study will help to find out the potential role of these plants in removal of pesticides which accumulate in soil.

Materials and Methods

Plant seeds, imidacloprid treatment and growth

Plant seeds

Cowpea (*Vigna unguiculata* L. Walp) and Sunflower (*Helianthus annuus* L.)viable seeds were procured from College of Agriculture, Pune.

Selection of Imidacloprid concentrations

A survey of famers was done to confirm the usage of imidacloprid on agricultural fields. The concentrations used in the study were taken higher than the recommended dosage as we wanted to check the effect of high imidacloprid concentration on the two plants (Table 1).

Growth Experiments

Ten seeds of each plant were germinated in petri plate on a germination paper moistened with different imidacloprid solutions (treated seeds) and distilled water (control seeds). Plates were kept in 65% humidity at 28±2°C for ten days. This experiment was performed in triplicates and seed germination was calculated on daily basis while the biochemical parameters were determined on the 10th day of experiment.

Germination studies

Seed germination of each plant was measured using Final Germination Percentage (FGP) as described by Kader (2005).

IC_{50} value determination of plants

The IC50 values of imidacloprid for seed germination of plants were calculated using linear regression method (Shakir *et al.* 2016).

Biochemical tests

All the protocols for biochemical analysis were performed after doing sufficient standardization experiments using a Double beam UV-Vis Spectrophotometer (Spectroscan UV2700, Chemito).

Estimation of Protein content

Protein content was estimated as described by Lowry *et al.* (1951) where Bovine Serum Albumin was used as a standard protein.

Estimation of Proline Content

Proline content was found out as described by Bates *et al.,* (1973). A standard graph of Proline amino acid was used to calculate the proline concentration in plant samples.

Estimation of Total Carbohydrate content

Total carbohydrate content was estimated as described by Scott and Melvin's (1953). D-glucose was used as a standard sugar.

Estimation of Catalase Enzyme

Catalase test was performed as described by Shakir *et al.,* (2018).

Estimation of Total Phenolic content

Total phenolic content was estimated by Folin-Ciocalteau colorimetric method according to Lee *et al.* 2014 and Phuyal *et al.* (2020). Gallic acid was used for standard assay.

Estimation of Photosynthetic Pigments

Total chlorophyll content was estimated as described by Arnon, 1949. Anthocyanin content was estimated as described by Macinelli, (1984).

Data analysis

A complete randomized block design was used to perform all the experiments. Data analysis was done using ANOVA and student t test at 0.05% level of significance using SPSS software version 23.

Results and Discussion

Effect of Imidacloprid exposure on Seed germination

Germination of Cowpea was minimum in the control. It recovered its FGP by 12%, 14%, and 11% at

Table 1. Different doses of imidacloprid (ppm) used in study

Recommended Dose for plants	Four times higher than Recommended dose	Eight times higher than recommended dose	Sixteen times higher than recommended dose	Thirty-two times higher than recommended dose
40	160	320	640	1280

160 ppm, 320 ppm and 1280 ppm respectively as compared to control. Sunflower had a variable response asit recovered its FGP at 160 ppm and 640 ppm by 17% and 14% respectively and then showed a reduction by 4% and 37% at 320 ppm and 1280 ppm respectively when compared to control (Figure 1). Stevens *et al.* (2008) reported that imidacloprid did not have any adverse effect on rice if applied in the pre-germinated stage, however prolonged exposure affected the rice crop quality.

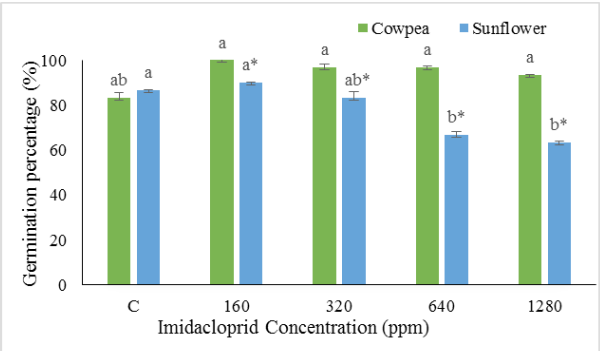

Fig. 1. Final Germination Percentage of two plants. Each bar is the mean value of triplicates and error bar shows the SD. Alphabets denote significant difference among the treatments as per ANOVA and Asterisk (*) denote significant difference among the plants as per students t test at 0.05% significance level.

IC_{50} values of plants

Based on germination percentage, IC50 values of imidacloprid for Sunflower and Cowpea were found out to be 614.58 and 629.0 ppm respectively. For further studies, the IC50 value of imidacloprid was used along with one concentration below and one concentration above it.

Effect of imidacloprid on Biochemical parameters of plants

Pesticide overdose causes an increase in the Plant's Protein content

The protein synthesis in imidacloprid exposed Cowpea and Sunflower was more as compared to control plants (Figure 2). It was seen to increase more in Cowpea as compared to Sunflower. In Cowpea and Sunflower, proteins increased by 63% and 57% at 640 ppm and by 61% and 65% at 1280 ppm respectively. These results differ from the ones reported by Shakir *et al.* (2018) which reported a decrease in protein content with increased imidacloprid concentration. The probable reason for higher protein content in plants exposed to pesticides is may be due to enhanced detoxification pathways and requirement by cell to repair its damaged membranes due to stress (Alharby *et al.* 2019).

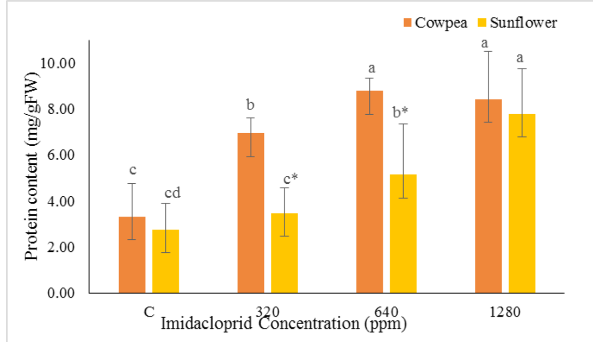

Fig. 2. Protein content in two plants. Each bar is the mean value of triplicates and error bar shows the SD. Alphabets denote significant difference among the treatments as per ANOVA and Asterisk (*) denote significant difference among the plants as per students' t test at 0.05% significance level.

Imidacloprid stress increases Proline content of plants

Imidacloprid increasedproline levels in plants at all the applied concentrations. It was seen to be more in Sunflower as compared to Cowpea. The proline content increased by 33%, 46% in Cowpea and 41%, 47% in Sunflower at 640 ppm and 1280 ppm respectively (Figure 3). Rise in proline content of imidacloprid treated plants can be associated with the activationof enzyme activities and increase in

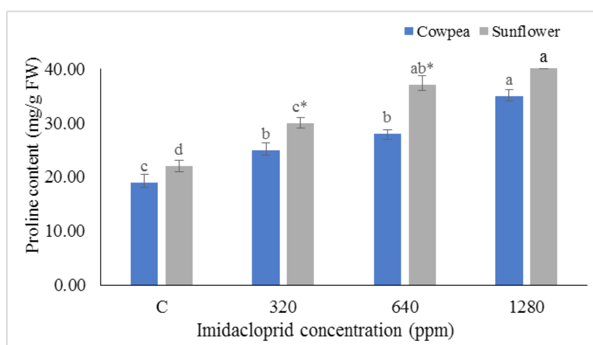

Fig. 3. Proline content in two plants. Each bar is the mean value of triplicates and error bar shows the SD. Alphabets denote significant difference among the treatments as per ANOVA and Asterisk (*) denote significant difference among the plants as per students' t test at 0.05% significance level.

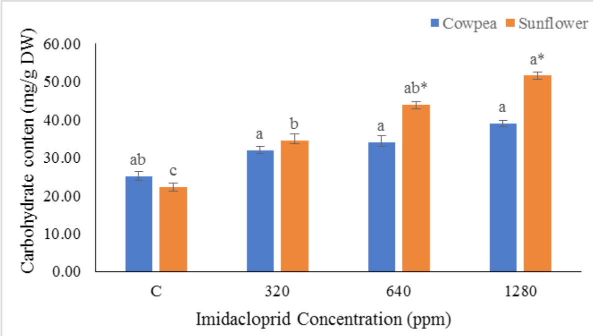

Fig. 4. Carbohydrate content in two plants. Each bar is the mean value of triplicates and error bar shows the SD. Alphabets denote significant difference among the treatments as per ANOVA and Asterisk (*) denote significant difference among the plants as per students' t test at 0.05 % significance level.

Phenolic content (Silva *et al.* 2018). Carbohydrate content regulates proline synthesis and causes its accumulation. It has been reported that increased proline content leads to an increased Catalase activity (Kaur and Asthir, 2015). Protection of Protein integrity, stabilization of proteins and protein aggregation are some of the important functions of proline (Fedotova, 2019).

The Carbohydrate content of plants increased with increase in imidacloprid stress

One of the indications that plants are under some kind of stress is increase in carbohydrate content. In the above study it was observed that, the application of pesticides to plants in increasing concentrations increased the carbohydrate content. This increase was more prominent in Sunflower than Cowpea (Figure 4). Rise in Sunflower carbohydrates was seen by 40%, 52%, 57% against 20%, 27% and 36% in Cowpea at 320 ppm, 640 ppm and 1280 ppm respectively.

The imidacloprid application alters the Catalase activity

When exposed to elevated levels of imidacloprid, Catalase increased in both the plants. The highest increase in Catalase activity in Cowpea was by 68% at 1280 ppm imidacloprid while in Sunflower it was by 60% (Figure 5). To protect the plants from harmful effects of oxidative stresses the enzymatic antioxidants get activated and stored in high amounts (Foyer and Shigeoka, 2011). Consistent with these facts, the results of the present work show profound

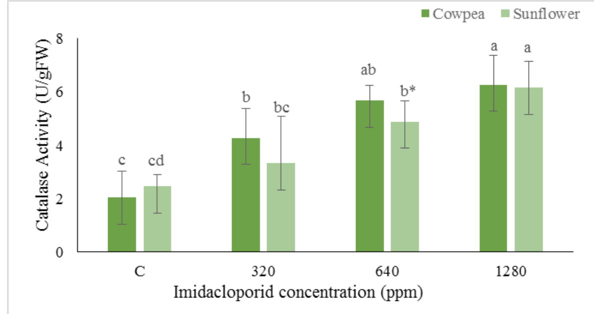

Fig. 5. Catalase activity in two plants. Each bar is the mean value of triplicates and error bar shows the SD. Alphabets denote significant difference among the treatments as per ANOVA and Asterisk (*) denote significant difference among the plants as per students' t test at 0.05% significance level.

biochemical changes in Cowpea and Sunflower in response to imidacloprid.

Effect of imidacloprid stress on Plants' Phenolic content:

Plants treated with imidacloprid showed an increase in the phenolic content in both the treated plants. Phenolic content of Cowpea and Sunflower increased by 70% at 1280 ppm. Maximum Phenolic content was seen in Sunflower at 640 ppm imidacloprid and in Cowpea it was seen at 1280 ppm (Figure 6). One of the possible reasons for increased phenolic content in plants is due to activation of phenylpropanoid pathway which is a producer pathway of phenols by carbohydrates (Homayoozadeh *et al.* 2020). Hence, an increase in total carbohydrates and Phenolic contents in imidacloprid treated plants show that these param-

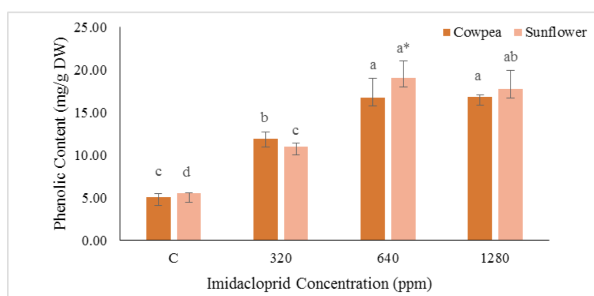

Fig. 6. Phenolic content in two plants. Each bar is the mean value of triplicates and error bar shows the SD. Alphabets denote significant difference among the treatments as per ANOVA and Asterisk (*) denote significant difference among the plants as per students' t test at 0.05% significance level.

eters can be interrelated with each other.

Effect of Imidacloprid on Photosynthetic pigments

Chlorophyll content was reduced by 4%, 35%, 38% in Cowpea and by 8%, 25%, 33% in Sunflower at 320, 640 and 1280 ppm imidacloprid when compared to control (Figure 7). Liang *et al.* 2015 reported increased chlorophyll content in oilseed rape with increase in imidacloprid concentration.

Anthocyanin content of Cowpea reduced maximum to 40% at 1280 imidacloprid concentration and in Sunflower the reduction was about 54% (Figure 8). Overall observations suggests that increased imidacloprid concentrations did not have a major negative impact on production of chlorophyll pigments in both the plants and the highest Chlorophyll

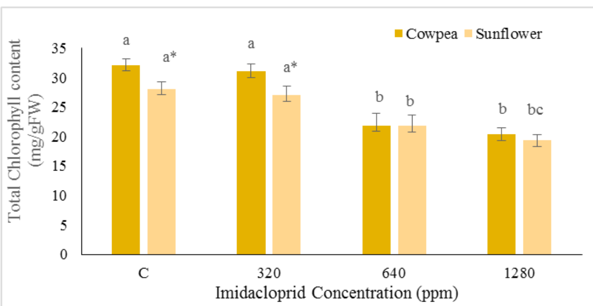

Fig. 7. Total chlorophyll content in two plants. Each bar is the mean value of triplicates and error bar shows the SD. Alphabets denote significant difference among the treatments as per ANOVA and Asterisk (*) denote significant difference among the plants as per students' t test at 0.05% significance level.

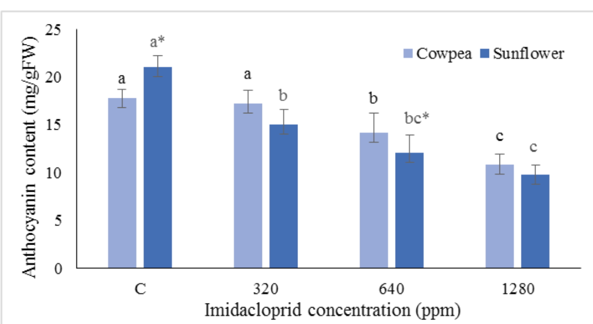

Fig. 8. Anthocyanin content in two plants. Each bar is the mean value of triplicates and error bar shows the SD. Alphabets denote significant difference among the treatments as per ANOVA and Asterisk (*) denote significant difference among the plants as per students' t test at 0.05% significance level.

and Anthocyanin content was seen in control plants. However, Sharma *et al.* 2016, 2017 reported increased pigment storage capacity of plants in order to alleviate imidacloprid induced toxicity.

Conclusion

The seed germination responses and biochemical tests of two plants viz. Cowpea (*Vigna unguiculata* (L.) Walp.) and Sunflower (*Helianthus annuus* L.) were studied in presence of imidalcoprid. The FGP of Cowpea increased with more imidacloprid while in Sunflower it reduced slightly at high imidacloprid concentration. To reduce the detrimental effects of imidaclorpid exposure, plants enhanced their basic biochemical parameters. The results of experiment strongly suggest that both plants may be programming their primary and secondary metabolite production in order to tolerate imidacloprid (Zhang *et al.* 2022). Our work underlines that fact that imidacloprid tolerant plants can be used as phytoremediators to reduce imidacloprid pollution in soil. Further studies needs to be done on molecular level to understand the exact tolerant mechanism in these plants.

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