CHAPTER 5: TOXICITY ASSESSMENT

5.1. General

The toxicity of a pesticide is influenced by a variety of factors. Pesticide toxicology takes into account the parameters such as dosage, exposure time, general health, organism biology, stresses on populations of living things, and environmental conditions (Connon et al., 2012). Pesticides end up in the soil in significant quantities after application, where filtering processes, like immobilization by clay minerals and organic matter, chemical breakdown, and microbial degradation, protect groundwater and surface water (Gavrilescu, 2005; Sarkar et al., 2021). The highest risk of acute or long-term harm is present when pesticides are administered directly to water sources or large areas of land. Under typical environmental conditions, persistent pesticides do not easily degrade and pose the greatest risk of negative consequences (Damalas and Eleftherohorinos, 2011; Rani et al., 2021). Imidacloprid may affect aquatic species and humans since it can be absorbed by crops and consequently enters the food chain. Only around 0.1% of the pesticides applied are believed to reach the target organisms, while the rest contribute to environmental pollution (Nayak & Solanki, 2021).

To assess the toxicity caused by imidacloprid, eco-toxicological studies have been conducted by a number of researchers (Zhang et al., 2014). Understanding the interactions between the biota and abiota of environmental systems as well as the threats associated with the introduction of organic and inorganic compounds into those systems is the main emphasis of ecotoxicology. To assess the toxicity of chemicals like drugs and pesticides in vitro, cytotoxicity assays and cell viability analysis on cultured cells are commonly used.

The toxicity of imidacloprid can be significantly decreased through biological degradation; however, total detoxification is not possible, which may be due to the presence of metabolites or partial mineralization of the drug. The decrease in toxicity following the breakdown of pesticides and other similar toxic compounds has not been the subject of many studies. Imidacloprid reduction has been studied through eco-toxicological assays, including tests on seed germination in plants, bioluminescent bacteria, and L929 mammalian fibroblast cell lines.

5.2. Phytotoxicity analysis

Although plants are generally not the target organisms of insecticides, they can nonetheless penetrate plant tissues when sprayed. The vulnerability of plants to chemical damage varies widely between and within species and is regulated by several factors.

5.2.1. Germination assay

The study aimed to assess the impact of imidacloprid on the growth of Cicer arietinum seeds. Four distinct samples, namely T1, T2, T3, and T4, were analyzed to evaluate their phytotoxicity. The sample T1, comprised of distilled water, served as the control. T2 represented the sample after undergoing degradation by the bacterial consortium CS1, and T3 was obtained after degradation by isolated bacteria, *Tepidibacillus decaturensis* strain ST1. The sample T4 was the untreated sample, containing 200 ppm of imidacloprid. Before the phytotoxicity assay, the samples were collected and filtered using a 0.22 μm syringe filter. The analysis revealed substantial removal of imidacloprid, with 84% and 80% removal from samples T2 and T3, respectively. The study found that imidacloprid exerted a detrimental effect on the germination process; however, a reduction in its toxicity was observed following bacterial degradation. Notably, the germination percentage was highest in the control sample (T1), followed by the sample treated with the consortium (T2), *Tepidibacillus decaturensis* strain ST1 (T3), and the untreated sample (T4). Furthermore, the root lengths of *Cicer arietinum* seeds in the case of sample T4 measured 3.6 ± 0.1 cm, while those of T2 recorded a length of 4.1 ± 0.2 cm, comparable to the control at 4.2 ± 0.1 cm. The root length for sample T3 was found to be 4.1 ± 0.1 cm. Additionally, the shoot lengths of Cicer arietinum treated by T1, T2, T3, and T4 were 14.2 ± 0.1 cm, 14.1 ± 0.1 cm, 14.2 ± 0.2 cm, and 13.4 ± 0.2 cm, respectively. The data obtained in the study have been presented in Table 5.1.

Table 5.1: Plant parameters examined during germination assay

S. No.	Sample	Germination%	Root length (cm)	Shoot length (cm)
1.	T1	100	4.2 ± 0.1 cm	14.2 ± 0.1 cm
2.	T2	95	4.1 ± 0.2 cm	14.1 ± 0.1 cm
3.	T3	95	4.1 ± 0.1 cm	14.2 ± 0.2 cm
4.	T4	80	3.6 ± 0.1 cm	13.4 ± 0.2 cm

The study shows that imidacloprid contamination reduces seed germination due to toxic by-products formed during its breakdown. Seeds exposed to untreated imidacloprid had a germination rate of 80%, indicating toxicity. However, seeds grown in water treated with bacteria had a much higher germination rate of 95%, suggesting that the bacteria effectively reduced the toxicity by

breaking down imidacloprid and its harmful by-products. This demonstrates the potential of bioremediation, using microorganisms to clean up environmental contaminants, as an effective and eco-friendly method to detoxify pesticide-contaminated environments. Higher seed germination rates improve crop yields and agricultural productivity while also protecting soil and water quality. The findings support using bioremediation techniques to manage pesticide pollution and promote sustainable agriculture.

5.2.2. Pot experiments

In addition to seed germination, the toxicity of imidacloprid was also examined through *Cicer arietinum* plant grown in soil. The germination rate of *Cicer arietinum* seeds was examined at varying imidacloprid concentrations. The morphological characteristics of plants, anti-oxidative enzyme assay, as well as the photosynthetic pigments (chlorophyll and carotenoid) of *Cicer arietinum*, were highly affected due to the treatment with different concentrations of imidacloprid. The various parameters examined during the study have been presented in Table 5.2.

Data showed that the shoot and root length were significantly decreased as compared to the control. Data analysis showed that the shoot and root length of *Cicer arietinum* treated with distilled water (S1) were 22.3 ± 0.4 cm and 6.2 ± 0.2 cm, respectively. Further, the shoot and root lengths were found to decrease with an increase in imidacloprid concentration, as presented in Table 5.2. The maximum shoot length and root length were found in control. It can be inferred from the results obtained that the toxicity increased with an increase in imidacloprid concentration.

The RL (Root Length)/SL (Shoot Length) ratio, which is crucial for maintaining a functional balance between photosynthesis and root water uptake, significantly decreased after treatment with imidacloprid, in a dose-dependent manner. The ratio of root and shoot length was found to be highest in the case of control and gradually decreased with increasing concentration, as shown in Table 5.2.

The growth performances of *Cicer arietinum* seeds varied according to different treatments. Similarly, the fresh and dry weights of roots treated by S1, S2, S3, S4 and S5 were recorded. The fresh and dry weights of the shoot system were obtained as (547.5 ± 1.2 and 74.2 ± 0.4), (550 ± 0.5 and 74.5 ± 0.2), (546.6 ± 0.4 and 73.8 ± 0.6), (465.3 ± 0.6 and 59.0 ± 0.4), and (416.4 ± 1.4 and 51.3 ± 0.5) for samples S1, S2, S3, S4 and S5 respectively. Similarly, the fresh and dry weights of the root system were also estimated and the results obtained have been presented in Table 5.2.

Table 5.2: The plant parameters examined during pot experiments at different concentrations of imidacloprid

S. No.	Parameter	Experimental results				
		S1	S2	S3	S4	S5
1.	Root length (cm)	6.2 ± 0.2	6.1 ± 0.1	5.7 ± 0.2	5.2 ± 0.1	4.4 ± 0.2
2.	Shoot length (cm)	22.3 ± 0.4	22.1 ± 0.2	21.8 ± 0.1	20.2 ± 0.4	17.5 ± 1.0
3.	RL/SL ratio	0.278	0.276	0.261	0.257	0.245
4.	Shoot dry weight (mg)	74.2 ± 0.4	74.5 ± 0.2	73.8 ± 0.6	59.0 ± 0.4	51.3 ± 0.5
5.	Root dry weight (mg)	20 ± 0.2	19.7 ± 0.4	19.8 ± 0.2	16.3 ± 0.1	14.8 ± 0.6
6.	Shoot fresh weight (mg)	547.5 ± 1.2	550.2 ± 0.5	546.6 ± 0.4	465.3 ± 0.6	416.4 ± 1.4
7.	Root fresh weight (mg)	141.5 ± 0.1	140.2 ± 0.1	140.6 ± 0.2	119.5 ± 0.1	105.4 ± 0.5

The % shoot elongation was found to be 100% in S1 and S2. However, with an increase in imidacloprid concentration, it was found to decrease gradually and was found to be 99.10% in S3 and 91.40% in S4, which further reduced to 80.20% in S5. Similarly, the % root elongation was also calculated for all the samples. The results obtained in the study indicate a gradual reduction in % root elongation with an increase in imidacloprid concentration, as presented in Table 5.3. In the control sample (S1), maximum germination was observed. The germination process was inhibited by imidacloprid at high dosages. The germination percentage was found to be inversely proportional to the imidacloprid concentration. It was found to be 100% in the case of S1 and reduced gradually to 55% in S5. The results obtained have been presented in Table 5.3.

Furthermore, the vigor index (VI) was also significantly affected by the elevated imidacloprid level (Table 5.3). A dose-dependent decrease in VI was noticed as imidacloprid concentration was increased. The vigor index was also calculated for all the samples. The increased imidacloprid

level adversely influenced the vigor index of *Cicer*. It was found to be maximum in S1 and minimum in S5. The % phytotoxicity (P%) was calculated separately for and shoots. It was observed that the % phytotoxicity increased with an increase in imidacloprid concentration in the case of roots as well as shoots. In the control, the P% of imidacloprid was 0, but with an increase in imidacloprid concentration, a statistically significant increase in P % was observed. It was found to be 1.61% in S2, 6.55% in S3, 8.77 % in S4 and 17.30 % in S5.

Table 5.3: Values of various parameters of *Cicer arietinum* plant obtained at different concentrations of imidacloprid

S. No.	Parameter	Experimental results				
		S1	**S2**	**S3**	**S4**	**S5**
1.	% shoot elongation	100	100	99.1	91.4	80.2
2.	% root elongation	100	98.38	93.44	91.22	82.69
3.	% germination	100	95	95	80	55
4.	Vigor index	2230	2099	2071	1616	962
5.	% phytotoxicity (root)	-	1.61	6.55	8.77	17.30
6.	% phytotoxicity (shoot)	-	0.89	1.35	7.33	13.36
7.	Tolerance index	100	98.38	91.93	83.87	69.35
8.	Germination index	100	98.38	88.77	72.98	45.48

Similar trends were observed in the tolerance index of seedlings treated with imidacloprid, as shown in Table 5.3. The results of this study show that the germination index (GI) of Cicer seeds decreased in a dose-dependent manner when treated with varying concentrations of imidacloprid. As the concentration of imidacloprid increased, the GI decreased significantly (as shown in Table

5.3). The G% decreased from 100% in the control to 88.77% at 150 ppm, further decreasing to 72.98% at 300 ppm, and finally to 45.48% at 500 ppm.

Stress tolerance index is a quantitative metric or indicator that measures the ability of plant species to tolerate and withstand a range of environmental stresses. Stress tolerance in plants exposed to varying concentrations of imidacloprid was calculated using various parameters. In the present study, stress tolerance index was calculated for root length, shoot length, fresh and dry root weight as well as fresh and dry shoot weight. The data obtained have been shown in Table 5.4

Table 5.4: Stress tolerance index in case of roots and shoots calculated at different concentrations of imidacloprid

S. No.	Parameter	Experimental results				
		S1	**S2**	**S3**	**S4**	**S5**
1	Root length STI	100	98.38	91.93	83.87	69.35
2.	Shoot length STI	100	99.1	97.75	90.58	78.47
3.	Root fresh weight STI	100	99.29	99.29	84.39	74.46
4.	Shoot fresh weight STI	100	100	99.63	84.85	75.91
5.	Root dry weight STI	100	98.5	99	81.5	74
6.	Shoot dry weight STI	100	100	100	79.51	69.13

The findings of the present study are consistent with earlier research that indicate negative impacts of imidacloprid on germination and early development of different crops, for example, rice (Stevens et al., 2008). A recent study, reported that high concentration of imidacloprid adversely affects the seed germination and growth of roots in onion (Fioresi et al., 2020). Additionally, the effects of imidacloprid may vary among different plant species (Li et al., 2019). Even though the treated samples were shown to have much lower phytotoxicity than the untreated sample, considerable variability was observed in the phytotoxicity experiments, which suggests that these assays cannot be used for precise quantification of toxicity.

Different types of pesticides exhibit negative impacts with a varying degree on the seed germination of various plants such as tomato, maize, Cowpea, and *Typha latofolia* (Shakir et al.,

2016). Seed germination of *Vigna unguiculata* treated with imidacloprid is increased at low concentration and decreased with higher concentration. This phenomenon could be attributed to the highly toxic effects of insecticides on meristematic cells at high concentrations (Bragança et al., 2018). In another study, seed germination of the tomato plant was decreased due to treatment with a high concentration of imidacloprid (Touzout et al., 2021). The seed germination of tomato was completely inhibited at the concentration of 160, 240, 2000, and 500 ppm from emamectin benzoate, lambda-cyhalothrin, imidacloprid, and alpha-imidacloprid, respectively after 27 h.

5.2.3. Enzyme activity assay

Plants possess an intricate and highly efficient antioxidant system that consists of enzymes such as CAT (catalase), POD (peroxidase), and SOD (superoxide dismutase). This system helps to mitigate and repair the damage caused by ROS (reactive oxygen species), which are highly reactive molecules that can cause oxidative stress in plants. There is mounting evidence to suggest that pesticides can be degraded by the elevated activity of oxidoreductase enzymes in plants. This reflects the level of toxicity to plants and their ability to combat stress caused by external factors. The oxidoreductase enzymes work by breaking down the pesticide molecules into less harmful compounds, thereby reducing the overall toxicity of the environment.

The use of pesticides can affect the physiology of plants in different ways. They can activate or inactivate various biochemical pathways in target and non-target plants. When plants are exposed to pesticides, they respond by activating their antioxidant defense systems. This activation happens through both enzymatic and non-enzymatic pathways. However, the toxicity of pesticides can cause oxidative stress to plants by producing ROS.

Antioxidant activities were estimated by measuring activity levels of catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD). Studies have revealed that pesticide application at higher concentrations significantly elevated ROS levels and caused membrane damage, increased cell injury and reduced cell viability both in root and shoot tissues compared with non-treated plants (Shakir et el., 2018). To cope with pesticide-induced oxidative stress, a significant increase in levels of antioxidants was observed in the plants exposed to higher doses of pesticides (Touzout et al., 2021).

In the current investigation, Cicer arietinum seedlings exposed to samples S1, S2, S3, S4, and S5 demonstrated statistically significant increase in activity of the anti-oxidative enzymes, including

CAT, POD, and SOD, indicating that enzyme activity increased with increase in the concentration of imidacloprid. It can be observed that the enzyme activity in the case of sample S2 is comparable to that of the control, which indicates the reduction in phytotoxicity due to the bacterial remediation of imidacloprid in S2.

5.2.3.1. Catalase activity (CAT)

The CAT activity in *Cicer arietinum* seedlings exhibited a significant dose-dependent increase. CAT activity was minimal in sample S1, which then increased gradually with increasing concentration of imidacloprid, and was found maximum at 500 ppm in sample S5 (Figure 5.1).

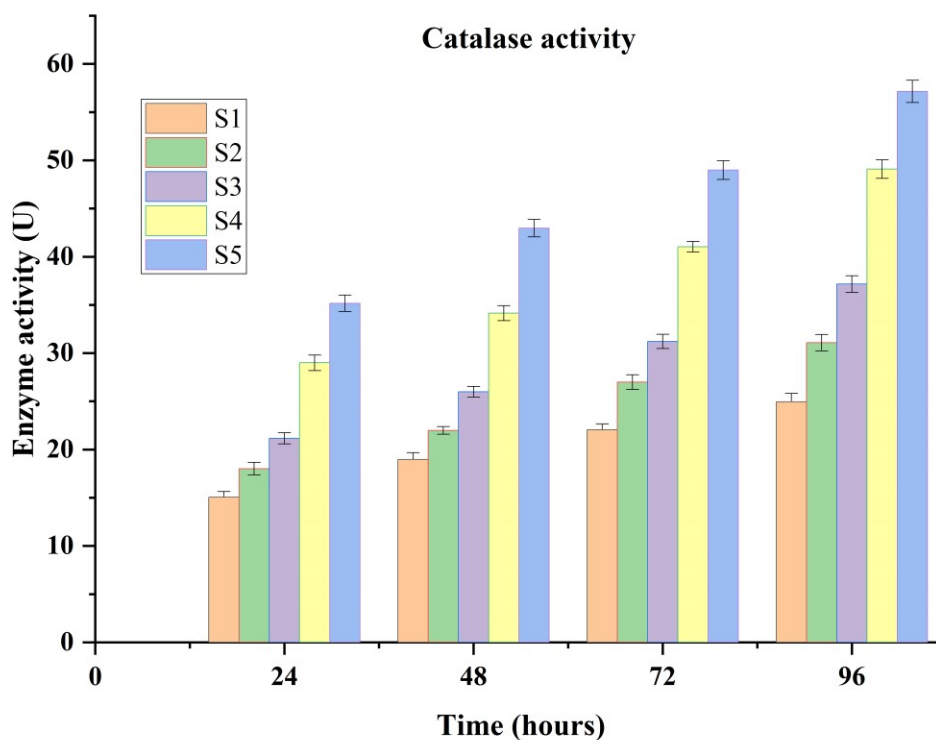


Figure 5.1: Anti-oxidative enzyme assay for catalase

5.2.3.2.Peroxidase activity (POD)

POD, an antioxidative enzyme involved in the removal of ROS, is another indicator of oxidative damage in plants. POD breaks down H_2O_2 and facilitates lignin production when H_2O_2 is present (García-Caparrós et al., 2021). POD activity increased dose-dependently as compared to the

control. The significance of POD in the detoxification of H_2O_2 during insecticide-induced oxidative stress is supported by its noticeable increase in activity, according to Shahid et al., 2021. POD activity of *Cicer arietinum* showed a gradual significant increase from S1 to S5 (Figure 5.2).

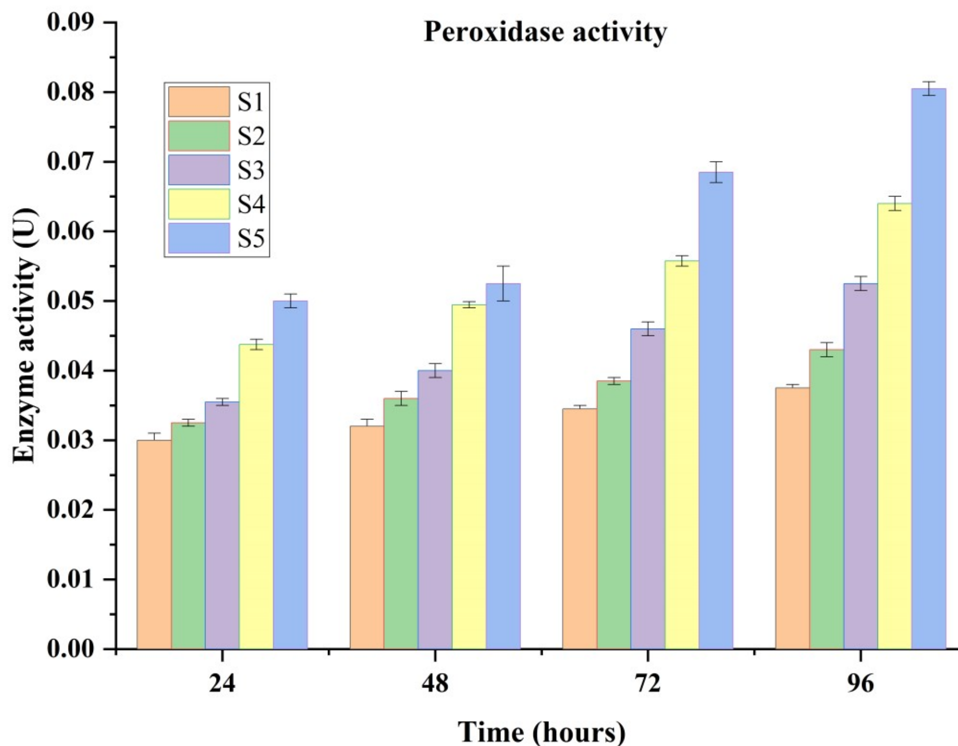


Figure 5.2: Anti-oxidative enzyme assay for peroxidase

5.2.3.3.Superoxide dismutase activity (SOD)

The Superoxide dismutase (SOD) enzyme activity assay was performed to study the toxic effect of treated and untreated samples with increasing imidacloprid concentration on *Cicer arietinum* plants. In contrast to the control, SOD activity considerably increases in the sample S5, due to the formation of superoxide anions in the sample thus triggering a defense mechanism, resulting in increased SOD activity. SOD activity was reduced the sample S4, as compared to S5, which further reduced in S3 and S2, eventually making it comparable to that of the control. The graphical representation of SOD activity is given in Figure 5.3.

The antioxidant defense system of plants is highly reliant on SOD. Free radicals may dismutate into H_2O_2 through the action of the SOD enzyme. The dose-dependent SOD activity was observed in the present study. This suggests that increased SOD enzymatic activity was induced by O_2 scavenging.

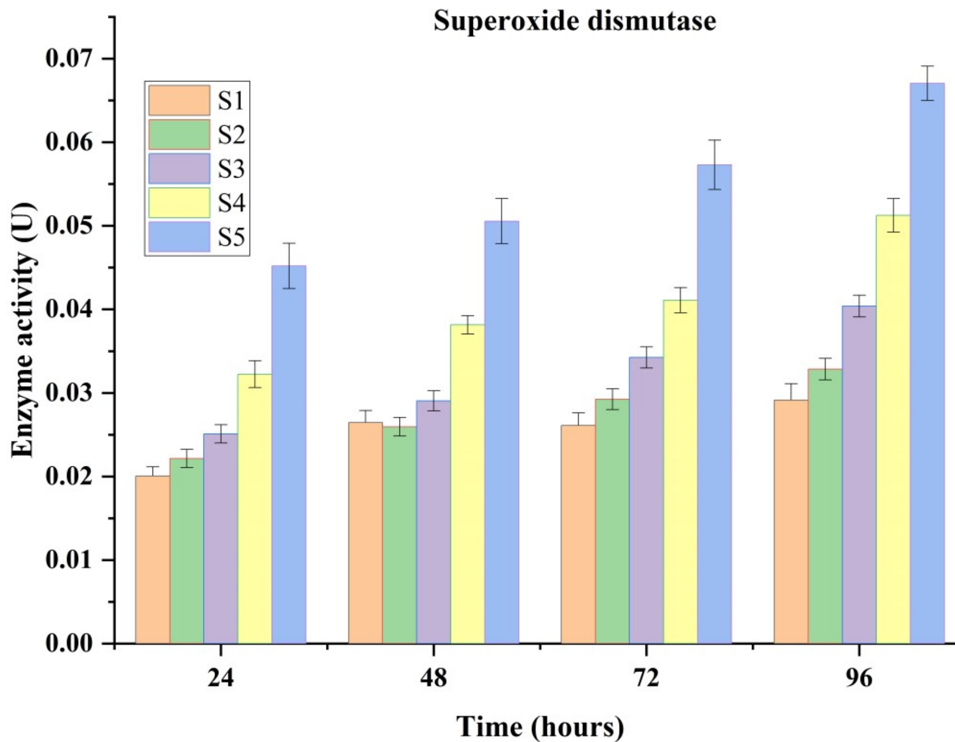


Figure 5.3: Anti-oxidative enzyme assay for superoxide dismutase

It can be concluded that *Cicer arietinum* seedlings in the presence of imidacloprid demonstrated a strong dose-dependent inhibitory effect on a number of growth parameters as well as explained the function of the imidacloprid metabolizing anti-oxidative enzyme system. To a certain amount, the increase in anti-oxidative enzymes can mitigate the effects of imidacloprid, although it varies between plant systems and among different pesticides (Wang et al., 2016). Evaluation of the level of phytotoxicity of particular pesticides and the capacity of the plant system to mitigate the effect are necessary due to the increasing use of pesticides over time, their persistence in the environment, and the gradual accumulation of their breakdown products (Zhang et al., 2020).

In order to investigate the differences in the obtained data, one-way ANOVA (Analysis of Variance) was used to test the significance of the data at a 0.05 level of significance.

In a nutshell, the results demonstrate that the growth and development of *Cicer arietinum* plants are significantly inhibited. This adverse consequence could be attributed to the decreased ability of the plant to utilize the environment and obtain the nutrients required for normal growth, which resulted in a range of phytotoxic indicators and a decline in biometric parameters, especially in the highest concentrations, as a result of imidacloprid toxicity (Ajermoun et al., 2022). The results obtained in the present study are comparable to the study conducted by Sharma et al., on imidacloprid toxicity in *Brassica juncea* L (Sharma et al., 2016).

5.2.4. Plant photosynthetic pigments

Chlorophyll content is essential for the production of organic molecules like proteins and carbohydrates in addition to promoting growth through the process of photosynthesis (Benavente-Valdés et al., 2016). In *Cicer arietinum* leaves, the impact of imidacloprid on total chlorophyll was investigated. The samples S1, S2, S3, S4 and S5 were also found to have impact on the plant photosynthetic pigments such as chlorophyll a, chlorophyll b and carotenoids. The total chlorophyll content was found to decrease with increase in imidacloprid concentration, however, carotenoid content had a directly proportional relationship with increasing concentration of imidacloprid.

A decline in chlorophyll-a, chlorophyll-b and total chlorophyll contents was noticed with increasing concentration of imidacloprid in soil, whereas carotenoids increased with the application of imidacloprid. Reduced levels of chlorophyll could be caused by the increased activity of the enzyme chlorophyllase, as well as by the degradation of chloroplasts and the oxidation of chlorophyll brought on by reactive oxygen. In contrast, imidacloprid toxicity increased pigments like carotenoid, possibly due to their antioxidant properties. Carotenoids have been acknowledged for their ability to scavenge reactive oxygen species, thereby protecting the photosynthetic system from abiotic stress (Sharma et al., 2016).

The findings from this study are consistent with those from earlier research projects. The amount of insecticide used was found to cause a steady decline in photosynthetic pigments. This decrease in chlorophyll under imidacloprid stress may be caused by an increase in the activity of the enzyme chlorophyllase, which leads to the breakdown of chlorophyll and a disturbance in the structure of

the chloroplasts (Ajermoun et al., 2022; Jan et al., 2020). The results of subsequent investigations have demonstrated that chemicals such as insecticides, have a deleterious impact on metabolic enzymes involved in the synthesis of photosynthetic pigments. Abiotic stresses can pose deleterious impacts on plant photosynthetic machinery including cellular membranes, cell division and cell elongation, biosynthesis of photosynthetic pigments, as well as electron transport chain (Sharma et al., 2019) The effects of imidacloprid on photosynthetic pigment contents in the leaves of *Cicer arietinum* have been presented in Figure 5.4.

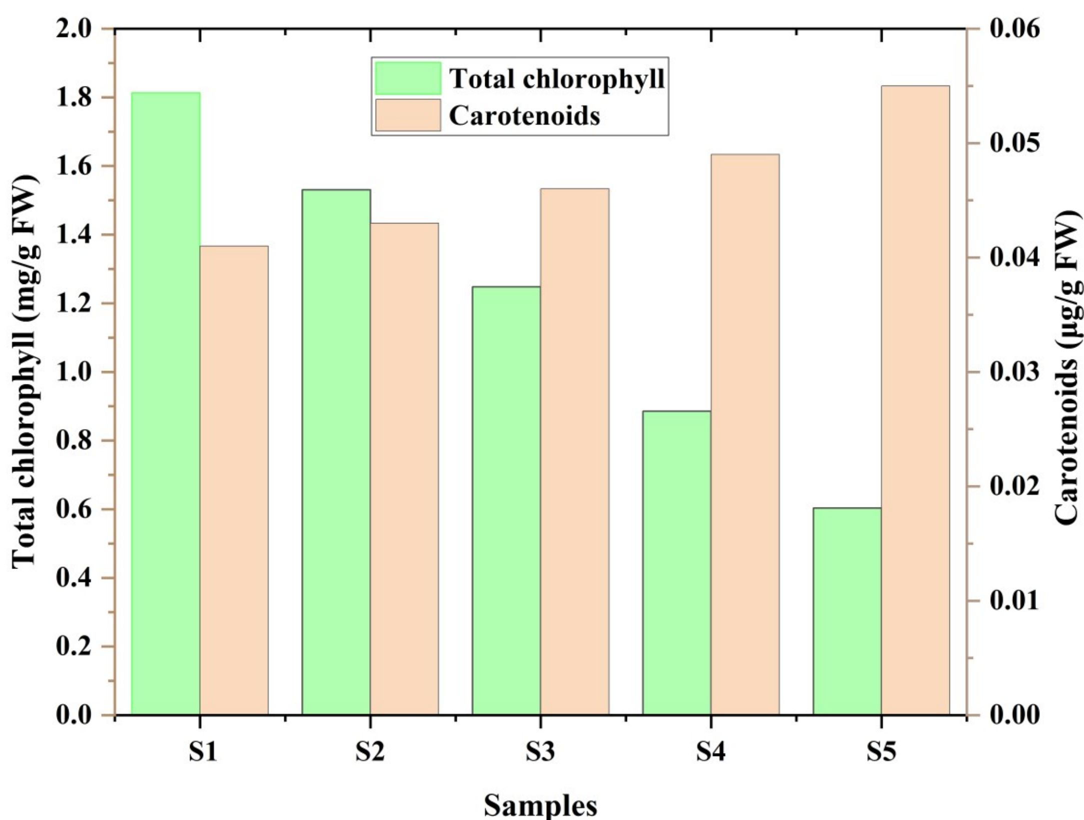


Figure 5.4: Effects of imidacloprid on photosynthetic pigment contents in the leaves of *Cicer arietinum*

5.3. Bacterial toxicity

The spectral intensities of luminescent bacteria *Photorhabdus luminescens* subsp akhurstii were assessed in different samples (T1, T2, T3 and T4) to study the toxicity caused to bacteria in the

presence of imidacloprid-contaminated sample. 1 ml of *Photorhabdus luminescens* subsp akhurstii was added to 5 ml of each sample. The bioluminescence intensity of bacteria in different samples was measured after 30 min and 24 h for the acute and chronic exposures. The maximum bacterial intensity was found at 466 nm in all the samples.

5.3.3. Acute toxicity

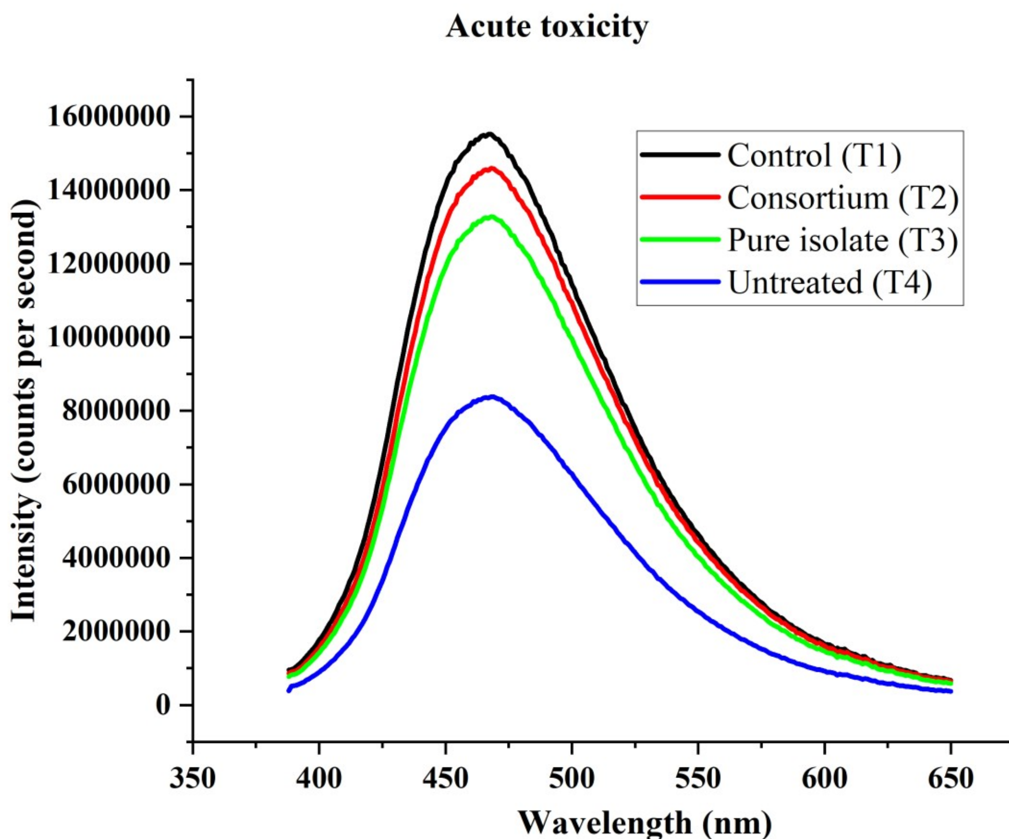


Figure 5.5: Acute bioluminescence intensities for samples T1, T2, T3 and T4

Figure 5.5 illustrates a noteworthy decline in bacterial intensity in the untreated sample compared to the control following 30 minutes of exposure. Specifically, the bioluminescence intensity exhibited a sharp decrease in sample T4, whereas it remained highest in T1. Conversely, treated samples T2 and T3 displayed higher intensities compared to T4, suggesting a reduction in toxicity. These observations indicate the potential effectiveness of the treatment in mitigating the adverse effects of the tested substance on bacterial activity.

It can be noted from Figure 5.6 that the luminescence intensity is negligible in the case of sample T4, which indicates a sharp decrease in the number of luminescent bacteria. In T2 and T3, the cells could survive due to a reduction in toxicity after bacterial treatment.

The % bioluminescence inhibition is indicative of the mortality of luminescent bacteria on exposure to treated and untreated samples. Sample T4 offered maximum inhibition of luminescent bacteria at 46% and 90.9% at 30 min and 24 hours, respectively. However, the bioluminescence inhibition improved in the case of treated samples, indicating a lesser reduction in the number of luminescent bacteria.

5.3.4. Chronic toxicity

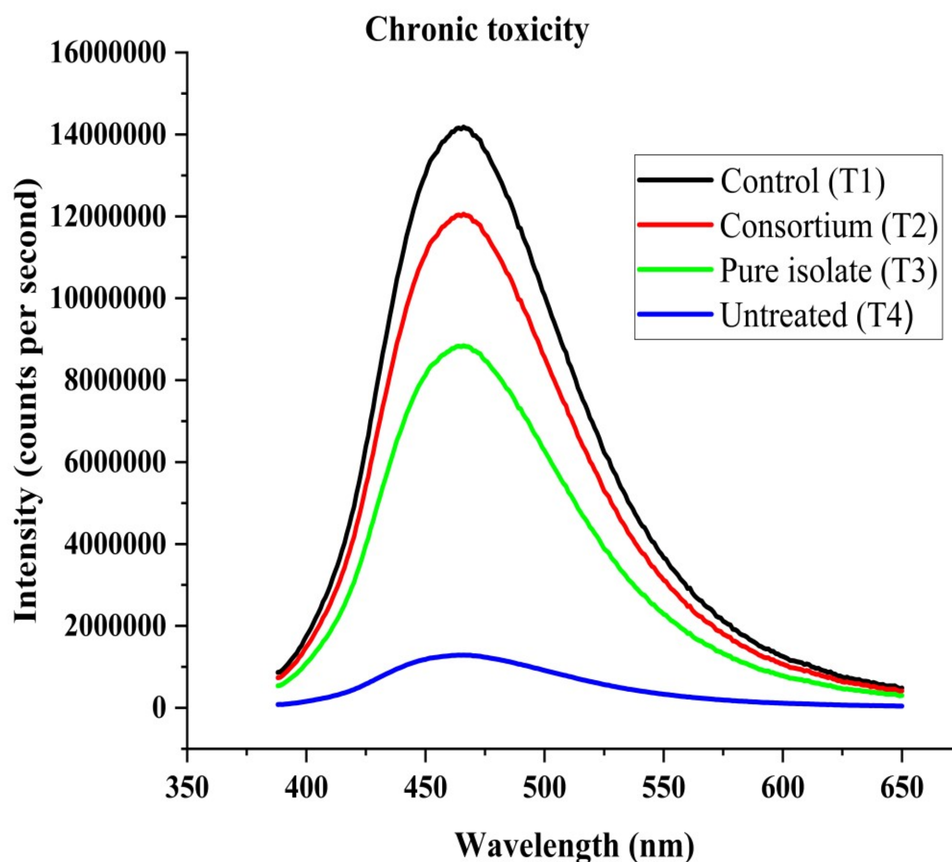


Figure 5.6: Chronic bioluminescence intensities for sample T1, T2, T3 and T4

Table 5.5: Bioluminescence inhibition for acute and chronic toxicity

Time of exposure	% bioluminescence inhibition		
	T2	T3	T4
30 min	6	14.5	46
24 hours	15	37.63	90.9

Similar studies have been conducted by researchers for assessment of bacterial toxicity of treated and untreated dye samples as well as insecticide-contaminated samples (Chaturvedi et al., 2021; Rodríguez-Castillo et al., 2019) and reported comparable conclusions.

The ecotoxicity studies done suggest that the toxicity of contaminated samples can be reduced to a large extent but some amount toxicity remains even after biological degradation. In contrast to the phytotoxicity investigation, the bioluminescence analysis provided a clear distinction between the toxicity of treated and untreated samples. Consequently, the bioluminescence assay can be utilized for a more quantitative and precise assessment of toxicity. Due to the possibility of the formation of harmful metabolites, the degradation and transformation of neonicotinoids are insufficient to ensure the eco-feasibility of the process. Ecotoxicological assessments were performed to ascertain whether the toxicity of contaminant decreased during biodegradation while taking into account the negative impact of neonicotinoids on non-target organisms.

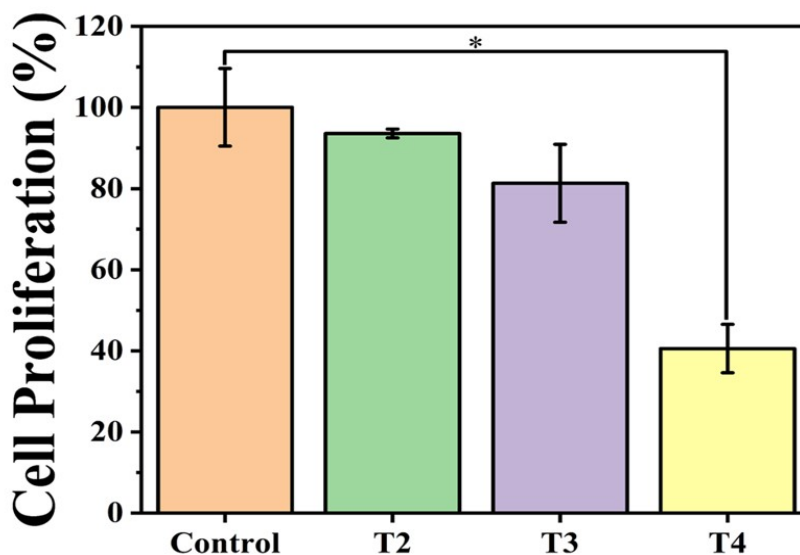
5.4. Cytotoxicity

Experiments were also conducted to study the impact of treated and untreated samples on cell proliferation. Study was conducted in the three groups. The cells treated with distilled water were taken as a control in the groups. Values are reported as (mean ± standard error) and significance level as ($*p < 0.05$).

5.4.1. MTT assay

MTT assay was conducted using all the samples. It can be observed from Figure 5.7 that control samples have maximum color intensity, which indicates the presence of more viable cells in the sample. The reduction in color intensity is due to the presence of mitochondrial dehydrogenase enzyme in lesser quantity in the samples as compared to the control. The color intensity was noted to be minimum in case of the untreated samples, due to the maximum toxicity exhibited by the

sample (T4). The reduction in color intensity is due to the presence of mitochondrial dehydrogenase enzyme in lesser quantity in the samples as compared to the control. It was observed that the cells grown in control without exposure to imidacloprid (T1) showed maximum proliferation, followed by samples treated with the consortium CS1, i.e., T2 and the sample T3 (treated by strain ST1). The cell proliferation in untreated samples (T4) was noted to be minimum, which indicates the toxicity of imidacloprid, resulting in inhibition of cell proliferation.



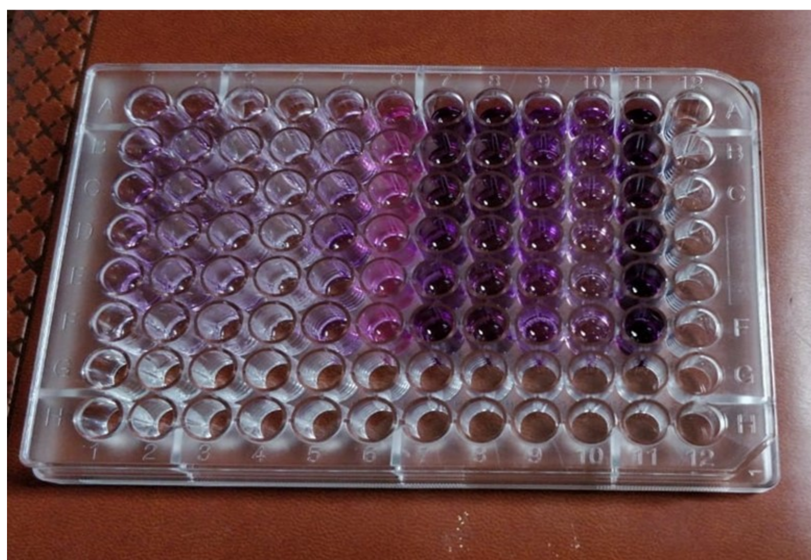


Figure 5.7: Cell toxicity studies of T1, T2, T3 and T4 on L929-RFP (mouse fibroblast cells) for 48 h of incubation and MMT assay in a 96-well microtiter plate

MTT assay of imidacloprid and its metabolites suggests that metabolites produced by the bacterial consortium are less toxic to L929 cell lines as compared to imidacloprid. Phugare et al. (2013) reported the imidacloprid metabolites degraded by *Klebsiella pneumoniae* strain BCH1 were less toxic as compared to imidacloprid. Reduction in cytotoxicity caused by metabolites has also been reported by Kumari et al., (2014), which is also in agreement with our results.

5.4.2. DAPI staining

DAPI stating was done for qualitative analysis of the cells. Figure 5.8 represents the images obtained through fluorescent microscopy when cells were stained using DAPI. Blue signals show the nucleus. After 48 hours of study, it can be observed that there is a significant decrease in the number of cells in sample T4, indicating the toxicity of untreated insecticide-contaminated samples to mammalian cell lines.

Experiments were also conducted to study the impact of treated and untreated samples on cell proliferation. It was observed that the cells grown in control without exposure to imidacloprid (T1) showed maximum proliferation, followed by treated samples. The cell proliferation in untreated samples (T4) was noted to be minimal, which indicates the toxicity of imidacloprid, resulting in inhibition of cell proliferation. The studies conducted suggest that the untreated samples (T4) are highly toxic to the mammalian cell lines, when exposed for 48 hours. There was a significant reduction in toxicity after the biodegradation of the imidacloprid-contaminated samples. The samples T2 and T3 were less toxic as compared to T4, due to the reduction in imidacloprid concentration. However, the cell proliferation and viability were found to be less as compared to the control sample T1, possibly due to the formation of some metabolites or due to incomplete degradation of imidacloprid.

A crucial outcome of the current investigation is the toxicological risk evaluation of imidacloprid and its bio-transformed metabolites in samples degraded by the bacterial consortium. Reduced toxicity of metabolites of compounds caused by microbial biotransformation has been used as a measure of the effectiveness of microorganisms in biological treatment (Awasthi et al., 2003). The toxicity analysis supports the potential use of the bacterial consortium as an approach to reduce the toxic impact of imidacloprid from the environment.

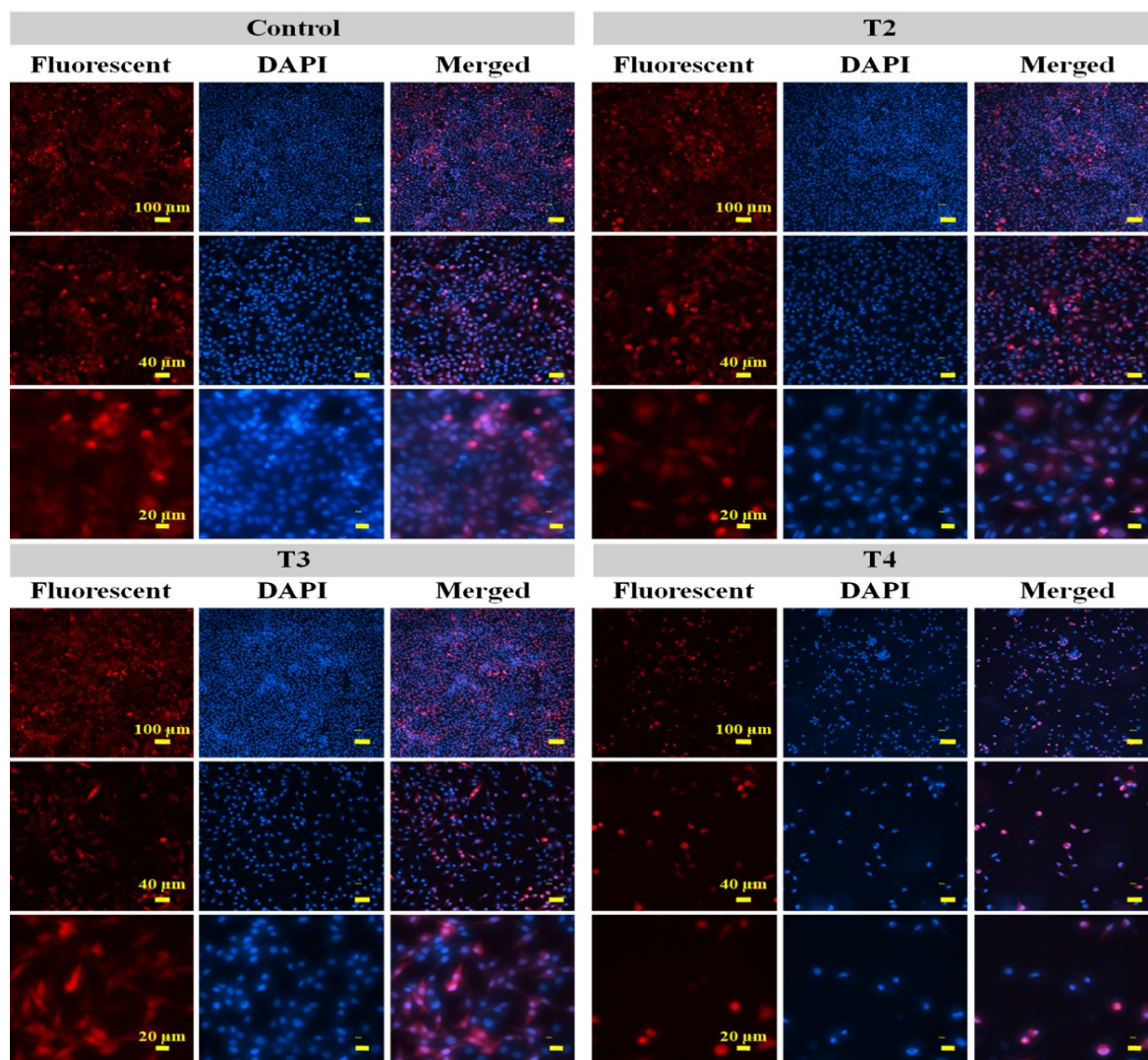


Figure 5.8: Fluorescence microscopy photographs of the L929-RFP (mouse fibroblast) cells after treatment with samples T1, T2, T3 and T4

5.5. Summary of the chapter

The study aimed to assess the toxicity of imidacloprid and biodegradable products on the growth and antioxidant enzyme activity of Cicer arietinum. The results showed that biodegradable products were less toxic compared to imidacloprid in its non-degraded form. The root length of *Cicer arietinum* treated with non-degraded imidacloprid was 3.6±0.02 cm, while that treated with distilled water was 4.2±0.05 cm. This study highlights the effectiveness of biodegradation in decomposing harmful materials in the environment. The process is eco-friendly, highly effective,

economically viable, and biocompatible. Furthermore, the study evaluated the effect of imidacloprid on seed germination, growth, and photosynthetic pigments in *Cicer arietinum*. High doses of imidacloprid were found to inhibit the growth of *Cicer arietinum*, affect pigment synthesis, promote oxidative stress, and disrupt antioxidant enzymes.

Apoptosis induction may be a possible outcome of imidacloprid exposure in high concentrations. Some studies also suggest the translocation and bioaccumulation of insecticides in different plant parts. The application of imidacloprid in agricultural fields and its subsequent leaching to the groundwater not only impedes soil quality and its fertility but also causes hazards to aquatic lives and non-target organisms. The application of *Tepidibacillus decaturensis* strain ST1 and consortium CS1 for the remediation of imidacloprid was found to be promising. % bioluminescence was found to be maximum in the case of untreated samples. Exposing cells to a cytotoxic compound may result in a reduction in cell viability and proliferation.