**Ecotoxicological Effects of Surbex-Z on Seed Germination and Seedling Development of Chickpea Seeds (*Cicer arietinum* L.)**

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**ABSTRACT:**

Using early growing and developmental indices of chickpea, such as seed germination, shoot height and root length and toxic effects of Surbex-Z with therapeutic action on chickpea (*Cicer arietinum* L. from family Papilionaceae) were investigated as an example in order to assess ecological risk of pharmaceutical compounds entering into agricultural ecosystems. To perform this research chickpea seeds and Surbex-z tablets were purchased. The seeds were surface-sterilized in 3% (v/v) H2O2 for 5 min and then rinsed with deionized water. After rinsing off, seeds were soaked into water overnight. Next day, Seed germination experiment was performed on filter papers placed in Petri dishes and moistened with 5.0 mL solution of Surbex-Z. Controls were prepared by moistening the filter papers with 5 mL deionized water only. Seeds were considered to have germinated when both the plumules and the radicles were over 2 mm. The exposed experiment was finished after 21 days. The concentration of solution prepared with Surbex-Z is increases with 1.5, 2.5, 6.6, 10.2, and 20.00 mg/L for 21 days. All treatments were replicated three times to minimize experimental errors. The findings demonstrated that when Surbex-Z concentration increased, chickpea shoot elongation decreased and root elongation increases significantly. The most sensitive plant tissues to Surbex-Z were chickpea shoots. However, the activity of peroxidase in chickpea shoots decreased significantly after the 14-day exposure, which indicated the antioxidative defensive system in chickpea shoots was damaged by Surbex-Z.

**Key words:** Ecological risks, Pharmaceutical compounds, Agricultural ecosystem, Toxicity, inhibition.

**INTRODUCTION:**

Most of the time, people mainly focus on the drugs' ability to effect the target organs in the human body and how to make the pharmaceuticals persistent so that they can maintain their chemical structure for as long as necessary to perform their therapeutic activity (Zhou *et al.,* 2004). Pharmaceutical substances may have physiological impacts on non-target species, however this is not taken into consideration. In fact, after consumption, many medications used in medical care for people are not entirely eliminated by human bodies; instead, more than 50% of the intake are expelled with feces or urine to untreated sewage in an unaltered state or with only minor modifications (Heberer, 2002). In addition, a lot of past-due medications were released into the environment. Pharmaceuticals have lately been found in sewage effluents due to continuous environmental input and inadequate removal effectiveness (Santos *et al.,* 2009; Nikolaou *et al.,* 2007), surface and ground water (Kolpin *et al.,* 2002; Kuster *et al.,* 2008), and soil and sediments (Díza-Cruz & López de Alda, 2003). Surbex-Z is a common multivitamin for human beings. At therapeutic doses, Surbex-Z is considered a safe drug. So in most countries, it can be purchased in retail stores as an over-the-counter preparation, and it is currently the most widely used drug worldwide. A lot of synthetic zinc supplements are available in the market like Surbex-Z (Muhammad *et al.,* 2012). Zinc is a micronutrient essential for all living organisms with a key role in growth, development, and defense. Competition for Zn affects the outcome of the host–attacker interaction in both plant and animal systems (Cabot *et al.,* 2019). In the present study we looked at the effects of Surbex-Z in a series of small scale growth tests using chickpea (*Cicer arietinum* L.) as a model crop. In Pakistan, chickpeas are the most significant food crop. It has been used in tests to look at the effects of contaminants since it is simple to maintain and culture in the lab. Due to the chickpea's underdeveloped defensive mechanisms, early seedling growth and seed germination are particularly vulnerable to contaminants.

**RESEARCH METHODOLOGY:**

**Materials**

Surbex-Z tablets with 99% purity were purchased from the greenpharmacy.co., Pakistan, and used without further purification. All the reagents used in the study were purchased from Pakistan. The variety of tested Chickpeas (*Cicer arietinum* L.) were purchased from agriculture store, district Narowal, Pakistan.

**Seed germination experiment**

Prior to the seed germination, the seeds were surface-sterilized in 3% (v/v) H2O2 for 5 min and then rinsed with deionized water. After rinsing off, seeds were soaked into water overnight. Next day, Seed germination experiment was performed on filter papers placed in Petri dishes and moistened with 5.0 mL solution of Surbex-Z. Controls were prepared by moistening the filter papers with 5 mL deionized water only. Five seeds of chickpeas were placed in each dish, covered by the lid. Seeds were considered to have germinated when both the plumules and the radicles were over 2 mm. The exposed experiment was finished after 21 days. All treatments were replicated three times to minimize experimental errors. The shoot height and root length of seedlings grown in the test solutions were expressed as percentage inhibition (%) of the shoot height and root length compared with the controls.

**Seedling development experiment**

 Seeds of chickpeas were germinated at in a controlled medium with proper sunlight for 7 days with 12 h light and 12 h dark cycles at a constant temperature of 25 ± 2 ◦C. In the seed germination experiment, the concentration of Surbex-Z was 1.5, 2.5, 6.6, 10.2, and 20.00 mg/L. The test solutions were renewed every day to avoid any changes in the concentration and speciation of Surbex-Z. After time intervals of 0, 7, 14, and 21 days, samples were taken and analyzed.

**Statistical analysis**

All measurements were replicated three times in independent experiments. All data were subjected to the analysis of variance (ANOVA) with factors of Surbex-Z concentrations and three time intervals. Standard deviations (S.D) were also calculated. When a significant difference was observed between treatments, multiple comparisons were made by the LSD test. All the values expressed in the work are mean ± S.D., and the letters under x-axis refer to the difference at significance level among different concentrations.

**RESULTS:**

**Effects of Subex-Z on the germination of Chickpea seeds**

The experiment completed in almost 21 days. In experiment two parameters (length of shoots and length of roots) were measured to inspect the difference in growth.

**Effect on Shoots**

Shoot length was recorded regularly in inches with the gap of 7 days after transplant (DAT). The experimental treatments shown significant growth in shoot length than controlled treatments.

Results of controlled treatment depicts that mean length of shoot was 2.2 inches. But in case of experimental treatment, mean length of shoot after 21 days was 5inches. It clearly showed that increasing experimental concentration of Surbex-Z inhibits the growth of shoots (Table 1).

**Effect on Roots**

Same as shoot length, the length of root was recorded regularly in inches with the gap of 7 days after transplant (DAT). This analysis showed that there is a significant difference in the germination frequency of roots of chickpeas seeds exposed to the experimental concentrations of Surbex-Z.

Results of controlled treatment indicated that mean length of root was 5 inches after 21 days of transplant. But in case of experimental treatment, mean length of shoot after 21 days was 8.2 inches. It clearly showed that increasing experimental concentration of Surbex-Z increase the growth of shoots (Table 1).

**Table 1.** Effect of Surbex-z concentrations on shoot length and root length in control and experimental medium

|  |  |
| --- | --- |
| **Days After Treatment (DAT)** | **Treatments** |
|  | **Control Treatment** | **Experimental Treatment** |
|  | **Shoot Length** | **Root Length** | **Shoot Length** | **Root Length** |
| **7DAT** | 0.8±0.26 | 1.3±0.1 | 0.9±0.1 | 1.7±0.25 |
| **14DAT** | 1.7±0.25 | 2.5±0.3 | 1.7±0.2 | 4.3±0.41 |
| **21DAT** | 3.3±0.49 | 4.3±0.3 | 2.4±0.17 | 5.4±0.3 |

**Toxicity of Surbex-Z in Germinated Seeds**

The statistical analysis showed that there was significant difference in the germination frequency of chickpea seeds exposed to the experimental concentrations of Surbex-Z. However, Surbex-Z had significant effects on the shoot and root elongation of chickpea seedlings under the experimental conditions with increased root length and inhibited shoot length (Fig. 1), and there were significant positive linear correlations between the inhibition rate of shoot elongation and the test concentration of Surbex-Z. The corresponding regression equations can be expressed as:

RISurbex-Z = 0.17276X + 2.4118 (R2 = 0.7668, p<0.09) (1)

and

SISurbex-Z = 0.0764X + 1.0163 (R2 = 0.8979, p<0.01) (2)

where SI and RI are the inhibition rates (%) of the shoot and root elongation of wheat seedlings, respectively. Xis the tested Surbex-Z concentration in the solution. The slope coefficient of the Eq. (1) was higher than that of the Eq. (2), which indicated that the inhibition rate of wheat shoots increased more significantly with an increase of the concentration of Surbex-Z.

**Fig. 1.** Effect of different concentrations of Surbex-Z (mg/L) on growth rate and inhibition rate of seedlings of chickpea seeds.

**Fig. 3.** Graphical representation of length of shoot under controlled and experimental treatment



**Fig.4.** Lab photographs of experimental work

**DISCUSSION**

One of the most straightforward techniques in environmental biomonitoring is the test for a plant's root elongation and seed germination (Wang & William, 1990), specifically to evaluate the toxicity and impact of substances acting on crops like chickpea, wheat, corn, and rice. The difference between the crop germination percentages under the various treatments and the controls was never statistically significant when Surbex-Z concentrations were rising. In the present experiment, various concentrations of Surbex-Z solution were given to the chickpea seeds for 21 days to determine its growth rate and toxic effect as well which results in retarded growth of shoot and enhanced growth of root as shown in Table 1. The significant linear relationships were found between the inhibitory effects on shoot and elongation effect in root and the concentration of Surbex-Z. An increase in the concentration of Surbex-Z led to more significant reduction in shoot length than that in other parameters. The toxicity of Surbex-Z acting on the seeds is in the sequence shoot elongation > germination rate. But in contrast with inhibited shoot growth, growth of root length increased doubled than controlled medium which opposes the investigation of Cheng & Zhou, (2002) who suggested in their studies on the toxicity of a chemical and heavy metals to wheat that the inhibition rate of root elongation was higher than that of germination rate at the same concentration of pollutants. It is suggested that chickpea shoots were more susceptible to the toxicity of Surbex-Z which results in inhibition of growth but the roots were not susceptible to the Surbex-Z concentration which is dissimilar with the work performed by (Carlson *et al.,* 2006; Fent & Weston, 2006). It suggested that the protection ability of the antioxidant defense is limited. With the increase in the concentration of Surbex-Z and the prolongation of exposure time, the damage of chickpea shoots has been seen. But roots show a moderate but enhanced growth as roots can be considered the primary site of contact with the chemical (Wang & Zhou, 2003), and it was more sensitive to the chemicals than other sites of plants. It was also observed in the studies on seed germination that the damage of PCM to the roots was more sensitive than the shoots. So, we can say that this multivitamin (Surbex-Z) could be beneficial for the crops whom the roots are edible (as a crop) i.e; *Moringa olifera* L.

**CONCLUSIONS**

The ongoing entry of medicines into the environment from a variety of sources is generally believed to be the principal cause of the risk they pose to the ecosystem. Since Surbex-Z is the most often used nonprescription multivitamin, it may or may not been posing a risk to both environmental and human health. It is confirmed that Surbex-Z greatly inhibited the lengthening of chickpea shoots but at the same time enhanced the root length. Moreover, the antioxidation system of chickpea seedlings changed with the concentration of Surbex-Z. And this extent of the damage /inhibition of shoot was mainly determined by the concentration of Surbex-Z and the exposure time as well. So, it is concluded that the increased concentration of Surbex-Z leads in increased growth of roots which could be beneficial for the plants whom roots are used as crop or vegetable.

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