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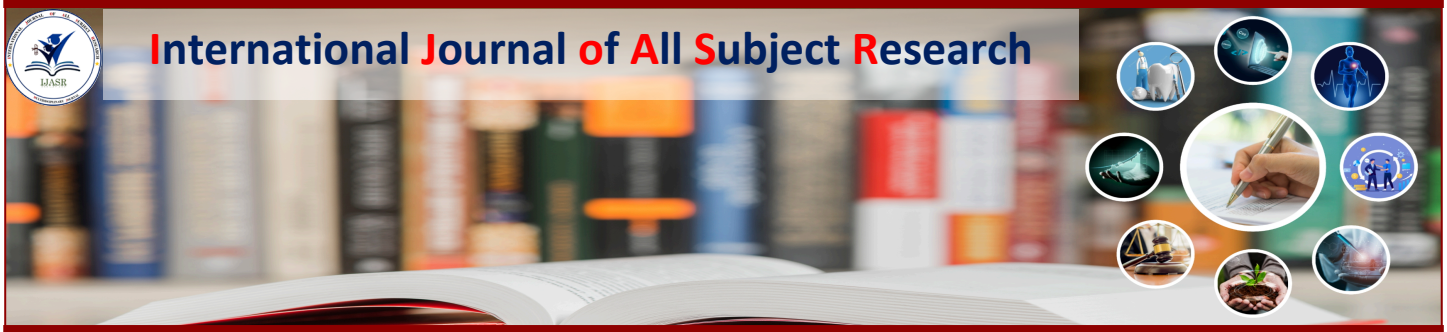

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# Penoxsulam Herbicide Microbiological and Toxicological Assessment on Soil Microorganisms and Animal Model

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**Abstract**

**Objective**: The aim of this study involves:

a) Pseudomonas isolates from soil samples are isolated and identified
b) Penoxsulam's bactericidal activity against Pseudomonas isolates is assessed,
c) Penoxsulam's animal model pre-clinical investigation,
d) Making sense of the facts to improve human welfare through raising awareness among the populace.

**Method:** Following the isolation and identification of Pseudomonas F from soil samples, four concentrations of penoxsulam-25 mg, 50 mg, 100 mg, and 200 mg-were tested for bactericidal efficacy. In order to assess an acute oral study in which animals were administered using an intubation cannula with a single dose level of 2000 mg/kg body weight and observed for 14 days, rabbits' eyes were exposed to Penoxsulam herbicide. This herbicide was carefully administered in the conjunctival sac of the left eye of the rabbit and examined for 7 days.

**Result**: No changes were found in body weight of rat and rabbit whereas Penoxsulam herbicide at a 100mg concentration had bactericidal activity and also toxic to rabbit eye rather than oral ingestion.

**Conclusion:** The results demonstrated that the herbicide Penoxsulam has bactericidal activity at a dosage of 100 mg. Overall, our results showed that penoxsulam did not appear to cause acute oral toxicity but was toxic to animal eyes and appeared to be damaging to eyes in the environment.

**Keywords:** Penoxsulam, bactericidal activity, wistar rats, oral toxicity

## Introduction

Herbicides are biologically active compounds, therefore accidentally employing them may significantly alter microorganism populations and behaviours. This could upset the microbial ecological balance in the soil and lower soil productivity. Herbicide sprays are now frequently employed to control weeds, despite the fact that they are extremely dangerous to both people and animals. The possibility that pesticide residues in soil could have an adverse impact on soil microorganisms is particularly worrying. Pseudomonas, one of the world's most diverse bacterial genera, has an important role in soil. The promotion of plant development and the reduction of pathogenicity depend on their distribution in the soil. Herbicides are thought to have no appreciable or lasting effects on microbial populations when applied at the typical field advised rates. According to reports, some microorganisms were able to break down the herbicide depending on application rates and the type of herbicide employed, while others suffered harmful effects. As a result, both positive and negative effects of herbicides on microbial development depend on chemical (type and concentration), microbial species, and environmental conditions. The 1940s saw the complete revolutionization of agriculture with the invention of 2,4-Dichlorophenoxyacetic acid (2,4-D), which also marked the beginning of the era of chemical weed control. More than 2000 herbicides with 15 different modes of action had been released on the market as of 2016. Penoxulam is currently one of the most promising herbicides out of all those in use. 2 Penoxsulam (2,2-difluoroethoxy) N-(trifluoromethy)-6-(5,8-dimethoxy1,2,4] triazolo[1,5-c] pyrimidin-2-yl)). In California, rice is treated with a brand-new triazolopyrimidine sulfonamide herbicide (Turner JA 2015) for post-emergence, all-purpose weed management (Oryza sativa L.). A systemic herbicide called penoxsulam kills plants by internally altering the weeds' phloem and xylem tissues as they develop. Penoxsulam is used to control grass, sedge, and broadleaf weeds in rice that has been transplanted, dry-seeded, or water-seeded. The detrimental effects of penoxsulam on plants depend on both their capacity to restrict development at the target site as well as how quickly they shed their leaves. The primary route of entry for this systemic herbicide is through the leaves, and the secondary route is through roots that are transported through the xylem and phloem. Weed growth will almost instantly stop following application of penoxsulam. Due to the warning signs, which include rapid growth obstruction, a chlorotic

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developing site, and the loss of the terminal bud, sensitive weeds will perish in two to four weeks. Its primary use would be as a post-emergence technique for rice that has been transplanted, water-seeded, or dried out. Herbicides are often used topically, orally, or inhaled by agricultural producers. However, they can also expose farmers during maintenance, sampling, trials, or any other activity. They mostly damage internal organs by entering the bloodstream.

The negative consequences of herbicides like Penoxulam on soil microorganisms and animal model need to be researched. Furthermore, safety standards for human exposure are being established through these research. However, relatively few research have been done to look at herbicide exposure in the eye and oral delivery in animal model as well as against benificial soil microorganisms. In order to close this gap and assess the risk of penoxsulam, the aim of this study involves:

a) Isolation and identification of *Pseudomonas* isolates from soil samples,
b) Bactericidal activity evaluation of penoxsulam against *Pseudomonas* isolates,
c) Pre-clinical study of penoxsulam on animal model,
d) interpretation of the facts to promote human wellbeing by raising people's level of awareness.

## Method and Material

### Pseudomonas Isolates from Soil Samples are Isolated and Identified.

Various soil samples were taken from a Delhi suburb for microbiological analysis. Cetrimide broth received 25g of soil samples, which were subsequently incubated for 48 hours at 370C. Gram's staining, a biochemical test in line with IS: 13428:2005 (Annexure-D), and 16s rRNA sequencing were used to validate the identity after looking for unique green colonies on Cetrimide agar plates. The Basic Local Alignment Search Tool (BLAST) technique is used to align the trimmed nucleotide sequences of several bacterial isolates from the National Center for Biotechnology Information (NCBI) (NCBI).

### Working Solution of Penoxsulam

Following four concentrations of penoxsulam were used for determination of bactericidal activity, viz, 25mg, 50mg, 100mg, 200mg.

### Inoculums Preparation

Using saltwater (0.85% NaCl), 24 hour-old isolated bacterial cultures were used to adjust the densitometer's 0.5 McFarland density to obtain 1.0 × 108 CFU/ml of bacteria.

### Zone of Inhibition Evaluation

Agar well diffusion methods were used to evaluate the bactericidal activity of penoxsulam concentrations. For 100 l of each adjusted culture, plates of sterile Nutrient Agar (NA) were punched to a 6mm diameter. Each concentration of penoxsulam solutions was put into the wells in portions of 100 l. Plates were incubated at 37°C for one night. Vernier callipers were used to measure the zone diameter on plates containing an inhibitory zone.

### Experimental Animals

Wistar rats that were 6 to 8 weeks old and weighed 130 to 190 gm as well as adult rabbits that were 2 to 3 kg as healthy were chosen for this experiment and housed in the testing area. All of the animals were handled in compliance with the standards set by the Indian government's CPCSEA agency. Every animal was housed in a space that was maintained at a constant 22°C and between 50 and 60% humidity. The space was ventilated at a rate of around 15 air changes per hour with 12 hours of artificial light each day (8 am to 8 pm) and controlled lighting. The water and meal were both freely available to the rats and rabbits, and since neither substance contained any poisons, there was no risk that the animals' behaviour would change. The floor of the testing chamber was routinely swept with a D-125-containing disinfectant solution. Before giving the doses during the study period, acclimatisation was carried out for 7 days. The study number, animal number, sex, dose, experiment start date, date of dosing, experiment conclusion date, and cage number were all labelled on the rat cages.

### Experimental design

### Penoxsulam herbicide acute oral toxicity investigation in Wistar rats

Six animals from steps I and II will be used in this experiment in stages. Wistar rats were used in the study, which was carried out in accordance with OECD Guideline No. 423. The trial was approved by the IAEC prior to starting. Six female rats were used in this experiment to perform a limit test (three animals per step). A single oral gavage dose of penoxsulam was prepared in maize oil and administered to rats by intubation cannula based on body weight. Following the dose, the feed was discontinued for four hours. A single dose of 2000 mg/kg body weight was administered to six female rats (Figure 1A and 1B). Initially, the animals were observed for 24 and 48 hours following medication. All the animals were thoroughly watched for clinical signs and mortality for the first 30 minutes, 2, 3, 4, and then twice daily for the following 14 days.

**Fig 1:** Oral administration of Penoxsulam Herbicide

### Penoxsulam Herbicide Study on Eye Irritation in New Zealand White Rabbits

The potential for significant eye discomfort in New Zealand white rabbits was also investigated (Oryctolagus cuniculus). The study was carried out in a GLP lab in accordance with OECD Guidelines (405) for Testing of Chemicals, Acute Eye Irritation/Corrosion. In this investigation, healthy male New Zealand white rabbits weighing between two and three kilogrammes were used. Injecting 100 mg of Penoxsulam into the conjunctival sac of the left eye in one animal required carefully moving the lower eyelid away from the eyeball. The lids were then carefully held together for one to two seconds to prevent compound loss.

**Table 1:** Application of Penoxsulam on rabbit eye (Initial Test)

| Step I | Step II | Step III |
|---|---|---|
| After gently pulling the lower eyelid away from the eyeball, the chemical was placed in the conjunctival sac of one eye of a single animal. | Buprenorphine (0.01 mg/kg subcutaneously) after 12 hours until the ocular lesion or clinical signs of pain and distress resolve. | The eyes were washed out at 24 hours after the chemical instillation. |

### Acute oral Toxicity Experiment

No treatment-related adverse signs, symptoms, or fatalities have been documented after the dose was administered in phase I. To confirm the preliminary results, three additional female rats were used in the second stage, and they too got the same amount of 2000 mg/kg body weight. The end point had been achieved and no further testing was required because no toxicity was discovered at this time, yielding a comparable result and no changes in the animals' body weights (Table 5). At the conclusion of the 14-day observation period, all of the animals were put to death and subjected to a gross pathological examination. There were no obvious pathological findings in the dead animals. Therefore, "Penoxsulam" has an LD50 value of 2000 mg/kg and a range of >2000-5000 mg/kg B.wt.

### Acute Eye Irritation Experiment

Because animal no. 1 displayed corneal opacity = 1, irritis = 1, conjunctival redness = 2, and conjunctival oedema (chemosis) = 2 after 24 hours of dose application in the eye, two additional rabbits (Animal nos. 2 and 3) were employed in the confirmatory test. By using the ocular method, 0.1 g of penoxsulam was injected into the conjunctival sac of both rabbits' left eyes. The untreated eyes of all three rabbits were unaltered throughout the entire 72-hour testing. At 24, 48, and 72 hours, the individual mean irritation ratings for animals nos. 1, 2, and 3 were, respectively, 1.33, 1.66, and 1.66 for corneal opacity, 1.33, 1.00, and 1.00 for irritation, 1.66, 1.66, and 1.66 for conjunctival redness, and 1.66, 1.66, and 1.33 for conjunctival oedema (chemosis). Animal number 1 displayed the clinical symptoms of lachrymation, miosis, conjunctivitis, iritis, and chemosis (Table 5), while animals numbers 02 and 3 also demonstrated these toxic symptoms were reversed in 5 days.

**Table 2:** Ocular response at 1, 24, 48, 72 hour and on day 7 after Penoxsulam application to the Rabbit eyes

| **Animal Number: 1** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Site of application: Left eye (Test)** | | | | | | | | |
| **Eye reactions** | **At hour** | | | | | **Day** | **Combined Index** | **Mean irritation score** |
| | **Before exposure** | **1** | **24** | **48** | **72** | **7** | | |
| Cornea | 0 | 0 | 1 | 2 | 1 | 0 | 4 | 1.33 |
| Iris | 0 | 0 | 1 | 2 | 1 | 0 | 4 | 1.33 |
| Conjunctivae | 0 | 0 | 2 | 2 | 1 | 0 | 5 | 1.66 |
| Chemosis | 0 | 0 | 2 | 2 | 1 | 0 | 5 | 1.66 |
| **Animal Number: 2** | | | | | | | | |
| **Site of application: Left eye (Test)** | | | | | | | | |
| **Eye reactions** | **At hour** | | | | | **Day** | **Combined Index** | **Mean irritation score** |
| | **Before exposure** | **1** | **24** | **48** | **72** | **7** | | |
| Cornea | 0 | 0 | 2 | 1 | 2 | 0 | 5 | 1.66 |
| Iris | 0 | 0 | 1 | 1 | 1 | 0 | 3 | 1.00 |
| Conjunctivae | 0 | 0 | 1 | 2 | 2 | 0 | 5 | 1.66 |
| Chemosis | 0 | 0 | 2 | 2 | 1 | 0 | 5 | 1.66 |
| **Animal Number: 3** | | | | | | | | |
| Site of application: Left eye (Test) | | | | | | | | |
| Eye reactions | At hour | | | | | Day | Combined Index | Mean irritation score |
| | Before exposure | 1 | 24 | 48 | 72 | 7 | | |
| Cornea | 0 | 0 | 2 | 1 | 2 | 0 | 5 | 1.66 |
| Iris | 0 | 0 | 1 | 1 | 1 | 0 | 3 | 1.00 |
| Conjunctivae | 0 | 0 | 2 | 2 | 2 | 0 | 6 | 2.00 |
| Chemosis | 0 | 0 | 1 | 2 | 1 | 0 | 4 | 1.33 |

$$\text{Mean Eye Irritation Score} = \frac{\text{Sum of the individual score for eye reactions}}{\text{Total Number of the observations (3)}}$$

Within 7 days, all of the eye lesions were completely cured. The test substance penoxsulam was determined to be "Mildly Irritating the Eyes (Slight Irritant)" to the eyes based on the obtained Mean score of corneal opacity, irritis, conjunctival redness, and conjunctival odoema (chemosis), which reversed after 7 days (Figure 3 & Table 6).

In light of this, the current document describes eye irritation, an oral study, and contact eye reactions to a herbicide called Penoxsulam Technical (used on rice crops). No other organ in the human body system is as easily accessible for observation as the eye, thanks to the application of a Penoxsulam as an allergen to the outer layer of eye testing. However, the eye also presents some distinctive and legitimate opportunities as well as obstacles for drug discovery. [13]

According to the OECD 423 Guideline, the tested Penoxsulam is categorised as a compound with a non-toxic oral impact to the GHS category (Globally harmonised system of classification and labelling of chemicals). The Environmental Protection Agency's [14] designation of LD 50 Range: >2000-5000mg/kg B.wt. is consistent with our findings. 5000 mg/kg B.wt is the LD50. [14] The ocular irritant seen with opthalmoscope equipment power, on the other hand, resulted in a combined score of three rabbits of cornea 1.55, iris 1.11, conjuctivea 1.77, and chemosis 1.55, all of which fell into category 2B. Our findings are consistent with those of the EPA, demonstrating that the chemical is a minor irritant to ocular responses.

The observation was similar to EPA's 2004 designation of Penoxsulam as a category IV substance, which signifies it is in the class of compounds with minimal ocular irritancy.

Body weight, according to Aleman and Gad et al, is the most sensible sign of an unfavourable effect, and it provides the most information in toxicological non-clinical research. In acute experiments, it was discovered that the usage of herbicides had no effect on the bodyweight of rats (Table 7 & 8). According to the evaluation, body weight increases in the species are typical; no deviations in the animals were identified

**Table 3:** Mean Body Weight recorded during single dose oral exposure of Female Wistar Rats

| Animal Number | Step | Dose mg/kgB.wt | Weight in gram on Day | | | |
|---|---|---|---|---|---|---|
| | | | Before dosing (day 1) | 7 | 14 | Before sacrifice (day 15) |
| 1 | I | 2000 | 197.6 | 206.7 | 213.9 | 214.7 |
| 2 | I | 2000 | 193.7 | 204.7 | 215.8 | 215.6 |
| 3 | I | 2000 | 194.7 | 205.6 | 217.3 | 219.9 |
| **Mean±S.D.** | | | 195.30±2.00 | 205.77±1.00 | 215.70±1.81 | 216.63±2.80 |
| 4 | II | 2000 | 205.7 | 213.3 | 223.2 | 224.1 |
| 5 | II | 2000 | 206.5 | 217.5 | 228.4 | 229.1 |
| 6 | II | 2000 | 208.3 | 217.4 | 230.6 | 232.5 |
| **Mean±S.D.** | | | 206.83±1.46 | 216.03±2.45 | 227.30±3.80 | 228.53±4.12 |

Toxicity in the eyes and oral is a significant source of worry and curiosity. Numerous pesticides are damaging to the user, their internal organs, and their eyes. Some pesticides, however, have negligible or no effects on the body. Both short-term and long-term usage of pesticides has the ability to change the body. Animal rights and welfare groups are angered when the test item comes into touch with the eye because it is a sensitive organ. [15] This experiment is essential for revealing details about what happens to xenobiotics following exposure through a particular route. New chemical agents have been created with eco-friendly planning to lessen the use and production of dangerous compounds.[16] In order to determine Penoxsulam's safety profile, this experiment used various methods for toxicological screening to evaluate a number of toxicity criteria, such as eye irritation and oral toxicity.[19] Herbicides can be handled by people safely when being used in the field, according to toxicological tests, however they are not safe for the eyes. Additional details on the security of using this on plants will come through irritation research. Last but not least, since the regulatory goal of these tests is to safeguard human health, a testing strategy based on human biology is necessary. [18]

In the end, we showed that Penoxsulam (an herbicide) appears to be safe for human usage when taken orally using a method that is acceptable to the environment. Although this compound is potentially irritating to the eyes, it is not immediately dangerous.

## Results & Discussion

### Bactericidal Activity of Penoxsulam

Soil samples from various Delhi locations were gathered for the current investigation. Pseudomonas isolates were searched for in these soil samples. Pseudomonas stutzeri, Pseudomonas aeruginosa, Pseudomonas fluorescence, and Pseudomonas baetica were four separate strains of Pseudomonas that were discovered throughout the study. The non-pathogenic Pseudomonas sequence found in the NCBI database showed 96 to 99% sequence similarity to all isolated strains discovered in this investigation, with the lowest E-value, most query coverage, and highest identity. The identification of the Pseudomonas isolate is illustrated in Figure 2A. Zone of inhibition was used to assess the bactericidal efficacy of the following four penoxsulam concentrations (25 mg, 50 mg, 100 mg, and 200 mg) against four isolated Pseudomonas bacteria. Table 4 and Figure 2B demonstrated the results of zone of inhibition of isolated four *Pseudomonas* strains against penoxsulam. It has been observed that 100mg and 200mg concentration of penoxsulam has shown bactericidal activity against isolated strains.

**Table 4:** Effect of Penoxsulam against *Pseudomonas* isolates

| Name of isolates identified with accession number | Concentrations of Penoxsulam | | | | |
|---|---|---|---|---|---|
| | 25 mg/ml | 50 mg/ml | 100 mg/ml | 200 mg/ml | Methanol as control |
| *Pseudomonas aeruginosa* NR_117678.1 | NZI | NZI | 17.25 ± 0.06 | 17.21 ± 0.14 | NZI |
| *Pseudomonas fluorescence* NR_113647.1 | NZI | NZI | 17.89 ± 0.04 | 17.75 ± 0.17 | NZI |
| *Pseudomonas stutzeri* NR_113652.1 | NZI | NZI | 17.96 | 17.94 | NZI |

| | | | | | |
|---|---|---|---|---|---|
| | | | ± 0.09 | ± 0.11 | |
| *Pseudomonas baetica* NR_116899.1 | NZI | NZI | 18.01 ± 0.09 | 17.99 ± 0.07 | NZI |

**Ethical Clearance**

IAEC approval was taken before initiated the experiment and all of the animals for this experiment were handled in compliance with the standards set by the Indian government's CPCSEA agency.

**Conclusion**

The results demonstrated that the herbicide Penoxsulam has bactericidal activity at a dosage of 100 mg. Penoxsulam has bactericidal effects on helpful soil bacteria like Pseudomonas, therefore taking it at this dosage will upset the soil ecosystem. This dosage has been used to study eye discomfort in a rabbit model since Pseudomonas can induce eye infections. The findings of a study on the acute oral toxicity and eye irritation of Penoxsulam Technical in Wistar rats and New Zealand white rabbits unequivocally demonstrate that the herbicide can cause eye damage when administered acutely. However, the herbicide is secure for human handling in agricultural applications once it has been consumed.

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