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# Comparative Germination Studies of Cowpea (*Vigna unguiculata* Linn. Walp) and Soy bean (*Glycine max* Linn. Merr) on Whole and Water Saturated Fractions of Hydrocarbon (Hexane)

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## ABSTRACT

*The effects of whole and water saturated fractions (WWSF) of hydrocarbon hexane on the germination of two leguminous crops: Vigna unguiculata (Linn) Walp and Glycine max (Linn) Merr were investigated at the green house of Botany and ecological department university of Uyo Akwa- Ibom State, using Petri dishes at room temperature (±28$^0$C), between July and September 2009. The results obtained in this study showed that the germinations of both cowpea (Vigna unguiculata) seeds and soybean (Glycine max) seeds were influenced differently by various concentrations of whole and water saturated fraction of hexane. At the highest concentration (75%) of water saturated fracture of hexane, 52.1 % of the cowpea seeds germinated compared to the 81.1% obtained when distilled water was used at 168HAP, while at the highest concentration (75%) of water saturated fracture of hexane, 50.5 % of the soybean seeds germinated compared to the 62.0% obtained when distilled water was used at 168HAP. The results also shown that at 168HAP, the mean radicle lengths of 8.8±1.6, 6.2±0.3, 6.1 ±0.9 and 5.4± 0.6 were obtained when distilled water, 25%, 50% and 75% water saturated fraction of hexane were introduced respectively into Petri-dishes containing cowpea , while there were slight reductions in the values obtained when whole hexane were used. The results also showed that 25%, 50% and 75% concentrations each of whole and water saturated fraction of hexane significantly affected the mean percentages of soybean (Glycine max) germination while significant reduction at (P=0.001) in the radicle lengths of soybeans at different concentrations (75, 50 and 25%) of whole and water saturated fraction of hexane were obtained .This therefore, suggested that cowpea and soybean will be affected if planted in an area contaminated with both whole and water saturated fraction of hexane.*

**Key Words**: Vigna unguiculata, Glycine max, Hexane, Germination, Hydrocarbon, HAP

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## INTRODUCTION

Cowpea (*Vigna unquiculata* L. Walp) is one of the world's dicotyledonous leguminous food crops and a major food crop of millions of people in the developing countries especially Nigeria [1].Cowpea belongs to the kingdom Plantae, division (Magnoliophyta), class (Magnollopsida), order (Fabales), family Fabaceae formally (Leguminiseae), Sub-family (Faboideae), genus (Vigna) and species (unquiculata). Cowpea provides an extremely significant portion of the dietary protein of the people and plays an important nutritional role in developing countries of the tropics and subtropics especially in sub-Saharan Africa [2].Cowpea young leaves, pods and pea contain vitamins and minerals which have fuelled its usage for human consumption and animal feeding [3].The roots of the cowpeas are eaten in Sudan and Ethiopia and the scorched seeds are occasionally used as a coffee substitute [4]. In Nigeria, cowpeas are used to make soups and bean mixes such as "moi-moi" and beans cakes [5]. Nigeria is reputed to be the highest producer of cowpea in the world [1,5]

*Glycine max* commonly known as soybean is an annual leguminous plant. It is a native to East Asia and was introduced to Nigeria in 1908 [6]. Soybean is classified as vegetables (garden) or field (oil). The vegetable types cook more easily, having a mild nutty flavor, higher in protein and lower in oil content than the field types. The oil seed is important for its high protein (38-45%) and oil content (20%). Soybeans are sources of complete protein and as recommended for vegetarians, vegans and people who cannot afford meat. Consumption of soybean may also reduce the risk of colon cancer, possibly due to the presence of sphingolipids [7]. They are also used in industrial product such as oils, cosmetics, resin, plastics as well as sources of biodiesels in the United States, accounting for 80% of domestic biodiesel production. Also soybean has been found to be effective as an insect repellant [8,9].

Germination studies on various food crops have been investigated at various sub-lethal levels [10, 11, 12, 13, 14, 15]. The major concern that has rocked most countries in recent time otherwise referred to as global food scarcity underscored reasons why these major crops of important values should be preserved. These traditional food plants in Africa have potential to improve nutrition, boost food security, foster rural development and support sustainable land care. These crops form major diets in Nigerian foods and any adverse effect such as pollution or contamination of various organic compounds on them should be properly investigated such that advice can be given to various stakeholders including government and other relevant bodies of possible consequences or benefits in case of pollution [15].

The rapid industrialization around the globe today has led to an ever increasing demand for petroleum and other organic chemical based product. Report indicated that large amount of these products are currently being consumed at an alarming rate and contamination of different environmental bodies such as soil, water and air with petroleum products as well as other organic chemicals associated with industries has become a global concern [16]. The components of crude oil that go into solution make up the water soluble saturated fraction (WSF) and these contain dispersed particulate oil, dissolved hydrocarbon and soluble contaminants [15,17] This is ecologically important because in event of oil spill into either soil or water, they are usually taken up, absorbed and metabolized by living organisms [18].Hexane is a very volatile colorless liquid occurring naturally in petroleum crude oil. The most preponderant sources of hexane are evaporation from gasoline and release through car exhaust. Several studies Have shown that plant up-take of hydrocarbons is negligible [19]. [20] found that Zucchin (*Cucurbita pepo*), Cucumber (*Cucumber sativus)* and squash (*Cucurbita pepo* ssp *ovifera*) all took up polycyclic

aromatic hydrocarbons and up take of pyrene and phenonthene by 12 plants resulted in adverse effect on their growth [21]. There is a paucity of information on the effects of water saturated fraction of hydrocarbon most especially hexane on the growth of crops in this environment. Thus, this research was undertaken to investigate the effects of whole and water saturated fraction (WSF) of hydrocarbon (hexane) on the seeds germination and radicle length of seedlings of cowpea and soy beans.

## MATERIALS AND METHODS

### Collection of Materials / Determination of Seeds Viabilities

Mature seeds of the two crops: Cowpea (*Vigna unguiculata*) and Soy bean (*Glycine max)* used for this investigation were obtained from Itam Market in Uyo, Uyo Local Government Area of Akwa Ibom State and the viabilities of the seeds were determined by floatation methods.

### Sources and Preparation of Water Saturated Fractions of Hexane (WSF):

The hydrocarbon (hexane) used in this research was purchased at Bong Chemical Store in Uyo, Akwa Ibom State. The water saturated fraction was prepared according to instruction with slight modifications [22]. 10mls of hexane (Raw) was slowly mixed with 90mls of distilled water in a conical flask. This was placed on gallen kamp table top magnetic stirrer with 7 inches magnetic rod and stirred for 24hours at room temperature. The oil to water mixture was allowed to stand overnight in a separating funnel. The water saturated fraction which was the filtrate was separated from the supernatant and was referred to as stock of 100% saturated water. The stock water saturated fraction was diluted serially with hexane and distilled water to give various concentrations respectively from the stock.

### Methodology (Procedure):

Ten viable seeds each of the two crops (*Vigna unquiculata* and *Glycine max)* were sown per Petri dishes paddled with tissue papers and maintained at temperature of 28-32°C, at the Botany and Ecological Green House, University of Uyo. The hydrocarbon (hexane) as prepared above (whole and saturated) were shaken properly after preparation and applied directly to the Petri dishes containing the ten viable seeds of each of the two crops and the control experiment was watered with distilled water only. The experiments were maintained under day and night conditions and readings taken every 24 hours.

### Determination of Parameters

Parameters such as number of germinated seeds and length of the seedling radicles were determined. The seeds were considered germinated with the emergence of radicles. The effects of the different concentrations of hexane (0, 25, 50, and 75%) on the seed germination was determined by counting the number of seeds that were growing while the length of radicle of germinating *Vigna unquiculata* and *Glycine max* that received the same treatment were measured to the nearest centimeters using a ruler. Measurements were taken at the interval of 24hours for both crops for seven days. The experiments were performed thrice and the data are shown as the mean ± standard deviation (SD).

**Statistic Analysis:** Data for each growth parameters obtained were analyzed separately using two ways analysis of variance (ANOVA)

## RESULTS AND DISCUSSION

The results obtained in this study showed that the germinations of cowpea (*Vigna unguiculata*) seeds were influenced differently by various concentrations of whole and water saturated fraction of hexane (Tables 1 and 2). At the highest concentration (75%) of water saturated fracture of hexane, 52.1 % of the cowpea seeds germinated compared to the 81.1% obtained when distilled water was used at 168HAP (Table 1). The results also shown that at 168HAP, the mean radicle lengths of 8.8±1.6, 6.2±0.3, 6.1 ±0.9 and 5.4± 0.6 were obtained when distilled water, 25%, 50% and 75% water saturated fraction of hexane were introduced respectively , while there were slight reductions in the values obtained when whole hexane were used (Tables 3 and 4). These results are in conformity with that of [12], in which *Abelmoschus esculentus* (okra), *Capsicum annum* (pepper), *Lycopersicon esculentus* (tomatoes), *Solanum melongena* (egg plant) germination were affected when planted in different concentrations of spent lubricating oil.

The results also showed that 25%, 50% and 75% concentrations each of whole and water saturated fraction of hexane significantly affected the mean percentage of soybean (*Glycine max)* seeds. (Tables 5 and 6).This is in agreement with [14, 15]. The emergence of radicle lengths of soybeans at different concentrations (75, 50 and 25%) of whole and water saturated fraction of hexane showed significant reduction in the radicle lengths of soybeans (Tables 7 and 8). The results obtained from this are in line with that of [14,15,23, 24] who reported serious reduction in germination and growth parameters of seeds in mineral soil with contamination level of 2-5%. [25] also reported the reduction in germination and growth parameters of crops when high level of polycyclic hydrocarbon (spent lubricating) were used. Conclusively. with increased concentrations of whole and water saturated fraction hydrocarbon (hexane), there is a significant reduction in seed germination, emergence of seedlings and radicle lengths of both cowpea and soybean seeds, therefore, cowpea and soybean will be affected if planted in an area contaminated with hexane,

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**Table 1: Mean Percentage of Cowpea seeds (*Vigna unguiculata*) Germination Grown in Different Concentrations of Water Saturated Fraction of Hexane and Distilled Water (Control)**

| Concentration (v/v) | 24HAP | 48HAP | 72HAP | 96HAP | 120HAP | 144HAP | 168HAP |
|---|---|---|---|---|---|---|---|
| Distilled Water | NG | 21.5 | 39.0 | 51.1 | 62.7 | 75.6 | 81.1 |
| 25% | NG | NG | 30.1 | 39.5 | 48.5 | 57.5 | 68.5 |
| 50% | NG | NG | 27.5 | 38.7 | 46.0 | 54.8 | 59.0 |
| 75% | NG | NG | 23.5 | 29.0 | 36.5 | 41.0 | 52.5 |

*Values are Mean percentages of three replicates ; HAP: Hours After Planting
NG: No observable growth

**Table 2: Mean Percentage of Cowpea Seeds (*Vigna unguiculata*) Germination Grown in Different Concentrations of Whole Hexane and Distilled Water (Control)**

| Concentrations (v/v) | 24HAP | 48HAP | 72HAP | 96HAP | 120HAP | 144HAP | 168HAP |
|---|---|---|---|---|---|---|---|
| Distilled Water | NG | 23.7 | 33.5 | 43.5 | 54.2 | 64.3 | 64.0 |
| 25% | NG | 22.5 | 32.7 | 42.7 | 53.5 | 63.5 | 63.7 |
| 50% | NG | NG | 22.0 | 32.3 | 43.5 | 55.0 | 55.5 |
| 75% | NG | NG | 20.5 | 32.5 | 42.5 | 50.5 | 51.7 |

**Values are Mean percentages of three replicates ; HAP: Hours After Planting*
*NG: No observable growth*

**Table 3: Mean Radicle Length of Cowpea Seedlings (*Vigna unguiculata*) Grown in Different Concentrations of Water Saturated Fraction of Hexane and Distilled Water (Control)**

| Concentrations (v/v) | 24HAP | 48HAP | 72HAP | 96HAP | 120HAP | 144HAP | 168HAP |
|---|---|---|---|---|---|---|---|
| Distilled Water | NG | 3.2±0.2 | 5.0±0.2 | 6.8±0.6 | 7.2±0.6 | 7.8±0.6 | 8.8±1.6 |
| 25% | NG | NG | 3.2±0.3 | 4.1±0.3 | 5.4±0.3 | 6.2±0.1 | 6.2±0.3 |
| 50% | NG | NG | 3.1± 0.4 | 3.9±0.4 | 4.7±0.3 | 5.3±0.8 | 6.1 ±0.9 |
| 75% | NG | NG | 2.5±0.9 | 3.1± 0.2 | 4.1± 0.2 | 4.4± 2.1 | 5.4± 0.6 |

**Values are Mean and Standard Variation of three replicates ; HAP: Hours After Planting*
*NG: No observable growth*

**Table 4: Mean Radicle Length of Cowpea Seedlings (*Vigna unguiculata*) Grown in Different Concentrations of Whole Hexane and Distilled Water (Control)**

| Concentrations (v/v) | 24HAP | 48HAP | 72HAP | 96HAP | 120HAP | 144HAP | 168HAP |
|---|---|---|---|---|---|---|---|
| Distilled Water | NG | 0.7±0.1 | 3.6±0.3 | 5.4±0.4 | 5.8±0.6 | 6.3±0.6 | 6.8±0.8 |
| 25% | NG | 0.5±0.1 | 2.9±0.3 | 4.1±0.2 | 5.0±0.7 | 5.2±0.7 | 5.8±0.7 |
| 50% | NG | NG | 1.4±0.4 | 3.5± 0.3 | 4.2±0.9 | 4.8±0.6 | 5.1±0.8 |
| 75% | NG | NG | 1.2±0.1 | 3.0±0.4 | 3.8±1.4 | 4.4± 1.6 | 4.7± 2.1 |

**Values are Mean and Standard Variation of three replicates ; HAP: Hours After Planting*
*NG: No observable growth*

**Table 5: Mean Percentage of Soybean seeds (*Glycine max*) Germination Grown in Different Concentrations of Water Saturated Fraction of Hexane and Distilled water (Control)**

| Concentrations (v/v) | 24HAP | 48HAP | 72HAP | 96HAP | 120HAP | 144HAP | 168HAP |
|---|---|---|---|---|---|---|---|
| Distilled Water | NG | NG | 25.0 | 35.5 | 46.7 | 57.7 | 62.0 |
| 25% | NG | NG | 22.0 | 34.5 | 44.5 | 52.5 | 62.0 |
| 50% | NG | NG | 21.0 | 33.5 | 42.5 | 46.5 | 56.7 |
| 75% | NG | NG | NG | 23.5 | 31.5 | 41.5 | 50.5 |

**Values are Mean percentages of three replicates ; HAP: Hours After Planting*
*NG: No observable growth*

**Table 6: Mean Percentage of Soybean Seeds (*Glycine max)* Germination Grown in Different Concentrations of Whole Hexane and Distilled Water (Control)**

| Concentrations (v/v) | 24HAP | 48HAP | 72HAP | 96HAP | 120HAP | 144HAP | 168HAP |
|---|---|---|---|---|---|---|---|
| Distilled water | NG | NG | 27.5 | 40.1 | 54.5 | 62.5 | 62.7 |
| 25% | NG | NG | 26.0 | 40.0 | 52.5 | 57.7 | 60.5 |
| 50% | NG | NG | 23.0 | 38.4 | 49.2 | 53.0 | 54.5 |
| 75% | NG | NG | NG | 21.0 | 35.5 | 44.0 | 54.1 |

**Values are Mean percentages of three replicates ; HAP: Hours After Planting*
*NG: No observable growth*

**Table 7: Mean Radicle Length of Soybean Seedlings (*Glycine max*) Grown in Different Concentrations of Water Saturated Fraction of Hexane and Distilled Water (Control)**

| Concentrations (v/v) | 24HAP | 48HAP | 72HAP | 96HAP | 120HAP | 144HAP | 168HAP |
|---|---|---|---|---|---|---|---|
| Distilled Water | NG | NG | 2.8±0.1 | 4.4±0.2 | 6.0±0.2 | 6.6±0.6 | 7.2±0.4 |
| 25% | NG | NG | 2.6±0.3 | 4.1±0.3 | 5.6±0.3 | 5.9±0.3 | 6.9±0.7 |
| 50% | NG | NG | 1.4±0.1 | 3.5± 0.4 | 4.3±0.9 | 5.5±0.3 | 5.8±0.8 |
| 75% | NG | NG | NG | 2.6 ±1.1 | 3.9±0.3 | 4.6±1.6 | 5.4± 0.2 |

**Values are Mean and Standard Variation of three replicates ; HAP: Hours After Planting*
*NG: No observable growth*

**Table 8: Mean Radicle Length of Soybean Seedlings (*Glycine max*) Grown in Different Concentrations of Whole Hexane and Distilled Water (Control)**

| Concentrations (v/v) | 24HAP | 48HAP | 72HAP | 96HAP | 120HAP | 144HAP | 168HAP |
|---|---|---|---|---|---|---|---|
| Distilled Water | NG | NG | 2.5±0.9 | 4.6±0.3 | 5.4±0.4 | 6.6±0.3 | 6.8±0.3 |
| 25% | NG | NG | 2.1±0.5 | 3.9±0.3 | 5.0±0.2 | 5.8±0.7 | 6.3±0.7 |
| 50% | NG | NG | 1.7±0.1 | 3.0± 0.2 | 4.8±0.3 | 5.0±0.6 | 5.1±0.6 |
| 75% | NG | NG | NG | 2.2 ±0.1 | 3.6±0.8 | 4.6±1.1 | 4.9± 2.1 |

**Values are Mean and Standard Variation of three replicates ; HAP: Hours After Planting*
*NG: No observable growth*

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