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# Effect of Crude Oil on Invertase and Amylase Activities in Cassava Leaf Extract and Germinating Cowpea Seedlings

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**Abstract:** The effect of crude petroleum oil on invertase and amylase activities was determined directly in the assay medium using a crude extract of cassava (*Manihot esculenta*) leaf as source of invertase and amylase as well as in germinating cowpea (*Vigna unguiculata* L. Walp.) seedlings. There was a decreasing cowpea seedling invertase and amylase activities with increasing crude oil concentration in the soil. After three days of germination, the invertase and amylase activities in seedlings from 5.9% crude oil-treated soil decrease to 42 and 15%, respectively, compared to the control. A similar profile was obtained with invertase and amylase activities when crude oil was added directly to cassava extract enzyme assay. Addition of 10% crude oil in the assay inhibits invertase and amylase activities by 5.1 and 29.6%, respectively.

**Key words:** Crude oil, invertase, amylase, soil enzymes, pollution

## INTRODUCTION

Nigeria is one of the major producers of crude oil and the impact of oil prospecting in the community has tremendously increased (Eriyamremu *et al*., 1999). Oil spills affect many species of plants and animals in the environment, as well as humans (Plohl *et al*., 2002). According to Baek *et al*. (2005) crude oil on soil makes it unsatisfactory for plant growth. This is due to insufficient aeration of the soil because of displacement of air from spaces between the soil particles by crude oil (Rowell, 1997). In Nigeria quite substantial amount of crude oil is spilled annually, for example, it has been observed from reports that more then 2,000 oil spillages occurred in Nigeria between 1976 and 1988, which amount to about 2 million barrels of crude oil discharged into the environment. Considering the large quantity of crude oil going into the environment, especially farmlands and rivers (Ossai *et al*., 1990) and the fact that the mainstay of the people living in this area are subsistent farmers and fishermen, there is urgent need for various agencies connected with oil production in Nigeria to pay more attention to the problem of oil leakage in the future (Osuji and Adesiyan, 2005).

The harmful effects of oil spills on the environment are many. Oil adversely affects plants and animals in the estuarine zone. Oil poisons algae, disrupts major food chain and decrease the yield of edible crustaceans. In a bid to clean up oil spills by the use of oil dispersants (Hoff, 1993), serious toxic effects will be excreted on planktons thereby poisoning marine animals. This can further lead to food poisoning and loss of lives (Rai and Chandra, 1992). Spilled oil can form coating on soil surface increasing temperature stress, reducing oxygen diffusion, resulting in more anaerobic soil condition and increasing low-oxygen stress to seeds.

Due to this substantial amount of crude oil released into the environment and considering the fact that a great proportion of the citizens are farmers, there is thus a need to study the effect of crude oil on plant's growth which depends on the activities of amylases and invertases which provides the simple carbohydrate required for plant growth. Many enzymes have been detected in soils but only

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a few assays have been evaluated thoroughly enough to be considered standard (Zahir *et al*., 2001). According to Dick and Tabatabai (1993), many of the enzymes detected in soils are hydrolases (they catalyze the hydrolytic cleavage of chemical bonds, oxidoreductases, transferases and lyases.

It has been observed that soil polluted with crude oil affects the germination of cowpea and the enzymes expressed during germination (Eriyamremu *et al*., 1999). It has been established that invertases and amylases are the major enzymes expressed during germination of seeds and they are the major starch hydrolyzing enzymes found in legumes (Kaur *et al*., 2002; Beers and Duke, 1990) during germination. The aim of this research is to determine the effect of crude oil pollution on activities of invertase and amylase in germinating cowpea seeds as well as in the soil.

## MATERIALS AND METHODS

### Planting of Cowpea

The study was conducted in 2006. Cowpea (*Vigna unguiculata* L. Walp) was purchased at Abraka main market, Delta State, Nigeria. The cowpea

seeds were then soaked in a bowl of water for about 2 min before planting. Loamy oil was collected from a garden at Delta State University, Abraka, Nigeria. 800 mL of the soil was then measured into 6 small size polyethylene bags. The first bag served as the control, while 10, 20, 30, 40 and 50 mL of crude oil (obtained from Escravos, Delta State, Nigeria) were added to the remaining five bags of soil and thoroughly mixed. Thereafter, six cowpea seeds were planted in each bag for duration of three days for germination to take place, before the seeds were uprooted and the enzyme extracted for analysis.

### Extraction

On the third day after planting, two plantlets were uprooted from each of the bags, these were washed. Thereafter, 1.0 g of cowpea plantlets were weighed from each of the samples and kept at 4°C for at least 1 h before extraction. The various samples were homogenized in pre-chilled mortar with pestle in the presence of 20 mL of 0.05 M phosphate buffer, pH 7.4. The homogenate was filtered with Whatman No. 1 filter paper. The filtrate was finally used for the determination of the invertase and amylase activities.

The activity of crude extract of invertase and amylase were determined in the presence of crude oil. The cassava (*Manihot esculenta*) leaves were collected from a farm in Abraka, Delta State, Nigeria. The leaves were thoroughly washed with clean tap water, allowed to drain and 1 g was extracted with 20 mL of 0.05 M phosphate buffer, pH 7.4. The cassava leaves were homogenized in pre-chilled mortar with pestle and the homogenate filtered using Whatman No. 1 filter paper. The filtrate was finally used for the determination of the invertase and amylase activities.

### Assay for Invertase and Amylase

Invertase and amylase activities was determined by the method of Lever (1972) using p-hydroxybenzoic acid hydrazide and 0.5% soluble starch solutions as substrate and reading the absorbance read at 410 nm. One unit of enzyme activity is the amount 1 nmol of reducing sugar released per min.

## RESULTS AND DISCUSSION

This study was carried out to ascertain the effect of crude oil pollution on the activities of invertase and amylase *in vitro* and *in vivo*. The effect of crude oil on invertase and amylase activities were determined directly in the assay medium using a crude extract of cassava leaf as source of invertase and amylase as well as in germinating cowpea seedlings from crude oil-treated soil. Invertase and amylase activities were measured in the cassava leaf extracts in the presence of crude oil to determine if there is any direct effect on the enzyme. The crude oil was added directly to the assay media in increasing amount and invertase and amylase activity was determined. The effect of crude oil on amylase activity *in vitro* using cassava extract as source of enzyme is presented in Fig. 1. When crude oil was added directly to enzyme assay, there was a gradual decrease in invertase and amylase activities as the amount of crude oil in the assay increases. This decrease is, however, more in amylase activities. Addition of 10% crude oil in the assay inhibits invertase and amylase activities by 5.1 and 29.6%, respectively.

Since it is also probable that crude oil pollution might affect enzyme activities in plants, we determined invertase and amylase activity in cowpea seedlings grown on crude oil-treated soil. High invertase and amylase activities were observed in the seedlings grown on the control soil (Fig. 2). But there was a sharp decrease in amylase activities in seedlings grown on the crude oil-treated soil. Increasing amount of crude oil in the soil led to decreasing amylase activities. After three days of

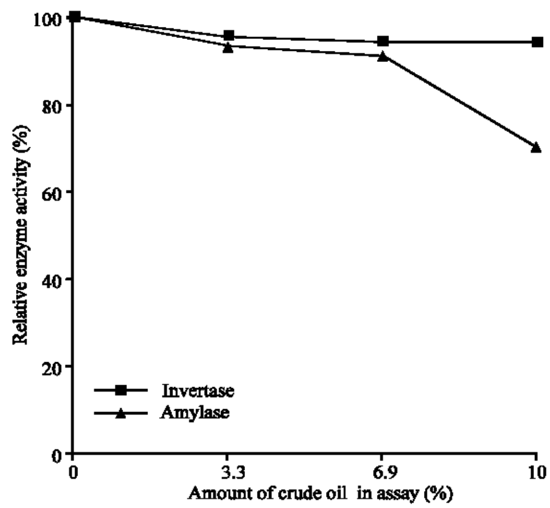


Fig. 1: Effect of crude oil on cassava leaf invertase and amylase activities *in vitro*

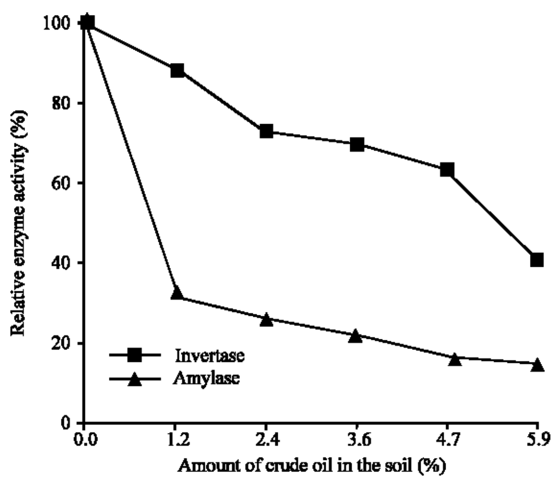


Fig. 2: Effect of crude oil on invertase and amylase in germinating cowpea seedling

germination, the invertase and amylase activities in seedlings from 5.9% crude oil-treated soil decrease to 42 and 15%, respectively, compared to the control.

The results of present study indicate that hydrolyzing enzymes, invertase and amylase, are present in germinating cowpea seeds and cassava leaves. They may play significant roles in supporting the growth of plant organs by converting sucrose and starch to monosaccharides to provide cells with fuel for respiration and with carbon for the synthesis of numerous compounds. Crude oil in soil inhibited the germination of seeds as well as invertase and amylase activities activity in *V. unguiclata*. Also, crude oil reduced invertase and amylase activities in cassava leaf extract. This may have been accompanied by reduction in nutrient mobilization. The mobilization of this sucrose for metabolic activities is essential to support growth in germinating seeds. Displacements of nutrients and nutrient leakage have also been suggested (Udo and Fayemi, 1975). The low germination observed in the crude oil treated soils may be related to reduction in soil nutrient availability.

During oil pollution, there is also reduction in water penetration into seeds, thus inhibiting spouting. Atuanya (1987) have previously shown that seeds grown in soil polluted by fresh crude oil failed to germinate even after 30 days of sowing. This could contribute to the low germination rate observed in the crude oil contaminated soil (data not presented). Fresh crude oil has a coagulatory effect on the soil, binding the soil particles into water impregnable soil work that seriously impair water drainage and oxygen diffusion (Atuanya, 1987).

Ilangovan and Vivekanandan (1992), working with blackgram (*Vigna mungo*), concluded that oil pollution in soil might deplete oxygen at the rhizosphere. Therefore, the depletion of soil oxygen by hydrocarbon degrading microorganisms and, therefore, oil polluted soil directly affects the overall physiology of the plant as evidenced by lower levels of macro and micro-biomolecules of the plant as well as polarity, thereby reducing plant growth. It is known that root stress reduce leaf growth via stomata conductance (Smith *et al*., 1989). According to Udo and Fayemi (1975), growth of plants growing on polluted soil was generally retarded and chlorosis of leaves results, coupled with dehydration of the plants indicating water deficiency. Also a significant increase in total phenolic content of leaves is observed during pollution.

An oil covered soil surface decreases oxygen movement resulting in more anaerobic soil conditions and increasing low oxygen stress on plant roots. In short term, spilled oil can form coating on plant foliage and the soil surface increasing temperature stress, reducing oxygen diffusion and also reducing photosynthesis. In conclusion, this study shows the adverse effect of crude oil on the germination, invertase and amylase activities of germination *V. unguiculata* and as well as the invertase and amylase activities of cassava leaf extract. Our future effort will concentrate on simple and economical methods of bioremediating crude oil-contaminated soils.

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