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EFFECTS OF SODIUM AZIDE ON THE SURVIVAL, GROWTH AND YIELD PERFORMANCE OF RICE (*ORYZA SATIVA,* FARO-57 VARIETY) IN A HYDROCARBON-POLLUTED SOIL

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ABSTRACT

The present study investigated the effects of sodium azide on the survival, growth and yield performance of rice (Oryza sativa, FARO 57 variety) in a hydrocarbon-polluted soil. Top soil (0 - 10cm) of known physiochemical parameters was collected randomly from a marked plot beside the Botanic Garden, University of Benin. Thereafter, 10 kg of sun-dried soil each was placed into large perforated buckets with 8 perforations made, at the bottom of each bucket, with a 2-mm diameter nail. Waste petrol-engine oil (WEO) was poured into the measured soil, and thoroughly mixed to obtain 2.5 % w/w oil in soil. Soils were then allowed to attenuate for two months. Rice seeds were pre-treated with various concentrations of sodium azide (NaN_3) solution on weight per volume basis (0.004, 0.008, 0.016, 0.032 and 0.064% w/v NaN_3 solution) for 6 hours before sowing in polluted and control soils. The untreated seeds were presoaked in distilled water. Results showed that tillering of rice plant was greatly enhanced by NaN_3 treatment. At 89 days after sowing (DAS), the average number of tillers observed was 6.70, 7.00 and 7.00 for the untreated plants in unpolluted soil, 0.004%w/v NaN_3 and 0.016%w/v NaN_3 treated plants respectively. Total number of grains obtained per rice stand was 81 in the untreated plants (control). This reduced to 64 grains/stand in the untreated rice plants sown in polluted soils. However, yield increased to a range of 103 – 159 grains/stand as levels of NaN_3 pre-treatments increased. Evidently, pre-treatment of rice seeds prior to sowing in polluted soils enhanced their vegetative growth and yield as well as plant survival.

Keywords: Heritability, Hydrocarbon-polluted soil, Oryza sativa, Sodium azide, Waste engine oil, Yield

INTRODUCTION

The environmental impact of the petroleum industry in Nigeria and other oil-producing countries has been on the increase. The increasing concern, however, of the environmental scientist is the destruction caused by oil spill both on cultivated and virgin lands. Oil spills on the land and sea

has been on the increase with explorative activities. According to Awobayo (1) , between 1978 and 1980 there were 734 cases of oil spills in Nigeria. This has become alarming in the past decades. Oil spills are destructive to both vegetations and animals in the soil not only because of their contact toxicity but also because hydrocarbons in the soil reduce oxygen tension and increase anaerobiosis which is harmful to plant roots (2). These oil mineral producing areas are in danger because the land is damaged and made infertile due to oil spill and other factors, and this prevents growth of crops for varying periods of time. The damaging effects are due to suffocation and toxicity of the crude oil (3).

The devastating effects of crude oil pollution has already been compared with that caused by waste engine oil, particularly considering the recent increase in the indiscriminate dumping of these oil waste by all manner of users (4). Disposal of spent engine oil into gutters, watercourses, open vacant plots and farmland are common practice among auto machine operators. This practice increases incidence of oil contamination of agricultural soils. Whisman *et al.* (5) reported presence of heavy metals such as vanadium, lead, aluminum, nickel and iron in unused lubricating oils, with high values in used ones. Oil-contaminated soils are of environmental concern because they are unsuitable for agricultural and recreational uses and are potential sources for surface and ground water contamination.

In Nigeria, about 20 million gallons of waste engine oil are generated annually from mechanic workshops and discharged carelessly into the environment (4), out of which only one liter is enough to contaminate one million gallons of fresh water (6). Apart from this, used engine oil renders the environment unsightly and constitutes a potential threat to humans, animals and vegetation (7). Several components of the oil, e.g. solvents and detergents added during the blending process, aliphatic hydrocarbons and PAHs distilled from crude oil, and heavy metals from engine wear are either toxic in themselves or can combine with products of combustion to generate carcinogens and endocrine disrupters (6).

Soil pollution by crude oil and crude oil based products depend on the type and amount of oil involved, the degree of its weathering, time of the year, species and age of plants involved (8). Odu (3) reported that pollution effects on plant system are a function of exposure time of the pollutant, the manner of disposal and the innate genetic response of the plant system as it is modified by environmental influences. Oil in soil makes the condition become unsatisfactory for plant growth (9), due to the reduction in the level of available plant nutrient or rise in toxic levels of certain elements such as Mn (10).

In spite of the adverse effects of oil in soil, Gudin and Syratt (11) reported that it is possible for oil in the soil to be degraded and release nitrogen and other mineral nutrients later on for plant growth resulting in growth stimulations in some plant species. Growth stimulation occurs in some plant species following the presence of crude oil. For example, Baker (12) found that crude oil can stimulate growth in *Pucinellia maritima* and *Festula rubra*. Plants respond differently to pollution effects due to an innate genetic response of the plant system as modified by environmental influences. Plant resistance to these environmental pollutants has been demonstrated in previous studies. Studies by Anoliefo and Edegbai (13) in *Solanum melongena* and *S. incarnum*, Dominguez-Rosado and Pitchel (14) in *Glycine max* and *Phaseolus vulgaris*, Ogboghodo *et al.* (15) in *Zea mays*, Ikhakiagbe (4) and Ikhajiagbe and Anoliefo (16) in *Vigna unguiculata*, have been shown to be tolerant to WEO and its heavy metal constituents. Because of plants' differential response to pollutants, such unhealthy environmental practices as improper WEO disposal would affect the distribution of plant species over time and space in affected areas.

Given that any impact of oil on crops would be very devastating (8,10,16,17) , it only goes to show the need for cultivation of crops that would possess, to some extent, some level of tolerance to these oil wastes so that the threat to crop production and food security would be laid to rest. This informs the basis of the present study. Among the various means available for breeding crops for resistance to environmental stress is the use of mutagenic agents like sodium azide (18-20). Induced mutations have been used to generate genetic variability and have been successfully utilized to improve yield and yield components of various crops like *Oryza sativa (21) Hordeum vulgare(22)* , *Triticum durum* (23), *Vicia faba (24)*, and *Vigna unguiculata (25)*. These reports show that mutagenesis is a potential tool to be employed for crop improvement. It is therefore the aim of the present study to investigate the impact of pretreatment of rice seeds with a mutagenic agent, sodium azide (20) on the survival and growth of rice in a waste engine oil-polluted soil.

MATERIALS AND METHODS

Top soil (0-10cm) of known physiochemical parameters (Table 1) was collected from an area measuring 50 m x 50 marked on a fallow land beside the Botanic Garden, University of Benin, Benin City. The soil was sun-dried to constant weight, and thereafter 10kg of soil was measured into buckets. Eight perforations were made at the bottom of each bucket, with a 2-mm diameter nail. Waste petrol-engine oil (WEO) was poured into the measured soil, and thoroughly mixed to obtain 2.5 % w/w oil in soil. Soils were then allowed to attenuate for two months. Rice seeds were pre-treated with various concentrations of sodium azide (NaN_3) solution on weight per volume basis (0.004, 0.008, 0.016, 0.032 and 0.064% w/v NaN_3 solution) for 6 hours before sowing in polluted and control soils. The untreated seeds were presoaked in distilled water.

Seeds of the rice (FARO 57) were subjected to the various concentrations of sodium azide for 6 hours. The treated seeds were removed from the solutions and washed in running water to remove excess chemicals and exudates from the seeds.

The set of pre-soaked seeds were immediately sown directly into polluted and control soils. Planting was done in the evening, just after sunset following the methods of Klu et al (26). Seeds were sown at the rate of 15 seeds per hole and at a depth of 3cm. These were thinned down to three per bucket one week later. Treatments were replicated four times. The experimental design chosen was the completely randomized design (CRD) following assumption of homogeneity of the experimental plot in use. As a result, treatments were randomized over the whole plot. Each treatment consisted of 4 replicates.

Genetic Studies

The genetic analysis was done on some yield parameters of rice in accordance with the methods of Mensah *et al (27)*. The mean squares at the treatment levels were taken as the phenotypic variance. Genotypic variance, which is the proportion of the phenotypic variance caused by variations in genes, the mean square at the error level, was subtracted from the corresponding phenotypic variance for all treatments used. The genetic parameters were as follows;

Heritability (%) = $\frac{\delta^2 g}{\delta^2 ph} \times 100$

Where $\delta^2 g$ = Genotypic variance, and $\delta^2 ph$ = Phenotypic variance

Genetic advance = $\frac{\delta^2 g}{\delta ph} \times k$

Where k = 2.06.

Genetic gain was calculated in terms of the genetic advance expressed as a percentage of the population mean. Genetic gain is expressed as GG=GA/Mean x 100/1. The phenotypic variance was used as the Treatment Mean Square value, while the genotypic variance was selected as the difference between the treatment mean square and the error mean square.

Statistics

Mean of data was calculated and presented on Tables. Other forms of statistics were those of ecological significance that required comparison with standard benchmark (28). Single factor analyses of variance was also conducted for some selected yield parameters of rice, using SPSS-16 statistical software.

Computation of Contamination Factor (CF)

CF expresses the ratio between the eventual concentrations of pollutant against its pre-contamination concentration [4].

$$CF = \frac{\text{Concentration of pollutant}}{\text{Pre-contamination reference}}$$

Pre-contamination concentration refers to the initial concentration of a particular contaminant (mg/kg) in the soil prior to exogenous application of the source of contaminant (4).

Computation of Hazard Quotient (HQ)

HQ expresses the possibility of the contaminant being an ecological risk or a contaminant of potential ecological concern (29). The hazards Quotient is expressed by the following equation:

$$HQ = \frac{\text{Measured concentration}}{\text{Toxicity reference value or selected screening benchmark.}}$$

When HQ > 1: Harmful effects are likely due to contaminant in question

When HQ = 1: Contaminant alone is not likely to cause ecological risk

When HQ < 1: Harmful effects are not likely

The Toxicity reference is provided by Efroymson *et al.* (29).

RESULTS AND DISCUSSION

The effects of sodium azide pretreatment on rice seeds before sowing in oil-polluted soil have been investigated in the present study. The study showed that there was reduction in heavy metal content during the period for which soil was allowed to naturally attenuate. At 1 week after pollution (Table 2), Fe content of soil was 1097.34mg/kg compared to 1326.42mg/kg at 2 months after pollution (2 MAP). Although Cd and Pb were not detected in the soil prior to exogenous application of WEO, hearin referred to as contaminant reference, concentrations of Cd and Pb were 1.58 and 1.03mg/kg respectively at 2 MAP. Total hydrocarbon content at 2 MAP was 2426.6mg/kg. This confirms earlier findings by Ikhajiagbe and Anoliefo (29,30). This reduction in heavy metal content of soil occurs as a result of a number of factors including inherent microbial action. Metals need immobilization or physical removal even though soil microorganisms can degrade organic contaminants. These metals are biotransformed by microbial activity to organic compounds, thereby ensuring their bio-unavailability (31). Microorganisms may possess reduction mechanisms that are not coupled to respiration, but

instead are thought to impart metal resistance. Reduction of metals can also occur through dissimilatory reduction where microorganisms utilize metals as a terminal electron acceptor for anaerobic respiration. For example, oxyanions of chromium (32,33) can be used in microbial anaerobic respiration as terminal electron acceptors. Microbial methylation is another mechanism of metal reduction. Pongratz and Heumann (34) earlier reported the biomethylation of Pb to dimethyl lead. Microbes may possess reduction mechanisms that are not coupled to respiration, but instead are thought to impart metal resistance. Methylation is another possible mechanism of metal reduction by microbial action. A number of different bacterial species including *Pseudomonas* sp., *Escherichia* sp., *Bacillus* sp., and *Clostridium* sp. have been implicated in the biomethylation of heavy metals (34).

Table-1. Physical and Chemical Properties of Soil before Waste Engine Oil Contamination.

Parameters	Units	Soil
pH	-	6.11
Electrical Conductivity	µs/cm	301
Total Org. Matter	%	0.61
Total Nitrogen	%	0.12
Exchangeable Acidity	meq/100 g soil	0.22
K	meq/100 g soil	1.43
Ca	meq/100 g soil	15.26
Mg	meq/100 g soil	10.97
P	mg/l	153.00
Clay	%	7.9
Silt	%	13.9
Sand	%	78.2
Fe	mg/kg	998.8
Mn	mg/kg	16.71
Zn	mg/kg	12.12
Cu	mg/kg	4.98
Cr	mg/kg	2.08
Cd	mg/kg	N.D
Pb	mg/kg	N.D
Ni	mg/kg	3.60
V	mg/kg	0.76
Total Hydrocarbon Content	mg/kg	224.06

ND: Not determined (≤0.001 mg/l)

Table-2. Heavy metals of soil after soil exposure to waste engine oil pollution prior to sowing of ice

	Fe	Mn	Zn	Cu	Cr	Cd	Pb	Ni	V	THC
	mg/kg									
Contaminant reference	998.8	16.7	12.1	4.98	2.08	ND	ND	3.6	0.76	224.06
1 WAP	1097.34	18.4	16.4	5.63	2.83	1.42	1.03	2.95	3.55	3425.63
2 MAP	1326.42	16.3	14.3	4.86	2.78	1.58	1.03	2.71	3.02	2426.6

WAP Weeks after pollution, MAP months after pollution, THC total hydrocarbon content

Apart from nickel, contamination factor of the heavy metal in all was greater than unity (CF > 1); This was due to exogenous application of WEO (Table 3). Contamination factor (CF) was less than unity at 2 MAP for Mn, Cu, Cd, Pb and Ni. The present study also showed a decrease in contamination factor of polluted soil from 1 week after pollution to 2 months, just before sowing. The CF explains the possibility for the inherent concentrations of contaminants in the soil to be primarily due to exogenous application of oil treatments (i.e. CF > 1), compared to pre-contamination levels (4). Considering hazard quotient(HQ) of soil after soil exposure to WEO pollution prior to sowing of rice, toxicity was indicated i.e. HQ > 1 for Fe, Cr, and V at 1 WAP and at 2 MAP (Table 4). There was an indication of considerable environmental risk factor at 2 MAP with Cd, Pb and V being less than zero, hence toxicity was indicated (Table 5).

Table-3. Contamination factor (CF) of soil after soil exposure to waste engine oil pollution prior to sowing of rice

	@Fe$^{998.8}$	Mn$^{16.7}$	Zn$^{12.1}$	Cu$^{4.98}$	Cr$^{2.08}$	CdND	PbND	Ni$^{3.6}$	V$^{0.76}$	THC$^{224.06}$
1 WAP	1.098	1.101	1.355	1.130	1.365	*ND	*ND	*0.819	4.671	15.288
2 MAP	1.328	*0.976	1.181	*0.975	1.336	*ND	*ND	*0.752	3.973	1.100

*CF < 1, contamination in soil is no longer due to exogenous application of waste engine oil (4).

@Values appearing as superscripts are contaminant references (mg/kg) of the respective pollutants.

Table-4. Hazard quotient (HQ) of soil after exposure to waste engine oil pollution prior to sowing of rice

	Fe@ ^{200}	Mn ^{500}	Zn ^{50}	Cu $_{100}$	Cr ^{1}	Cd ^{4}	Pb $_{50}$	Ni ^{30}	V ^{2}
1 WAP	*5.48	0.03	0.32	0.05	*2.83	0.35	0.02	0.09	*1.77
2 MAP	*6.01	0.02	0.27	0.03	*2.18	0.29	0.01	0.06	*1.39

@ *Toxicity references are provided in superscripts (28)*

Toxicity is indicated (i.e. HQ > 1). WAP Weeks after pollution, MAP months after pollution

Table-5. Environmental Risk Factor (ERF) of soil after soil exposure to waste engine oil pollution prior to sowing of rice

	Fe	Mn	Zn	Cu	Cr	Cd	Pb	Ni	V	THC
1 WAP	1097.34	18.4	16.4	5.63	2.83	1.42	1.03	2.95	3.55	3425.63
2 MAP	997.47	15.72	10.91	4.00	0.74	ND	ND	2.84	*-3.21	213.22

*ERF < O, toxicity is indicated.WAP weeks after pollution, MAP months after pollution.

Results showed delayed seedling emergence, though at 26 days after sowing, seeds in all the treatments apart from the 0% w/v (Polluted soil) treatment had 100% emergence (Table 6).

Chandra and Tarar (35) earlier reported that germination is inversely proportional to the dosage of mutagenic agents used in mutagenic experiments; the higher the mutagenic dosage, the lower the germination. It was apparent that the control plants had the highest percentage seedling emergence when compared with other concentrations of the mutagen, particularly within 10 days of sowing of pre-treated and control seeds. This is consistent with results of previous studies (20,25,36).

The reduction in seed germination in mutagenic treatments had been explained to be due to delayed or inhibition of physiological and biological processes necessary for seed germination which include enzyme activity, hormonal imbalance and inhibition of mitotic process. The inhibitory effect of NaN_3 on germination could be traced to the azide anions which are strong inhibitors of cytochrome oxidase, which in turn inhibits oxidative phosphorylation (37). In addition, it is a potent inhibitor of the proton pump and alters the mitochondrial membrane potential (38). These effects together may hamper ATP biosynthesis resulting in decreased availability of ATP which may slow the germination rate and reduce the germination percentage. Cheng and Gao (39) treated barley seeds with NaN_3 and reported significant reduction in the percentage germination.

Table-6. Effects of NaN_3 pretreatment on percentage seedling emergence of rice (FARO-57) in oil-polluted soil

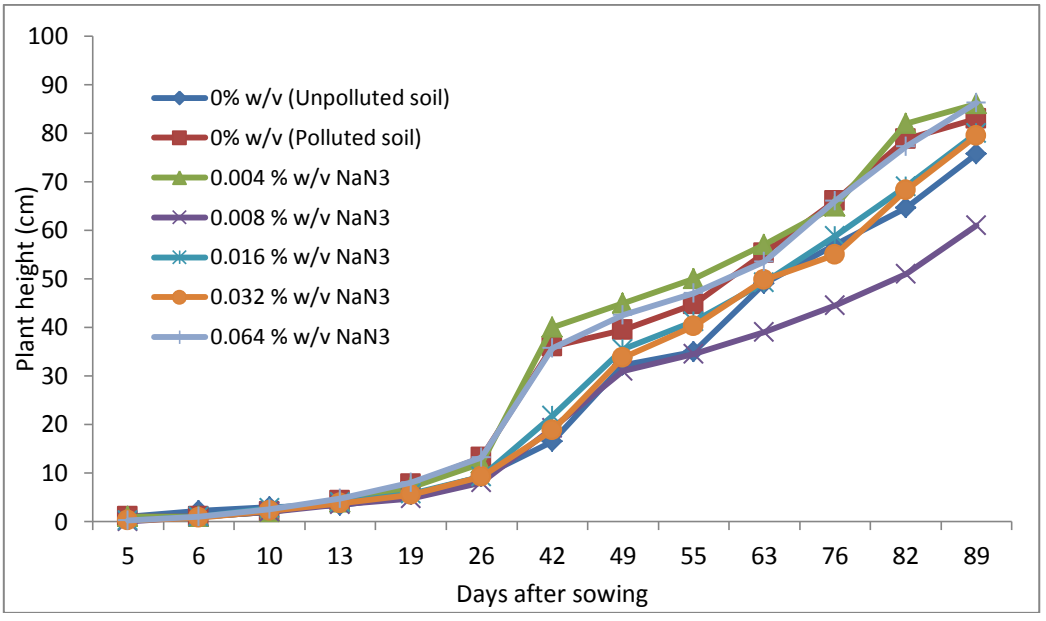
	Number of days after planting					
	5	6	10	13	19	26
0% w/v (Unpolluted soil)	75	12.75	95.00	14.75	100.00	100.00
0% w/v (Polluted soil)	46.67	51.67	53.33	69.23	75.63	77.87
0.004 % w/v NaN_3	46.67	63.33	90.00	95.00	100.00	100.00
0.008 % w/v NaN_3	0	23.33	68.97	76.67	95.00	100.00
0.016 % w/v NaN_3	23.33	53.33	87.87	93.65	100.00	100.00
0.032 % w/v NaN_3	23.33	51.67	53.33	95.00	100.00	100.00
0.064 % w/v NaN_3	23.33	58.34	75.23	96.67	100.00	100.00

Results show mean of 4 replications.

Plant height at 26 DAS was 9.25cm, 13.25cm, and 13.25cm for 0% w/v unpolluted soil, 0% w/v NaN_3-treated rice plants and 0.064% w/v NaN_3 treatment respectively. Also at 55 DAS plant height was 35cm, 44.75cm, and 47cm for 0% w/v treated unpolluted soil, 0% w/v NaN_3-treated rice plants and 0.064% w/v NaN_3 treatment respectively. At 89DAS, plant height observed was 75.67cm, 83cm and 86.25cm for 0% w/v unpolluted soil, 0% w/v NaN_3-treated rice plants and 0.064% w/v NaN_3 treatment respectively (Figure 1). Shoot length increased steadily with increase in concentration of NaN_3 in oil-polluted soil, compared with the control soil. Mshembula *et al.* (20) also reported increased growth in plant height of NaN_3-treated cowpea seedlings.

However, Mensah *et al.* (27) reported reductions in the growth of cowpea with increase in mutagenic level.

Fig-1. Effects of NaN_3 pretreatment on the development in height of rice (FARO-57) in oil-polluted soil



Tillering of rice plant was greatly enhanced by NaN_3 treatment indicated in Table 7. At 49 DAS the average number of tiller observed was 2.00 for 0% w/v unpolluted soil, 0.75 for 0.004% w/v NaN_3 respectively while at 89 DAS; 6.70, 7.00 and 7.00 for 0% w/v (unpolluted soil), 0.004%w/v NaN3 and 0.016%w/v NaN_3 treatment was observed. In spite of the environmental stress on the rice plant, chlorophyll content index (CCI) was 6.889 and 7.033 in 0.064% and 0.008%w/vNaN_3 treatments respectively, compared to the unpolluted soil (0% NaN_3w/v) with 10.317 CCI. Chlorophyll content index (CCI) was reportedly highest in the untreated plants in the unpolluted soil. Mshembula *et al.* (20) reported that high concentration of chlorophyll was observed at lower Sodium azide concentration in cowpea plants while reduced levels were recorded in higher concentrations of the mutagenic treatments in three of the five accessions studied compared to the control. This confirms most of the earlier reports in mungbean by Khan, (40) and Dixit and Dubey (41). It was also observed that for rice seedlings in the oil-polluted soil, those seedlings that were pre-treated in NaN_3 solution had slightly higher CCI compared to the untreated seedlings. Obviously, pretreatment with NaN_3 may have positively impacted on the CCI in the stressed rice plants occasioned by oil pollution. The number of leaves was more in 0.004%w/v NaN_3 treatments (51.00) and in 0.064%w/v was 47.00 compared to unpolluted soil with 45.25 leaves. However the percentage plant survival was 100% in all treatments and the percentage

leaf survival was highest with 59.57 in 0.064%w/v NaN_3 treated plants and 57.84 in 0.004%w/v treatment compared to 59.66 in 0% w/v NaN_3 treated rice plants (Table 8).

Table-7. Effects of NaN_3 pretreatment on number of tillers per rice plant in oil-polluted soil

	Number of days after planting					
	49	55	63	76	82	89
0% w/v (Unpolluted soil)	2.00	3.50	4.70	6.70	6.70	6.70
0% w/v (Polluted soil)	0	3.25	5.00	6.75	6.75	6.75
0.004 % w/v NaN_3	0.75	4.25	5.25	7.00	7.00	7.00
0.008 % w/v NaN_3	2.25	4.25	4.25	4.50	4.50	4.50
0.016 % w/v NaN_3	2.50	4.75	6.00	7.00	7.00	7.00
0.032 % w/v NaN_3	2.00	5.00	5.50	5.50	5.50	5.50
0.064 % w/v NaN_3	1.50	4.00	5.25	6.25	6.25	6.25

Results are mean of 4 replications

Table-8. Effects of NaN_3 pretreatment on some stress parameters of rice (FARO-57) in oil-polluted soil

	Chlorophyll content index	No. of leaves @ 90DAS	Leaves survived @ 90DAS	Percentage plant survival (%)	Percentage leaf survival (%)
w/v (Unpolluted soil)	10.317	45.25	27.00	100	59.66
w/v (Polluted soil)	5.500	48.25	31.25	100	64.76
04 % w/v NaN_3	6.578	51.00	29.50	100	57.84
08 % w/v NaN_3	7.033	40.75	23.50	100	57.66
16 % w/v NaN_3	6.711	47.50	27.00	100	56.84
32 % w/v NaN_3	6.400	42.25	23.75	100	56.21
64 % w/v NaN_3	6.889	47.00	28.00	100	59.55

Considering the yield parameter, 0.016%w/v NaN_3 treated plant was observed to have weighed more with 2.110g (100 seed wt), 1.228g (fresh wt per panicle) compared to 1.618g (100 seed wt) 0.941g (fresh wt per panicle) for 0% w/v (unpolluted soil). Seeds treated in 0.016%w/v NaN_3 had the highest number of grains per plant (159.24) (Table 8), as compared to 81.23 f0r 0% w/v NaN_3-treated rice plants. In the untreated rice plants, the presence of oil in soil caused a reduction in plant yields expressed as number of grains per plant from 81.23 in the unpolluted soil to 64.54 in the polluted soil. Similar yield reductions have been reported by Ikhajiagbe and Anoliefo (29,30,42). Mechanisms of toxicity of metals tend to be dependent on the nature of the reactivity of the metal itself. They may alter or inhibit enzyme activity, interfere with deoxyribonucleic acid (DNA) synthesis or electron transport, or block uptake of essential elements (28). Variability in response to `toxic' levels of metals by different plants is due to a number of defenses. These include exclusion from the root, translocation in nontoxic form, sequestering in nontoxic form in the root or other plant parts, and formation of unusable complexes containing metals that may otherwise be inserted into biomolecules instead of the proper element (e.g as replacing P) (43). Number of soybean seeds produced per plant was decreased by 67 % when plants were grown in an average garden soil to which 10 mg/l Cd was added as CdCl (44). Cadmium at 5 mg/l had no

effect. Plants were grown from seed to maturity. However, when seeds in the oil-polluted soils were pretreated in NaN_3 solution grain yield was positively impacted. This supports earlier reports of Mensah *et al (19)*.in groundnut (*Arachis hypogaea* L.), Mensah *et al (27)* in sesame seed (*Sesame indicum L.*), and Mshembula *et al.* (20) in cowpea (*Vigna unguiculata*).

Table-9. Effects of NaN_3 pretreatment on some yield parameters of rice (FARO-57) in oil-polluted oil

Treatments	100 seed weight (g)	Fresh weight per panicle (g)	Number of grains per plant	Fresh weight of whole plant(g)	Dry weight of seeds per panicle(g)	Dry weight of whole plant (g)	Weight of root (g)
0% w/v (Unpolluted soil)	1.618	0.941	81.23	17.595	0.394	7.324	1.2669
0% w/v (Polluted soil)	1.428	0.843	64.54	14.038	0.667	8.002	1.4972
0.004 % w/v NaN_3	1.486	0.970	103.25	21.265	0.668	8.789	0.734
0.008 % w/v NaN_3	1.187	0.807	139.68	20.068	0.654	8.648	1.3933
0.016 % w/v NaN_3	2.110	1.228	159.24	18.394	0.709	8.519	0.8128
0.032 % w/v NaN_3	1.649	0.925	144.25	19.919	0.833	8.412	0.7117
0.064 % w/v NaN_3	1.673	1.067	158.71	21.857	0.601	7.383	0.6556

Results are mean of 4 replications

Heritability was 99.46% for number of grains per plant while genetic gain was 139.96, indicating that the phenotypic expression of fruiting is genetic as occasioned by NaN_3 pretreatment. Heritability for 100 seed weight was 97.76%, and 96.59% for dry weight of seed; whereas the genetic gain was 91.33 for dry weight of seeds per panicle; also indicating that the NaN_3 treatment had significantly enhanced the formation of seeds (Table 10). Genetic parameters were highest for number of grains per plant, having a heritability of 99.46%, a GA of 150.14 and a GG of 139.96.

Table-10. Genetic parameters of some yield parameters rice subjected to sodium azide pre-treatment after exposure to waste engine oil pollution.

Character	**Mean**	**Phenotypic Variance (δ^2ph)**	**$\sqrt{(\delta^2 ph)}$ or δph**	**Genotypic Variance (δ^2g)**	**Heritability (%)**	**Genetic Advance**	**Genetic Gain**
No. of grains per plant	107.27	5369.140	73.274	5340.373	99.46	150.14	139.96
Dry wt. of seeds/panicle(g)	0.646	0.088	0.297	0.085	96.59	0.59	91.33
Whole plt Wt. (g)	8.154	1.795	1.339	1.312	73.09	2.02	24.77
100 seed weight (g)	1.593	0.401	0.633	0.392	97.76	1.28	80.35

CONCLUSION

Considering the results emanating from the present study, , it is enough to conclude that NaN_3 as a mutagen has impacted positively on the survival, growth and yield performance of rice under stress occasioned by waste engine oil pollution.

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