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Investigation of Acute Toxicities of Nigerian Crude Oil, Dispersant, Sodium Dodecyl Sulphate and a Mixture of Crude Oil-Plus-Dispersant to *Desmocaris trispinosa*

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Abstarct: Toxicity bioassay was conducted to compare the toxicities of four toxicants: a Nigerian crude oil (Bonny Light), oil dispersant (Nalco-D4106), reference compound (Sodium dodecyl sulphate) and a mixture of crude oil-plus-dispersants to Freshwater shrimp, *Desmocaris trispinosa*. Tests were conducted over a 96-hr period after acclimatization of the test organisms in the laboratory. The test organisms (*D. trispinosa*) were exposed to the following concentrations of Nigerian Bonny Light crude oil (0, 40, 80, 160, 240 and 320 mg/l), dispersant (0, 500, 1000, 2000, 4000 and 8000mg/l), reference compound (0, 5, 25, 50, 75 and 100mg/l) and mixture of crude oil-plus-dispersants (0, 540, 1080, 2160, 3240 and 4320mg/l). The 96-hrs LC_{50} of Bonny Light crude oil, dispersant (Nalco- D4106), sodium dodecy sulphate and crude oil-plus-dispersants to *D. trispinosa* were 126mg/l, 1585mg/l, 56mg/l and 1995mg/l respectively. The toxicity factor of dispersant (Nalco-D4106) to *D. trispinosa* was 28 while the synergistic factor of crude oil-plus-dispersants to *D. trispinosa* was approximately 16. This study shows that Bonny light crude oil is more toxic to *D. trispinosa* than dispersant (Nalco-D4106) and also that the dispersant caused a reduction in the toxicity of the crude oil to *D. trispinosa*.

Key words: Bonny light · Nalco-D4106 · Sodium dodecyl sulphate · Desmocaris trispinosa · 96-hr LC50 · Toxicity factor · Synergistic factor

INTRODUCTION

Acute toxicity bioassay are widely used to assess the effects of pollutants on one or more organisms usually based on the determination of acute lethal toxicity and sub- lethal toxicity test using sensitive species or organisms based on their economic and ecological importance, availability and ease of handling. Although, the tests are laboratory based, simple, of single variable and do not necessarily simulate the field situations, they nonetheless provide useful information on the potential of the pollutant to harm the biota [1].

The decision making process surrounding the use of dispersants in oil spill response is necessarily an environmental cost-benefit analysis; both must be assessed and the consequences of both use and non-use of dispersants must be weighed for these costs and benefits to be identified. The effects of dispersants on inherent oil toxicity must be understood to provide insight into the consequences of their use as well as guidance about when they may, or may not, be appropriate. This understanding can only come when the toxicities of the dispersant and oil are evaluated separately and in combination [2].

However, most toxicity studies in Nigeria have focused on the toxicity of Nigeria crude oil alone to aquatic fauna [3, 4] and toxicity of dispersant alone to Fauna [5]. Very few [6, 7] have examined the toxicities of Nigeria crude oil and dispersants to indigenous aquatic Fauna separately and in combination in one experiments in order to establish the efficacy of dispersants to remedy oil spills and their effects on indigenous aquatic Fauna. Therefore, this study attempts to bridge this gap by examining the toxicities of Nigerian crude oil (Bonny Light), dispersant (Nalco-D4106) and a mixture of the crude oil and dispersant to a commercially viable species (*Desmocaris trispinosa*) endemic to Nigeria.

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MATERIALS AND METHODS

Experimental Set - up and Fish Management : Bioassay was conducted in the laboratory of the Department of Wildlife and Fisheries Management, University of Ibadan, Nigeria. The method of bioassay employed was the one outlined by [8] and [9]. The toxicities of the following toxicants to Desmocaris trispinosa were compared:

- Nigerian Light Crude Oil (Bonny Light)
- Oil Dispersant (Nalco - D4106)
- Reference Compound (Sodium dodecyl Sulphate)
- Crude oil - Plus - Dispersant

Acclimatization of Test Organisms: The test specimens (*Desmocaris trispinosa*) were collected from Epe, Lagos, Nigeria in thick transparent polyethylene bags with artificial oxygen to sustain the freshwater shrimp during transportation to the laboratory. The shrimps were held in glass tanks (60 x 30 x 45cm^3) for 14 days prior to the start of the experiment. Each acclimatization tank had the habitat water from where the shrimps were collected. The habitat water in the tanks was replaced every 2 days. During acclimatization, the temperature was maintained at 29±2°C while aeration was continued throughout the period with aquarium pumps. The photo-period was 12 hour light and 12 hours darkness.

Preparation of Test Medium and Application of Test Chemicals

Experiment 1: A preliminary (range-finding) test as described by [10] and [11] was conducted to determine the main experimental concentrations for the crude oil (Bonny Light), dispersant (Nalco-D4106), reference compound (Sodium dodecyl sulphate) and Crude oil - Plus - Dispersant. The main experimental concentrations for the toxicants (crude oil, dispersant, reference compound and Crude oil - Plus - Dispersant) above were determined based on 0% - 100% mortality of Desmocaris trispinosa in 24 hours.

Experiment 2:

Definitive Test: The second stage of the experiment gives details of the main experimental concentrations for the toxicants as described by [10] and [3].

(A)crude Oil (Bonny Light) Against *D. Trispinosa*.: The followings were set up: 0.0, 0.1, 0.2, 0.4, 0.6 and 0.8ml crude oil (Specific gravity: 0.7942g/ml) in 2L of freshwater corresponding to 0, 40, 80, 160, 240 and 320 mg crude oil respectively per litre of water.

(B)dispersant (Nalco - D4106) Against *D. Trispinosa*.: The following were set up: 0.0, 1.0, 2.0, 4.0, 8.0 and 16.0ml of dispersant (Specific gravity: 1.0006g/ml) in 2L of freshwater corresponding to 0, 500, 1000, 2000, 4000 and 8000mg of dispersant respectively per litre of water.

(C)sodium Dodecyl Sulphate (Sds) Against *D. Trispinosa*.: The followings were set up: 0.0, 10.0, 50.0, 100.0, 150.0 and 200.0 mg SDS in 2L of freshwater corresponding to 0, 5, 25, 50, 75 and 100mg SDS respectively per litre of water.

(D)crude Oil (Bonny Light)-plus-dispersant (Nalco - D4106) Against *D. Trispinosa*.: The followings were set up: 0.0, 0.1, 0.2, 0.4, 0.6 and 0.8ml Bonny Light crude oil and 0.0, 1.0, 2.0, 4.0, 6.0 and 8.0ml dispersant in 2L of freshwater corresponding to a combined concentration of 0, 540, 1080, 2160, 3240 and 4320mg crude oil-plus-dispersant respectively per litre of water.

Selection of Organisms (*D. Trispinosa*) for the Bioassay

(A)crude Oil (Bonny Light): One hundred and twenty (120) specimens of *D. trispinosa* of mean length 3.93±0.23cm and mean weight 5.25±1.43g were randomly assigned in equal number (20) into six test tanks (60 x 30 x 45cm^3) separately containing the following concentrations of the crude oil 0mg/l (control), 40mg/l, 80mg/l, 160mg/l, 240mg/l and 320mg/l. Each of these experimental units was replicated thrice to give a total of eighteen (18) experimental units (test tanks) containing three hundred and sixty (360) specimens of *D. trispinosa*. The control (0mg/L) contained only 20 specimenss of *D. trispinosa* without the test toxicant (Bonny Light crude oil). During the bioassay, the test solution in each tank was renewed every 24 hours.

(B)dispersant (Nalco - D4106), Sodium Dodecyl Sulphate and Crude Oil-plus-dispersant.: A similar experiment as the one described above was set up for dispersant, sodium dodecyl sulphate and crude oil-plus-dispersant using the concentrations determined after the preliminary tests (Experiment 1). Six experimental units were each set up for the toxicants (dispersant, sodium dodecyl sulphate and crude oil-plus-dispersant). These were the concentration of the toxicants: 0 mg/l (Control) and 500 mg/l, 1000 mg/l, 2000 mg/l, 4000 mg/l and 8000 mg/l for

dispersant; 0 mg/l (control), 5 mg/l, 25 mg/l, 50 mg/l, 75 mg/l and 100 mg/l for sodium dodecyl sulphate; and 0 mg/l (control), 540 mg/l, 1080 mg/l, 2160 mg/l, 3240 mg/l and 4320 mg/l for the crude oil-plus-dispersant. Each of these experimental units was replicated thrice to give a total of fifty four (54) experimental units containing one thousand and eighty (1,080) specimens of *D. trispinosa*. The controls (0 mg/l) contained twenty (20) specimens of *D. trispinosa* without the toxicant (dispersant, sodium dodecyl sulphate or crude oil-plus-dispersant). In all, there were seventy two (72) experimental units containing one thousand four hundred and forty (1440) specimens of *D. trispinosa*.

Monofilament nettings were used to cover the tanks to prevent the shrimp specimens from jumping out of test solutions. The behaviour of specimens was observed and death was recorded for the 24-hr, 48-hr, 72-hr and 96-hr test periods. Death was defined as complete immobility [12].

Statistical Analysis: Each test concentration was converted into a logarithm and the corresponding percentage (%) mortality was transformed into probit [13]. The median lethal toxicity (LC_{50}), median lethal time (LT_{50}), minimum lethal concentration and minimum lethal time were determined according to the method described by Finney [14]. Analysis of Variance (ANOVA) was used to test for significant differences in the number of survivors in the concentrations of the test toxicants (crude oil, dispersant, sodium dodecyl sulphate and crude oil-plus-dispersant).

The toxicity factor for dispersant and synergistic or joint action factor for crude oil-plus-dispersant was determined using the formula described by Odiete [15].

$$\text{Toxicity Factor For dispersant} = \frac{\text{96hr } LC_{50} \text{ value of dispersant}}{\text{96hr } LC_{50} \text{ value of reference Compound (SDS)}}$$

$$\text{Synergistic or joint action factor} = \frac{\text{96hr } LC_{50} \text{ of dispersant-plus-crude oil}}{\text{96hr } LC_{50} \text{ of crude oil alone}}$$

RESULTS AND DISCUSSION

The 96 - hr median lethal concentrations (LC_{50}) of the toxicants; dispersant (Nalco- D4106), reference compound (Sodium dodecyl sulphate), Nigerian crude oil (Bonny Light) and a mixture of crude oil-plus-dispersant to *Desmocaris trispinosa* are shown in Figure 1, 2, 3 and 4 respectively. The coefficient of determination (r^2) between log concentration of toxicants and probit mortality showed that there was a strong, positive and significant correlation between the toxicant and mortality values except for the reference compound where ($r^2 = 0.73$; N = 5; $\alpha = 0.05$) (Fig. 2). The coefficient of determination (r^2) for dispersant, crude oil and crude oil-plus-dispersant are ($r^2 = 0.99$; N = 5; $\alpha = 0.05$) (Fig. 1), ($r^2 = 0.98$; N = 5; $\alpha = 0.05$) (Fig. 3) and ($r^2 = 0.97$; N = 5; $\alpha = 0.05$) (Fig. 4) respectively. The 96 - hr LC_{50} values obtained from graphical illustrations are dispersant (1585 mg/l), reference compound (56 mg/l), crude oil (126 mg/l) and crude oil-plus-dispersant (1995 mg/l) and they are presented in Table 1.

The 96-hr LC_{50} value (126mg/l) of Bonny light crude oil obtained in this study is consistent with the value obtained in previous studies by Daka and Ekweozor [3] which studied the effect of size on the acute toxicity of a Nigerian crude oil (Egbogoro Liner II) to the mangrove oyster, *Carasostrea gasar*. The study revealed that

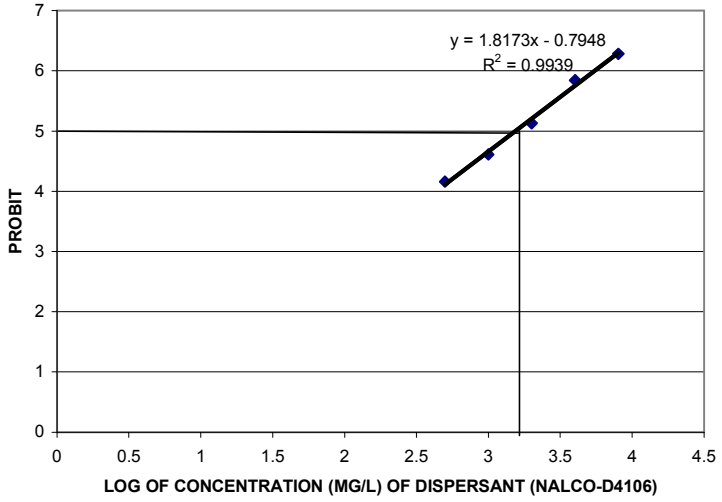

Fig. 1: 96-hr Lc_{50} of Dispersant (Nalco-d4106) to *Desmocaris Trispinosa*

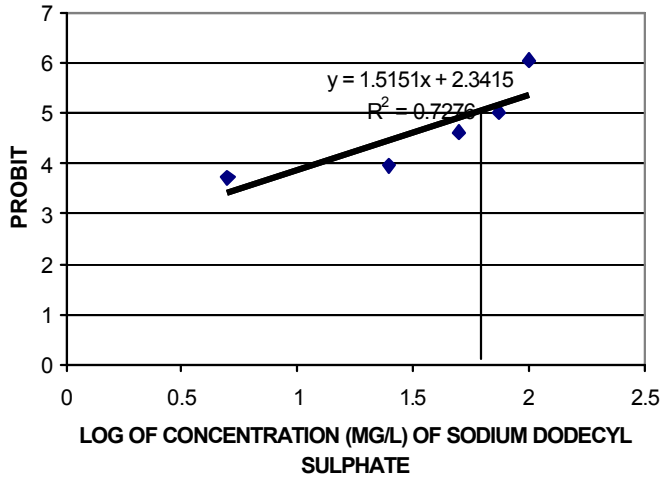

Fig. 2: 96-HR LC_{50} of Reference Compound (Sodium Dodecyl Sulphate) to *Desmocaris trispinosa*

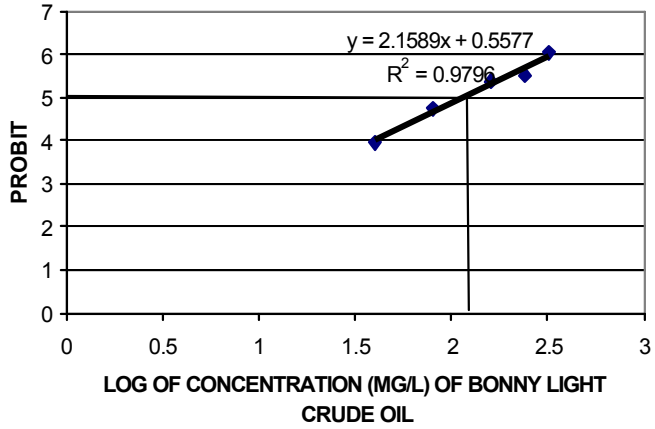

Fig. 3: 96-HR LC_{50} of Crude Oil (Bonny Light) to *Desmocaris trispinosa*

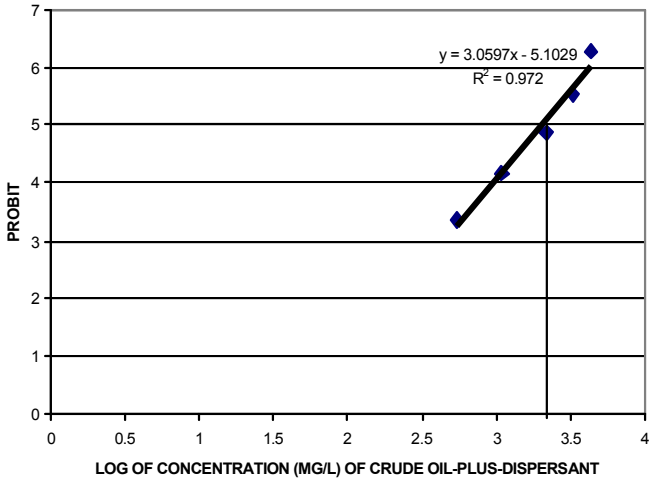

Fig. 4: 96-HR LC_{50} of Crude Oil (Bonny Light) -Plus- To Dispersant (Nalco-D4106) *Desmocaris trispinosa*

Table 1: 96-hr LC_{50} of Toxicants to *Desmocaris trispinosa*

Toxicant	96hr LC_{50} (Mg/l)
Dispersant (Nalco-D4106)	1585
Reference Compound (Sodium dodecyl sulphate)	56
Crude oil (Bonny Light)	126
Dispersant (Nalco-D4106)-plus- Crude Oil	1995

the 96-hr LC_{50} of Egbogoro Liner II crude oil to *C. gasar* of size range of 10 - 30mm, which is similar to the size range of *D. trispinosa* used in this study, was 135mg/l. However, Singer *et al* [16] used a flow through exposure system to assess the effects of Prudhoe Bay oil to *Holmesimysis costata* and *Atherinops affinis* and got LC_{50} values ranging from 14.28-16.84mg/l and 5.77-12.17mg/l respectively. The reason for this low median lethal concentration might be due to the bioassay method used and the type of oil administered [17, 18].

The median lethal concentration of the four toxicants {dispersant (Nalco - D4106), reference compound (sodium dodecyl sulphate), crude oil (Bonny light) and mixture of crude oil - plus - dispersant} showed that the most toxic to *Desmocaris trispinosa* is the reference compound (sodium dodecyl sulphate) with an LC_{50} value of 56mg/l. This value obtained in this study is similar to the value (46mg/l) reported in Kikuchi and Wakabayashi [19] in which the 48-hr LC_{50} of sodium dodecyl sulphate to *Oryzias latipes* was investigated.

Bonny light crude oil only was more toxic to *D. trispinosa* than dispersant only as shown by their LC_{50} values which are 126mg/l and 1585mg/l respectively. This result agrees with the study of Fuller *et al* . [18]. The study investigated among other things, the toxicities of a dispersant, corexit 9500 and Arabian medium crude oil to a shrimp, *Americamysis bahia* (formerly *Mysidopsis bahia*). The LC_{50} values of corexit 9500 and Arabian medium crude oil to *A. bahia* obtained in that study were 0.62mg/l and 32mg/l respectively showing that Arabian medium crude oil is more toxic than the dispersant, corexit 9500. However, Otitoloju and Popoola [7] reported that Biosolve (a dispersant) was about 27,284 times more toxic than crude oil while OSD 9460, another dispersant was found to be about 4 times more toxic than crude oil when acting alone against *Clarias gariepinus*.

Desmocaris trispinosa was more sensitive to crude oil only than the mixture of crude oil - plus -dispersant as shown in LC_{50} values which is 126 mg/l and 1995 mg/l for Bonny light crude oil and the mixture of Bonny light crude oil - plus - dispersant (Nalco- D4106) respectively. This result agrees with the study of Fuller *et al* [18] in which the median lethal concentrations (96- hr LC_{50}) of water-soluble fraction of Arabian medium crude oil only and crude oil- plus- dispersant (Corexit 9500) to a shrimp, *Americamysis bahia* was investigated. The LC_{50} of crude oil only to *A. bahia* was 0.62 mg/l while the LC_{50} of crude oil- plus - dispersant to *A. bahia* was 0.65mg/l. However, the result of this study does not agree with the study of Singer *et al.* [16] where the LC_{50} of water- soluble fraction of Prudhoe Bay crude oil only and crude oil - plus - dispersant (Corexit 9500) to *Holmesimysis costata* was 15.28mg/l and 14.62mg/l respectively. The result in this study and the previous studies cited shows that in the remediation of crude oil spillage by chemical dispersion, the type of crude oil involved, the dispersant applied and the resident organisms are some of the important factors to be considered before a decision on the use or non-use of a dispersant for a particular oil spill scenario can be taken.

Table 2 shows the median lethal time (LT_{50}) of toxicants to *D. trispinosa*. The highest LT_{50} (94 hrs) for the dispersant (Nalco - D4106) occurred at a concentration of 2000 mg/l, while the lowest (39 hrs) was observed at 8000 mg/l. The highest LT_{50} of reference compound (sodium dodecyl sulphate), crude oil (Bony Light) and crude oil - plus - dispersant occurred at concentrations of 75 mg/l, 160 mg/l and 63 mg/l respectively while the lowest LT_{50} values of 34 hrs (sodium dodecyl sulphate), 49 hrs (Bonny Light Crude Oil) and 31 hrs (crude oil-plus-dispersant) occurred at 100 mg/l, 320 mg/l and 4320 mg/l respectively.

The minimum concentrations of each toxicant that can cause the death of *D. trispinosa* and the minimum time within which these concentrations can cause the death of this crustacean are presented in Table 3. Among the four toxicants investigated, the mixture of crude oil - plus - dispersant caused the death of *D. trispinosa* within the shortest time, 32 hrs, while the highest minimum time of 94 hrs was recorded in the dispersant (Nalco - D4106). The dispersant had the highest minimum concentration (8128 mg/l) that could cause the death of *D. trispinosa* while the lowest minimum concentration (74 mg/l) was observed in the reference compound (sodium dodecyl sulphate).

Table 4 shows the result of the survivors of *D. trispinosa* exposed to different concentrations of the toxicants. In all the toxicants, the number of survivors in each concentration differs significantly ($p < 0.05$) from others. The toxicity factor of the dispersant (Nalco - D4106) was 28.19 while the synergistic or joint action factor of the mixture of crude oil-plus-dispersant was 15.85.

Table 2: Median Lethal Time (LT_{50}) of toxicants to *Desmocaris trispinosa*

Concentration (Mg/l) of dispersant)(Nalco - D4106)	LT_{50} (HR
0 (control)	0
500	0
1000	0
2000	94
4000	78
8000	39
Concentration (Mg/l) of reference compound (Sodium dodecyl sulphate)	**LT_{50} (HR)**
0 (control)	0
5	0
25	0
50	0
75	96
100	34
Concentration (Mg/l) of Crude oil (Bonny Light)	**LT_{50} (HR)**
0 (control)	0
40	0
80	0
160	90
240	80
320	49
Concentration (Mg/l) of Crude oil - plus - dispersant (Nalco - D4106)	**LT_{50} (HR)**
0 (control)	0
540	0
1080	0
2160	0
3240	63
4320	31

Table 3: Minimum concentration and minimum time of toxicants to *Desmocaris trispinosa*

Toxicant	Minimum concentration (Mg/l)	Minimum time (HR)
Dispersant (Nalco - D4106)	8128	94
Reference Compound (sodium dodecyl sulphate)	74	34
Crude oil (Bonny Light)	324	90
Dispersant (Nalco - D4106)-plus-Crude oil	3236	32

Table 4: Survivors of *Desmocaris trispinosa* exposed to different concentration (Mg/l) of toxicants

(A) Dispersant (Nalco - D4106)	
Concentration (mg/l)	Survival (Mean ± S. E.)
Control (0)	20.00 ± 0.00^{a}
500	15.67 ± 3.33^{b}
1000	12.33 ± 0.58^{c}
2000	8.33 ± 0.58^{d}
4000	3.67 ± 3.33^{e}
8000	2.00 ± 0.00^{f}
(B)Dispersant compound (sodium dodecyl sulphate)	
Control (0)	20.00 ± 0.00^{a}
5	17.67 ± 0.33^{b}
25	16.67 ± 0.33^{c}
50	12.33 ± 0.58^{d}
75	9.67 ± 0.33^{e}
100	2.33 ± 0.58^{f}

Table 4: Continued

(C)Dispersant (Bonny Light)	
Control (0)	20.00 ± 0.00 [a]
40	16.67 ± 0.33 [b]
80	11.67 ± 0.33 [c]
160	6.33 ± 1.15 [d]
240	5.63 ± 0.33 [e]
320	2.33 ± 0.58 [f]
(D)Dispersant (Nalco - D4016) and crude oil (Bonny Light)	
Control (0)	20.00 ± 0.00 [a]
540	18.67 ± 0.33 [b]
1080	15.67 ± 0.33 [c]
2160	10.33 ± 0.58 [d]
3240	5.33 ± 0.58 [e]
4320	1.67 ± 0.33 [f]

The number of *D. trispinosa* that survived after 96 hours in each concentration of Bonny light crude oil was significantly different ($p<0.05$). Similarly, the numbers of *D. trispinosa* that survived after 96 hours in each concentration of the other toxicants also differ significantly ($p< 0.05$).

The toxicity factor of the dispersant (Nalco -D4106) to *D. trispinosa* was 28.19, which implies that the reference compound (sodium dodecyl sulphate) is about 28 times more toxic to *D. trispinosa* than Nalco -D4106. The synergistic or joint action factor of the crude oil-plus-dispersant to *D. trispinosa* was 15.85 (≅ 16) which means that the Bonny light crude oil only is about 16 times more toxic than the mixture of crude oil - plus - dispersant. This also implies that the dispersant (Nalco-D4106) reduce the toxicity of Bonny light crude oil by a factor of 16. Alade and Anyachukwu [6] reported the potentiation of toxicity of crude oil by two chemical dispersants (Teepol and Conco-K) on *Barbus* sp fingerlings and *Clarias* eggs. Otitoloju and Popoola [7] also reported that toxicity evaluations of the mixture of crude oil / dispersant varied, depending largely upon the proportion of addition of the mixture components. The interaction between mixtures of crude oil and a dispersant, Biosolve at the test ratios of 6:1, 9:1 and 12:1 were found to be synergistic (SR: 7655, 14876 and 8792 respectively), indicating that the mixture of crude oil and dispersant was more toxic than crude oil only. They also reported a synergistic action between crude oil and another dispersant, OSD 9460 at ratios: crude oil: OSD 9460 of 6:1 and 9:1 (SR: 2.2 and 1.84 respectively). However, at ratio of 12:1, an antagonistic action was observed (SR: 0.84).

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