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Assessment of the protective effects of *Moringa oleifera* leaf extract against Neem- Oil induced toxicity in zebra fish, *Danio rerio*

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Abstract
Now a day, some botanicals have been used in crop fields and home applications as alternative to synthetic pesticides, to reduce the negative impacts to the environment. Present study was aimed to investigate the acute toxicity of biopesticide, Neem- Oil (NO) and the effective concentrations of Moringa leaf extract (MLE) on the mortality of zebra fish, *Danio rerio*. The four day acute bioassay test was performed using direct interpolation method and probit analysis. 130 adult zebra fishes were randomly selected and exposed to different concentrations of neem- oil and mortality rates were recorded. In direct interpolation method graphs were plotted between % mortalities and concentrations of toxicant at 24, 48, 72 and 96 hrs and LC_{50} values were found as 3.2 ml/l, 2.6 ml/l, 2.2 ml/l and 1.8 ml/l respectively. Obtained data from direct interpolation method were computed by the probit analysis (statistical method) using Finney's table. The LC_{50} values at different exposure periods were found to be 3.155 ml/l for 24 hrs, 2.582 ml/l for 48 hrs, 2.162 ml/l for 72 hrs and 1.717 ml/l for 96 hrs. The upper confidence limits were 3.703, 2.971, 2.484 and 1.995 whereas lower confidence limits were 2.607, 2.193, 1.840 and 1.439 respectively. To assess the effective concentrations of MLE 110 fishes were exposed to different concentrations of MLE alongwith 24 and 96 hrs LC_{50} of NO. On increasing the concentrations of MLE from 2-8 ml/l mortality rate decreased. Fishes remain alive at 8 ml/l and 6 ml/l of MLE against 24 and 96 hrs LC_{50} of NO respectively. In the light of results, MLE can be suggested as the effective remedy against NO –induced mortality in zebra fishes.

Keywords: Neem- oil, Moringa leaf extract, acute toxicity, zebra fish

Introduction
Synthetic pesticides are frequently used in agricultural practices to promote the crop production and high yield by eradicating, preventing, repelling insects and pests. Continuous application of synthetic pesticides has caused accumulation of residues of pesticides in the environment which results in ecological degradation. It causes lethal effects on non- target organisms of agro-ecosystem indirectly and direct toxicity to users (Bansode and Patil, 2016) [8]. These pesticides enter into aquatic ecosystem through run off and cause negative effect on aquatic inhabitants like fishes which may be lethal or sub- lethal (Shankar *et al.*, 2013; Sabra and Mehana, 2015; Srivastava *et al*., 2016) [52, 50, 55].

To overcome the hazardous effect on environmental damage caused by synthetic pesticides and the growing need for alternative strategies of pests control emphasizing the use of natural pesticides for crop protection. Recently, the use of bio-pesticides has secured attention as they are easily available, less-expensive, more readily biodegradable, leave no residue in the environment, safe for human beings (Ramanujam and Ratha, 2008; Chaudhary *et al*., 2017) [45, 11] and more eco-friendly than synthetic pesticides (Reza and Glolamreza, 2012; Roy Choudhury, 2016; Pant *et al*., 2016) [48, 49, 41]. Among the plant families, Meliaceae and Rutaceae have attracted most of the entomologist's and phytochemist's attention because they produce various toxins collectively called as "triterpenes". In family Meliaceae, *Azadirachta indica* (Neem) has a great potential to eliminate harmful insect and pests. It has insecticidal active ingredients, "tetranortriterpeneazadirachtin" which extract from their seeds and other parts also in the form of oil (Morgan, 2009) [40]. Neem- Oil has antifeedant, antiviral, antibacterial and antifungal properties. It has been used successfully in aquaculture and very effective remedy to control fish parasites and predators of fish fry (Kreutzweiser *et al.*, 2004; Winkaler *et al*., 2007; Ramachandra Mohan, 2018) [30, 62, 44]. Due to insecticidal properties, adverse effect of neem-oil and other ingredients of Neem-tree against beneficial organisms including fishes have also been reported at multiple exposure (Koul and Wahab 2004; Miller *et al.*, 2006) [29, 37]. Exposure of azadirachtin causes behavioral changes to fishes and their young ones, great mortality and other toxic effects by altering several physiological,

hematological, biochemical parameters (Stalin *et al*. 2008; Bhat *et al*., 2012) [56, 9]. But it has also been shown that Neem-Oil have greater margin of safety to fishes as compared to other synthetic chemical pesticides (Kumar *et al*., 2012) [31].

After entering into the body of organisms, high concentration of bio-pesticide may impaired oxygen free radical scavenging system and generates Reactive Oxygen Species (ROS) more frequently. Excess amount of ROS elevate the process of lipid peroxidation thereby increasing oxidative stress and inflicting cellular damage (Jerobin *et al*., 2015) [23]. Thus, the use of therapeutically active compound against the toxicity of pesticides is very essential to enhance scavenging free radical system (Refaie *et al.,* 2017) [47].

Moringa oleifera (Moringaceae) is a wide spread tropical and subtropical, multipurpose, medicinal plant. *Moringa oleifera* is commonly known as "Miracle tree" or "Drumstick tree". It has rich combination of therapeutically active ingredients such as rhamnetin, rutin, quercitin, apigenin, chlorogenic acid, Kaempferol. It is also enriched in exogenous supply of carotenoids, ascorbic acid which are prominent antioxidant compounds. It has several polyphenols, thiocarbamate glycosides, flavonoid glycosides, amino acids such as lysine, methionine, cysteine, tryptophan (Pari *et al.,* 2007; Karthivashan *et al*., 2013; Torres-Castillo *et al*., 2013; Fitriana *et al*., 2016) [42, 26, 59, 19]. The leaves of *Moringa oleifera* have anti-diabetic, antiepileptic, antiseptic, antiviral, antiparalytic, anti-cancer, anti- aeging and anti-inflammatory properties (Patel *et al*., 2010; Raut *et al.,* 2014; Kumar *et al*., 2015; Karthivashan *et al.,* 2016) [43, 46, 32, 25]. The leaf extract of Moringa regulates hormone levels, hypercholesterolemia and hyperglycemia (Khan *et al*., 2017; Vergara-Jimenez *et al*., 2017) [28, 60]. Several works have been carried out to investigate the ameliorative effect of *Moringa oleifera* leaves extract against heavy metal like cadmium, arsenic, and drugs such as acetaminophen induced toxicity in mammals (Maduka *et al*., 2014; Sheikh *et al*., 2014; Al-Otaibi, 2016) [33, 53, 5]. It has also been reported that the leaves extract of *Moringa oleifera* are used for improving growth, boosting the immunity, reducing oxidative stress in fishes (Khalil and Korni, 2017; Hamed and El-Sayed, 2019) [27, 21].

Recently zebra fish (*Danio rerio*) has become the most popular experimental organism for the toxicological research (Tanguay, 2018) [58]. Several works have been carried out to investigate the effects of biopesticides in fishes but the protective effect of *Moringa oleifera* Leaf Extract on biopesticde toxicity in zebra fishes are scanty. Therefore, our study was conducted to evaluate the acute toxicity of Neem-Oil (NO) and impact of Moringa Leaf Extract (MLE) against the toxicity in zebra fish, *Danio rerio*.

Materials and Methods

Test animal

Healthy, sexually mature adult zebra fish (*Danio rerio*) with an average weight of 2-4g were purchased from local aquarium shop of Jhansi district, U. P., India. Fishes were brought to the laboratory into polythene bags. They were shifted into well oxygenated glass aquarium. The fishes were exposed to a prophylactic treatment by bathing in 0.1% potassium permanganate (KMnO4) for 1-2 minutes to check dermal infections. They were acclimatized for 10-15 days under laboratory conditions before the initiation of all experiments. During acclimatization, the conditions of water such as Temperature (25^0-28^0), hardness (220mgl^{-1}), dissolve oxygen (5.0-6.5mgl^{-1}) and pH (7.2 ± 0.2) were maintained. Fishes were fed twice daily with commercial diet. The water was renewed, faecal matter and other waste products were siphoned off daily. The feeding was stopped 24hrs prior to experiments. All the experiments were carried out in rectangular glass aquaria (2′×1′×1′) separately according to experimental groups.

Test chemical

Biopesticide, Perfect$^+$ Neem- Oil was purchased which is manufactured by PerFarmers company, India was used in the current study. Oil needs emulsifier for dissolving its fatty substances. Complete preparation of soluble form of oil for its application during experiments was 98% Neem- oil and 2% of emulsifier (Mild soap: godrej Ezee manufactured by Godrej Consumer Products Ltd, Mumbai).

Preparation of stock solution

Stock solution (0.1ml/ml) was prepared by dissolving 10 ml emulsified Neem- Oil in 100 ml distilled water. According to experimental exposure, different dilutions were prepared by adding required amount of distilled water.

Acute toxicity test

For the acute toxicity bioassay two exploratory experiments and one definitive test were carried out using direct Interpolation method. Initial exploratory experiment was performed taking two random concentrations such as 0.5 ml/l and 5.0 ml/l in separate glass aquaria containing five fishes each. Thereafter, second exploratory experiment was carried out by four concentrations as 1.0, 2.0, 3.0 and 4.0 ml/l. After exploratory experiments the definitive test was conducted using the concentration of neem- oil viz 1.4, 1.8, 2.2, 2.6, 3.0, 3.4 and 3.8 ml/l and 10 fishes were exposed for each concentration in IInd exploratory and definitive test in separate aquarium. Behavior of the test fishes was observed and mortality was recorded from time to time up to 96 hrs exposure periods. Dead fishes were removed quickly and the water in all aquaria were renewed every 24 hrs. All the concentrations of Neem- Oil were maintained during all exposure periods (24, 48, 72 and 96 hrs). To assess the Lethal Concentration at 50% for all exposure periods, a dose response curve was plotted between % mortalities and concentrations of bio-pesticide.

To find out actual LC_{50}, Probit analysis was carried out as suggested by Finney (1971) [18]. Initially all concentrations and percent mortalities from definitive test were transformed into their logarithmic values and correct percent as reported by Ghosh (1984) [20]. The value of correct percent moralities were converted into their empirical values. Subsequently, Regression lines of probit against logarithmic transformations of concentrations were made for all exposure periods Log concentrations were obtained from the regression line by drawing a perpendicular at 5 probit corresponding to the 50% mortality for 24, 48, 72 and 96 hrs intoxication and inverse of these log concentrations were the actual LC_{50} values. The standard errors of LC_{50} and confidential limits were also calculated (Ahmad *et al.,* 2019) [3].

Standardization of Effective Concentrations of Moringa Leaf Extract (MLE) on Neem- Oil (NO) toxicity

Collection and preparation of Moringa Leaf Extract

Fresh leaves of *Moringa oleifera* were harvested from Bundelkhand region. The plant leaves were collected, washed in running tap water, air dried at room temperature (24^0C) for a day. The dried leaves were grinded into fine powder and stored in airtight container. 25 g of the powdered leaves were

poured into conical flask containing 250 ml hot (98°C) distilled water. The mixture was kept for 24 hrs and then filtered using filter paper (Khalil and Korni, 2017) [27].

Experimental design

For standardization of Effective Concentrations of Moringa Leaf Extract (MLE) against 24 hrs and 96 hrs LC_{50} of Neem-oil (NO) fishes were divided into several groups of 10 fishes each-

For 24 hrs LC_{50}:

Group I: exposed with 24 LC_{50} of NO (3.155ml/l) alone
Group II: exposed with LC_{50} of NO (3.155ml/l) + 2 ml/l of MLE
Group III: exposed with LC_{50} of NO (3.155ml/l) + 4 ml/l of MLE
Group IV: exposed with LC_{50} of NO (3.155ml/l) + 6 ml/l of MLE
Group V: exposed with LC_{50} of NO (3.155ml/l) + 8 ml/l of MLE
Group VI: exposed with LC_{50} of NO (3.155ml/l) + 10 ml/l of MLE

For 96 hrs LC_{50}:

Group I: exposed with LC_{50} of NO (1.717ml/l) alone
Group II: exposed with LC_{50} of NO (1.717ml/l) + 2 ml/l of MLE
Group III: exposed with LC_{50} of NO (1.717ml/l) + 4 ml/l of MLE
Group IV: exposed with LC_{50} of NO (1.717ml/l) + 6 ml/l of MLE
Group V: exposed with LC_{50} of NO (1.717ml/l) + 8 ml/l of MLE

Results

Acute toxicity of Neem- Oil (NO):

During initial exploratory experiment, Neem- Oil caused 100% mortality of zebra fishes at 5.0ml/l concentration and no mortality occurred at 0.5ml/l concentration whereas II[nd] exploratory experiment showed 20% mortality at 1.0ml/l, 60% at 2.0ml/l, 100% at 3.0ml/l conc. after 96 hrs and 100% at 4.0ml/l after 48hrs exposure period (Tables-1 and 2). The data related to mortality at all exposure periods after definitive test are indicated in table- 3. After plotting the graphs the LC_{50} values were estimated as 3.2, 2.6, 2.2 and 1.8 ml/l after 24, 48, 72 and 96 hrs exposure periods respectively (Figure- 1)

Statistically LC_{50} values were calculated by Probit analysis. Concentrations obtained from definitive test were transformed into log concentrations, percent mortalities into correct % and their corresponding probit values using Finney' table (1971) are indicating in table- 4 and 5. Graphs were plotted between the log concentrations and probit values. The logarithmic value of neem-oil corresponding to 50% mortality to all exposure are representing in figure 2-5. The LC_{50} values, SE and their upper and lower confidential limits are illustrated in table- 6.

Table 1: I[st] Exploratory Experiment

Conc. (ml/l)	No. of fishes	24 hrs		48 hrs		72 hrs		96 hrs	
		M	% M	M	% M	M	% M	M	% M
0.5	5	--	--	--	--	--	--	--	--
5.0	5	5	100%						

Table 2: IInd Exploratory Experiment

Conc. (ml/l)	No. of fishes	24 hrs		48 hrs		72 hrs		96 hrs	
		M	% M	M	% M	M	% M	M	% M
0.1	10	--	--	--	--	--	--	2	20
0.2	10	1	10	2	30	1	40	2	60
0.3	10	4	40	2	60	2	80	2	100
0.4	10	9	90	1	100	--	--	--	--

Table 3: Definitive test for Direct Interpolation Method

Conc. (ml/l)	No. of fishes	24 hrs		48 hrs		72 hrs		96 hrs	
		M	% M	M	% M	M	% M	M	% M
1.4	10	-	-	-	-	1	10	2	30
1.8	10	1	10	1	20	1	30	2	50
2.2	10	1	10	2	30	2	50	2	70
2.6	10	2	20	3	50	2	70	2	90
3.0	10	4	40	2	60	2	80	2	100
3.4	10	6	60	2	80	1	90	1	100
3.8	10	8	80	1	90	1	100	-	-

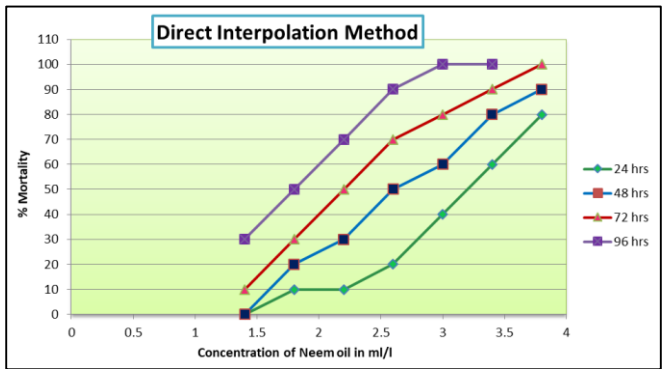

Fig 1: Determination of LC_{50} at different exposure periods using Direct Interpolation Method

Table 4: Log concentrations and Probit values of Neem- Oil in zebra fishes after 24 and 48 hrs

Conc. (ml/l)	Log conc.	No. of fishes	24 hrs			48 hrs		
			% Mortality	Correct %	Empirical value	% Mortality	Correct %	Empirical value
1.4	0.146128036	10	0	0.25	3.04	0	0.25	3.04
1.8	0.255272505	10	10	10	3.72	20	20	4.16
2.2	0.342422681	10	10	10	3.72	30	30	4.48
2.6	0.414973348	10	20	20	4.16	50	50	5
3.0	0.477121255	10	40	40	4.75	60	60	5.25
3.4	0.531478917	10	60	60	5.25	80	80	5.84
3.8	0.579783597	10	80	80	5.84	90	90	6.28

Table 5: Log concentrations and Probit values of Neem- Oil in zebra fishes after 72 and 96 hrs

Conc. (ml/l)	Log conc.	No. of fishes	72 hrs			96 hrs		
			% Mortality	Correct %	Empirical value	% Mortality	Correct %	Empirical value
1.4	0.146128036	10	10	10	3.72	30	30	4.48
1.8	0.255272505	10	30	30	4.48	50	50	5
2.2	0.342422681	10	50	50	5	70	70	5.52
2.6	0.414973348	10	70	70	5.52	90	90	6.28
3.0	0.477121255	10	80	80	5.84	100	97.5	6.96
3.4	0.531478917	10	90	90	6.28	100	97.5	6.96
3.8	0.579783597	10	100	97.5	6.96	100	97.5	6.96

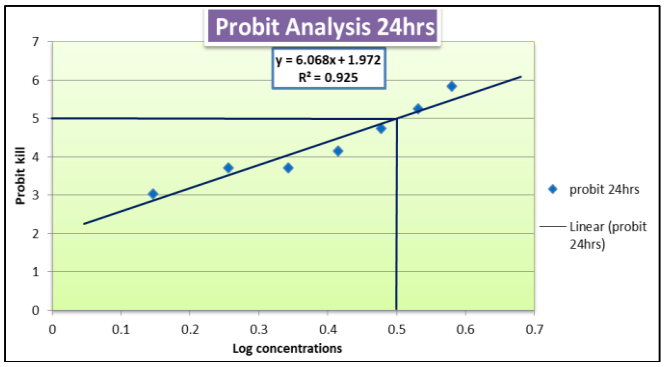

Fig 2: Plot of log concentrations versus Probit values after 24 hrs intoxication of Neem-Oil from table- 4.

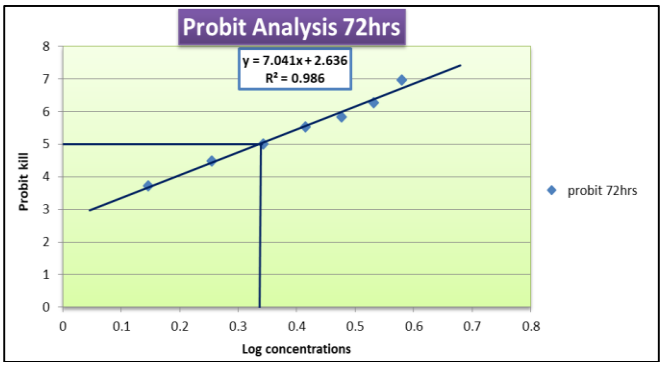

Fig 4: Plot of log concentrations versus Probit values after 72 hrs intoxication of Neem-Oil from table- 5

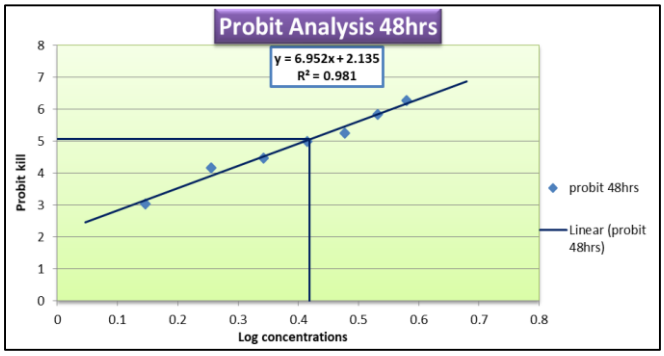

Fig 3: Plot of log concentrations versus Probit values after 48 hrs intoxication of Neem-Oil from table- 4.

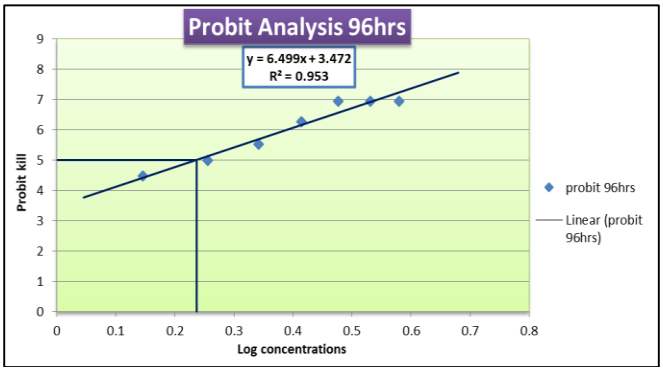

Fig 5: Plot of log concentrations versus Probit values after 96 hrs intoxication of Neem-Oil from table- 5.

Table 6: LC_{50} values, Regression equations, 95% confidential limits (Upper and Lower), for the Neem- Oil at different exposure periods for zebra fish, *Danio rerio.*

Exposure periods (hrs)	LC_{50} value (ml/l) ± SE	Regression Equation y = bx+c	95% Confidential Limits	
			Upper	Lower
24	3.155 ± 0.548	6.068x + 1.972	3.703	2.607
48	2.582 ± 0.389	6.952x + 2.135	2.971	2.193
72	2.162 ± 0.322	7.041x + 2.636	2.484	1.840
96	1.717 ± 0.278	6.499x + 3.472	1.995	1.439

During experimental periods, some ethological changes were also observed in Neem-Oil intoxicated fishes. The behavioral alterations were increased with the concentrations and duration of exposure of toxicant. The intoxicated fishes exhibited erratic and jerky swimming, loss of equilibrium and hyperactivity. After addition of neem-oil fishes showed respiratory trouble by abnormal opercular movement and came to surface to engulf air. Mucous secretion was seen at higher concentration of neem-oil which is the sign of distress.

Standardization of Effective Concentrations of Moringa Leaf Extract (MLE) on Neem- Oil (NO) toxicity-

During standardization, 50% mortality occurred when the 24 and 96 hrs LC_{50} concentrations of Neem-oil (NO) were administered along with 2 ml/l MLE. Mortalities of fishes were decreased when concentrations of MLE were increased. No mortality found at 8 ml/l of MLE in combination with LC_{50} concentration of 24 hrs (3.155 ml/l) whereas all fishes remain alive at 6 ml/l of MLE with LC_{50} concentration of 96 hrs (1.717 ml/l) exposure period. Death rate was elevated on further increasing concentrations of MLE after both exposure periods (Figure 6 and 7). Therefore the effective concentrations of MLE against Neem- Oil toxicity were found to be 8 ml/l and 6ml/l after 24 and 96 hrs exposure periods respectively.

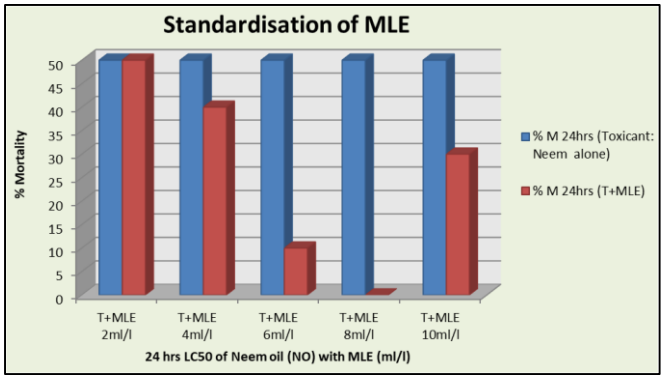

Fig 6: Assessment of Effective Concentrations of Moringa leaf extract (MLE) on Neem- Oil (NO) induced toxicity after 24 hrs exposure periods.

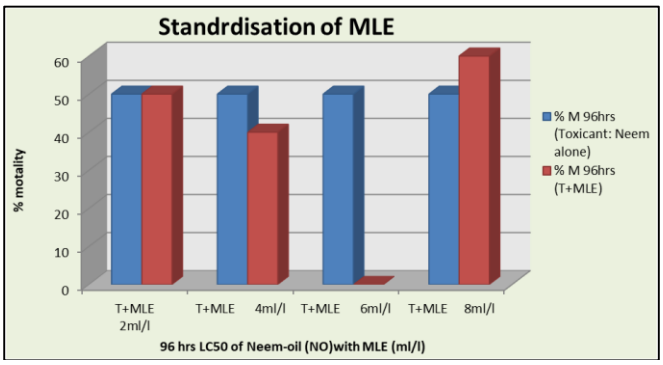

Fig 7: Assessment of Effective Concentrations of Moringa leaf extract (MLE) on Neem- Oil (NO) induced toxicity after 96 hrs exposure period.

Discussion

Biopesticides contain active principle component with low half life and their toxicity is not detrimental for our environment. Azadirachtin is a most active compound of neem showing insecticidal properties. It has been reported that the neem formulations are target specific and comparatively less toxic to fishes (Bhat *et al.*, 2012) [9]. Exposure of neem based pesticides for a prolonged period can cause harmful effects on organisms. Several workers reported the LC_{50} value in different fish species using neem based pesticides (Maranho *et al.*, 2014; Bansode and Patil, 2016; Adamu *et al.*, 2017) [36, 8, 1]. Winkaler *et al.* (2007) [62] have been reported the neem leaf toxicity for juveniles of *Prochilodus lineatus* as 4.8 g/l. This result was just similar to the findings of Cruz *et al.* (2004) [12] in Piaractus *mesopotamicus* intoxicated by neem pesticide. Cagauan *et al.* (2004) [10] reported that the median lethal concentration of neem to *Oreochromis* was 12.4 ml/l and *Gambusia affinis* was 8.31 ml/l and the corresponding 96 h LC_{50} Conc. were 2.57 and 3.0 ml/l. The toxicity of two neem- based pesticides, Neemgold and Nimbecidine on fresh water loach, *Lepidocephalichthys guntea* has been reported which were 0.525mg/l and 0.135mg/l for 96 hrs respectively (Mondal *et al.*, 2007) [39]. Kumar *et al.* (2012) [31] evaluated LC_{50} values in ozoneem induced *Heteropneutes fossilis* after 24-96hrs exposure periods which were found to be 173.06 mg/l, 80.69mg/l, 58.57mg/l and 52.35mg/l. Hamdy and Okail, (2008) [22] assessed the LC_{50} (96 h) of neem pesticide as 112 mg/l in grass carp. Stalin *et al.* (2008) [56] have been examined that LC_{50} concentration of azadirachtin to fish, *Poecilia reticulata* are 0.02mg/l for 24 hrs, 0.017mg/l for 48 hrs, 0.014mg/l for 72 hrs and 0.011mg/l for 96 hrs exhibiting less toxicity of biopesticide. Davoodi and Abdi (2012) [13] reported that 96 hrs LC_{50} value for neem gold to *Cyprinus carpio* was 75.49 mg/l. Saravanan *et al.* (2010) [51] reported median lethal concentration of *Azadirachta indica* leaf extract was 1.035g/l after 96 hrs intoxication in *Labeo rohita.* The median lethal concentration for Neem-on (Neem-seed kernel) induced fish, *Labeo rohita* was found to be 42.66 mg/l (Bhat *et al.*, 2012) [9]. Maitra *et al.* (2014) [34] have reported that the acute toxicity on major carp *Labeo rohita* was found to be 44.61 ppm after 96 hrs exposed by Neemsheilds (azadirachtin- based biopesticide). Acute toxicity of neem pesticide Bioneem for fresh water fish *Gara mullya* was determined by Bansode and Patil (2016) [8]. Suresh Babu *et al.* (2013) [57] investigated the median lethal concentration at different exposure periods as 165.72 mg/l for 24 hrs, 95.17 mg/l for 48 hrs, 62.48 mg/l for 72 hrs and 55.76 mg/l for 96 hrs in fresh water cat fish, *Pangasius hypophthalmus* intoxicated by Azadirachtin. Acute toxicity of Neem (*Azadirechta indica*) leaf extract for Snake headed fish *Channa gachua* was reported by Dhara and Karmakar (2016) [15] and found to be LC_{50} values as 21.80, 19.59, 13.95 and 11.18 g/l after 24, 48, 72 and 96 hrs exposure periods respectively. They have also been reported the LC_{50} concentrations for neem leaf extract induced fish fry of *O. mossambicus* which were found to be 3.29 g/l, 2.62 g/l, 2.19 g/l and 1.67 g/l after 24, 48, 72 and 96 hrs respectively. These concentrations were reported after 24-96 hrs exposure periods as 4.96 g/l, 3.56 g/l, 2.74 g/l and 2.27 g/l for juvenile stage and 7.58 g/l, 7.00 g/l, 6.28 g/l and 5.83 g/l for adults. The LC_{50} values of neem leaf extracts to various stages of fishes exhibited elevating concentrations suggesting that increased age showed resistance of fishes against toxicant exposure.

Some researchers reported the acute toxicity of neem formulations in zebra fishes (Ahmad and Ansari, 2011; Maranho *et al.*, 2014) [2, 36]. Ansari and Sharma (2009) [7] reported that Achook (96 h LC_{50}- 0.025µgdm^{-3}) was found toxic to adult zebra fishes. The Value of LC_{50} (96h) was reported as 0.22ml/l in *Danio rerio* exposed to bioneem by Maranho *et al.* (2014) [36]. Ahmad and Ansari (2011) [2] investigated the neem-pesticide on embryos of zebra fishes as 0.06µg/l whereas 96h LC_{50} was 0.05µg/l for fingerlings showing embryo are more sensitive. Ansari and Ahmad (2010) [6] reported the median lethal concentration of Neemgold was 23.125µg/l after 24h, which decreased to 2.980µg/l after 96h intoxication in zebra fishes showing time dependent responses. Singh and Ansari (2010) [54] has been reported median lethal concentration of two neem based pesticides, Nimbecidine and Ultineem to zebra fishes which were 2.37µg/l and 0.83µg/l respectively. The results of the present study are between the findings of other researchers taking zebra fishes as well as other fishes. The variations in LC_{50} probably due to variation in selected fish species, their size, age, sex and water quality also (Dhara *et al.*, 2016) [14]. Differences related to sensitivity to different formulations of neem pesticides for several fish species may also be attributed to variation in the amount of active compound as azadirachtin (Ahmad and Ansari, 2011; Suresh Babu *et al.*, 2013) [2, 57].

Moringa oleifera leaf extract possesses anticancer, antioxidant and hepatoprotective properties (Johri *et al.*, 2011; Sheikh *et al.*, 2014; El- bakry *et al.*, 2016) [24, 53, 16]. It has a potential to reduce more reactive oxygen species (ROS). Consumption of *Moringa oleifera* leaf enhances free radical scavenging activity diminishing pesticidal stress. Protective effects of *Moringa oleifera* have been studied against many drugs, heavy metals and pesticide by several researchers (Mallya *et al.*, 2017; Akpanyung *et al.*, 2018) [35, 4].

Administration of different concentration of MLE in groups treated with 24 hrs and 96 hrs LC_{50} in this study showed that 8ml/l and 6ml/l MLE are the effective concentration at which all fishes remain alive. It was investigated in previous works that besides beneficial impacts high concentration of flavonoids can also produce reactive oxygen species (Watjen *et al.*, 2005; El-Gendy *et al.*, 2010; Mohabbulla *et al.*, 2016) [61, 17, 38] which is indicated in the current research as increasing the concentration of MLE mortality occurred again. Therefore, administration of effective concentration of flavonoids can improve the free radical scavenging system due to antioxidant properties.

Conclusion

The present study reveals that Neem- Oil has significant potential of causing toxicity by induction of oxidative stress. Moringa Leaf Extract has the great potential to eliminate the Neem- Oil induced toxicity in zebra fishes exhibiting its detoxification capacity. These protective effects of MLE may be due to high antioxidants contents enhancing antioxidant defense system of the fishes.

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