International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 12-07-2021, Accepted: 26-07-2021, Published: 12-08-2021
Volume 6, Issue 4, 2021, Page No. 145-152

The acute toxic effect of azoxystrobin 23% SC, copper sulfate and their combine synergism on the freshwater fish *Labeo rohita*

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Abstract
The industries only provide the data of single chemical toxicity during the introduction of particular chemicals. But no one pesticide could survives alone in this nature; it would interact with other pesticides and exhibits their toxic nature more than individual. The previous reports of several research works have indicated that the synergistic interactions of various pesticides and heavy metals cause severe impact on the aquatic, terrestrial animals and as well as human beings when compared with their individual effects. In the current work the freshwater fish *Labeo rohita* were exposed to two toxicants Azoxystrobin, Copper sulfate individually and 1:1 ratio of their combine lethal toxicity synergistically. The estimated 24 hrs, 48 hrs, 72 hrs and 96 hrs LC_{50} values for Azoxystrobin were 5.7 mg/L, 4.9 mg/L, 4.1 mg/L, and 3.3 mg/L; Copper sulfate were 5 mg/L, 4.3 mg/L, 3.6 mg/L, and 2.9 mg/L; while the mixtures were 2.62 mg/L, 2.30 mg/L, 2.02 mg/L, and 1.75 mg/L, respectively. The data obtained were statistically evaluated using Finney's probit analysis method and the survival time decreased with increasing concentration. The present study was undertaken is to provide an existing knowledge on the combine synergistic effects of pesticide and heavy metals in living systems.

Keywords: azoxystrobin, copper sulfate, combine synergism and *Labeo rohita*

Introduction

The improper management of pesticides in agriculture crops could result in contamination of water bodies. When pesticide reaches the aquatic environment, it might present there for several days or weeks, depending upon its solubility, morphological and physiological changes; produce several effects and mortality in the organisms. If a fish exposed to a small amount of pesticide on many occasions over a long period of time that might cause chronic poisoning. Exposure of fish and other aquatic animals to pesticides and heavy metals depends on its Bioavailability, bioconcentration, biomagnifications, and persistence in the environment. The toxicity mechanism of heavy metals and pesticides has been reported in individual doses separately. However, the synergistic toxicity mechanism of xenobiotic has not been reported clearly. Till now there is few studies are found that reported as how heavy metals and pesticides influence or affect the toxicity of each other. Because some reasons are identified such as the form of exposures, complex interactions among chemicals, preparation of work also difficult and multifaceted. Therefore, more study should be focused on this area. The information retrieved from the extensive literature survey indicates that the combinations of pesticides with pesticides, pesticide with heavy metal, and heavy metal to heavy metal acts synergistically and exhibit more toxicity than a single molecule alone (Nitika *et al.*, 2017) [24].

In the present study I have chosen the strobulirin fungicide Azoxystrobin 23% SC which are used throughout the world and heavy metal copper sulfate. Fungicides are substances used to control fungal organisms causing crop damage and prevent fungal plant diseases. According to the Bartlett *et al.*, 2002[7] strobilurin natural compounds were found unsuitable as agricultural fungicides because they are unstable under natural sunlight conditions. Azoxystrobin is a strobilurin of growing concern in aquatic environments because it is the most sold fungicide worldwide; however, the information available about its effect on aquatic non-target organisms is scarce. Almost all types of heavy metals are present in the aquatic environment and fishes are extremely sensitive to some of these metals (Ali Muhammad *et al.*, 2017) [3].The bioaccumulation of toxic metals can happen in the body and food chain. So, the toxic metals generally exhibit chronic toxicity (Hadeel M Huseen and Ahmed J Mohammed 2019) [15].

Azoxystrobin was the most frequently detected fungicide (45%, 1.13 µg/L maximum concentration). It was found in samples from 17 sites in 11 states and with 11 of these sites having detections in more than one sample. Its relatively widespread occurrence may be due to its high water solubility and its rapidly increasing use in a wide variety of agricultural and non-agricultural settings (US Environmental Protection Agency 1997; Gianessi and Reigner 2006[13]; US Department of Agriculture 2009b) [27]. According to William A. Battaglin *et al.*, 2011[32] Water samples were collected from 29 streams in 13 states in 2005 and/or 2006, and analyzed for 12 target fungicides. Nine of the 12 fungicides were detected in at least one stream sample and at least one fungicide was detected in 20 of 29 streams. At least one fungicide was detected in 56% of the 103 samples, as many as five fungicides were detected in an individual sample, and mixtures of fungicides were common. Azoxystrobin was detected most frequently (45% of 103 samples). The current research work consists of two related experiments; to estimate the lethal concentrations (LC) of Azoxystrobin and copper sulfate individually; and to estimate the lethal concentration of their mixture on the freshwater fish *Labeo rohita*.

Materials and methods

1. Test Chemical

A. Fungicide: Azoxystrobin

Azoxystrobin 23% SC is a broad spectrum systemic fungicide recommended for the control of diseases infecting grapes, chilli, cucumber, cumin, mango, Tomato and Potato crops as under. It contains 23% active ingredient which is equivalent to 250g/l of the product. Azoxystrobin is the first of a new class of pesticidal compounds called ß-methoxyacrylates, which are derived from the naturally-occurring strobilurins. Not only in India throughout the world, has the use of Azoxystrobin increased tremendously in the recent past.

B. Heavy Metal: Copper Sulfate

Copper sulfate is an inexpensive treatment for farmers. it is approved by the EPA as a general use material like algaecide, insecticide, water treatment, molluscicide and it is not a restricted pesticide. Aqua farmers had used it to control ich on fish and fungus on fish eggs (Harry K. Dupree 2017) [16].

2. Collection and acclimatization of test fish

The fingerlings of *Labeo rohita* caught from a soil fish pond from Kuchipudi, Tenali, Guntur (Dst), A.P. with average weight 6.235 ± 0.25g. Then the fish acclimatized to the laboratory conditions in large tub (200L) thank with sufficient dechlorinated ground water for 15 days at room temperature 28±2^{0}C. During the conditioning period, the fingerlings were only supplied with air, at the same time water was renewed every day and no food was given to the fish. The percent mortality was recorded daily and dead fish was removed. After the one week of acclimation, the fish was transferred randomly into the plastic aquaria to test the lethal concentrations of selected pesticides separately and the combine mixture.

3. Stock and Test solution

The fungicide Azoxystobin stock solution was prepared by mixing 1g of the soluble semi liquid in 1 L of distilled water, while the heavy metal copper sulfate stock solution was prepared by making up 3.929g to 1 L. Stock solution of the pesticide mixture was prepared by mixing 1 g of Azoxystrobin and 3.929g of copper sulfate and making up the mixture to 1 L. Mixture was prepared based on equi-toxic ratio. Test solutions of the required concentration were prepared by dilution the stock solutions for the range finding and definite test.

4. Acute Toxicity test

During acute toxicity trails, the fish were subjected to 12-hr photo period and were not fed. Prior pilot experiments were conducted using 1L bottles and one fish for each bottle was introduced to choose the mortality concentration, at which concentration the fish respond Experiments were conducted to select mortality range from 10% to 100% for 24, 48, 72 and 96 hours to determine LC_{50} values of in Azoxystrobin 23% SC, Copper Sulfate and combine mixture of Azoxystrobin 23% SC + Copper Sulfate exposed to the freshwater fish *Labeo rohita*. Four groups of fish juveniles, 10 individuals in each group, in four separate well aerated plastic tubs were exposed. i). Control group (C), exposed to toxicant free lab water, ii). Experimental Group 1 (EG-1), exposed to Azoxystrobin iii). Experimental Group 2 (EG-2), exposed to Copper sulfate and iv). Experimental Group 3 (EG-3), exposed to Azoxystrobin + Copper sulfate in 1:1 ratio. The physicochemical characteristics of the laboratory water were: Capacity of each tub = 20 L; temperature= 25.0±1.0°C, pH= 6.5-6.8.

All experiments were carried out in triplicate in static renewal system of water after every 24h interval, with the regular addition of fresh solution of toxicant with same concentration to sustain the nominal concentrations of Azoxystrobin, copper sulfate and combine mixture. The dead fish were removed from test chambers immediately after death; the data regarding the fish mortality was recorded from the tests at the end of each specific time period. Care was taken during handling of fish to avoid stress. The physic-chemical characteristics of water were determined by standard methods of APHA (2005) [5]. The stock solutions of Azoxystrobin and Copper sulfate were freshly prepared which was renewed after every 24 hours. The lethal concentrations ensure death even before noticing the behavioural abnormalities.

Statistical Analysis

The concentration-response data of each pesticide was analyzed using the probit Analysis Method (Finney, 1971 method) [12]. Similarly, the probit method was also used to analyse toxicological data for the pesticide mixture. The MS Excel 2007 was used to find regression equation (Y=mortality; X=concentrations) and 95% CL. The toxicity factor (TF) was derived from the 96hr LC_{50} values. Several mathematical models have being developed and utilized to estimate interaction in the present study I have used the synergistic ratio (SR) and isobolograms. Synergistic actions were also calculated (Altenburger, R *et al*., 2003) [4].

Results

Determination of LC_{50} values

Toxicity of pesticides, HM, other chemical substances is influenced by physical factors like temperature, pH and biological factors like size, national status, species specificity and chronobiology of the animal. The toxicity of a pesticide and heavy metals could vary from species to species and this variation is due to differential tolerance of animals to exposure. The data was computed according to Probit Analysis Method (Finney, 1971) [12] and the LC_{50} values were determined. The time-response relationship indicated that the fishes responded in a time-dependent manner to the exposed pesticides. In present study the individual LC_{50} values of *Labeo rohita* for 24hr, 48hr, 72hr and 96hrs exposed to Azoxystrobin are 5.7mg/l, 4.9mg/l, 4.1mg/l and 3.3mg/l and the 96hrs reported values were given in the III.Table.1; Copper sulfate 5mg/l, 4.3mg/l, 3.6mg/l and 2.9mg/l and the 96hrs reported values were given in the III.Table. 2. The combined synergism of Azoxystrobin + Copper sulfate caused 50% mortality to *Labeo rohita* for 24hr, 48hr, 72hr and 96hrs at 2.62mg/l, 2.30mg/l, 2.02mg/l and 1.75mg/l and the 96hrs reported values were given in the III.Table. 3. The confidence Levels of 95% and Regression values for combine synergism (Azoxystrobin 23% SC + Copper sulfate) exposed to fish *Labeo rohita* at different exposed periods were given in the III.Table. 4. The type of interactions between Azoxystrobin and Copper sulfate was given in the III.Ttable. 5.

The graphical representation of percent mortality versus Log concentration of Azoxystrobin, Copper sulfate and their combined synergism showed in III.Fig.1, 2 and 3 respectively. The percent mortality gradually increased with the increased concentrations of the fungicide Azoxystrobin, Copper sulfate and Azoxystrobin + Copper sulfate. Data from the static bioassays were then fitted into the synergistic ratio (SR) and isobologram model to determine the type of interaction either antagonistic or synergistic between the different classes of pesticides.

Table: 1. Observed per cent mortality, probit mortaliy and LC_{50} value of the fish *Labeo rohita* exposed to Azoxystrobin 23% SC for static 96 hrs.

S.no.	Dose in mg/ml	Mortality	Probit value(Y)	Log(100*Dose) =X	X*X	Y*Y	XY
1	2.5	10%	3.7184	2.39794	5.75012	13.8265	8.9165
2	2.9	30%	4.4756	2.4624	6.0634	20.031	11.0207
3	3.3	50%	5	2.51851	6.34291	25	12.5926
4	3.7	70%	5.5244	2.5682	6.59566	30.519	14.1878
5	4.1	90%	6.2816	2.61278	6.82664	39.4585	16.4125
			25	12.5598	31.5787	128.835	63.13

X 2.51197; Y 5; SXX 0.02883; SYY 3.83499; SXY 0.33083; SLOPE B 11.4757; VARIANCE B 3.46879; VARAINCE A 21.988; M 2.51197; ANTILOG 3.25065; LC_{50} 3.3.

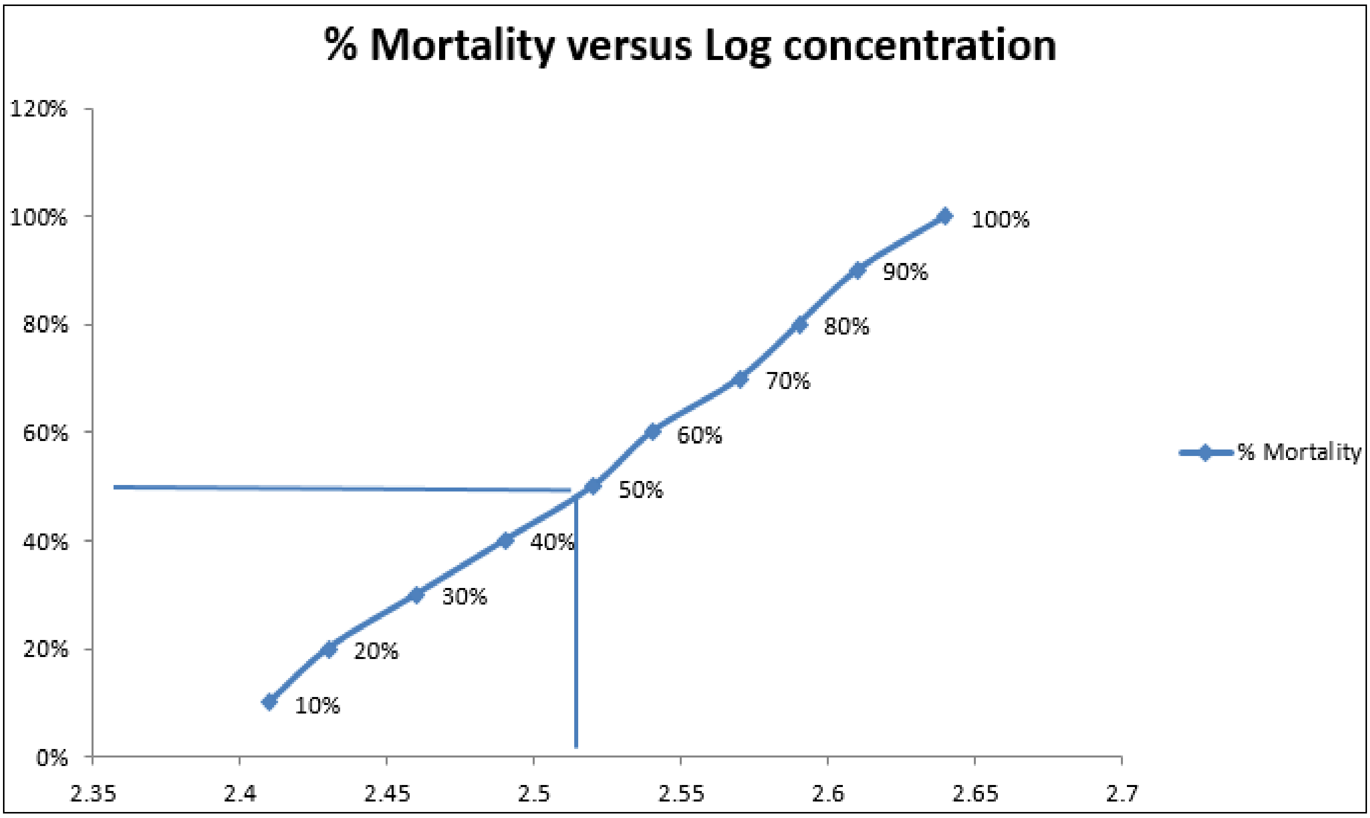

Fig 1: Relationship between the Log conc. of Azoxystrobin 23% SC and percent mortality of *Labeo rohita* for 96 hrs.

Table 2: Observed per cent mortality, probit mortaliy and LC_{50} value of the fish *Labeo rohita* exposed to Copper sulfate for static 96 hrs.

S.no.	Dose in mg/ml	Mortality	Probit value(Y)	Log(100*Dose) =X	X*X	Y*Y	XY
1	2.18	10%	3.7184	2.33846	5.46838	13.8265	8.69532
2	2.54	30%	4.4756	2.40483	5.78323	20.031	10.7631
3	2.90	50%	5	2.4624	6.0634	25	12.312
4	3.26	70%	5.5244	2.51322	6.31626	30.519	13.884
5	3.62	90%	6.2816	2.55871	6.54699	39.4585	16.0728
			25	12.2776	30.1783	128.835	61.7272

X 2.45552; Y 5; SXX 0.0303; SYY 3.83499; SXY 0.33911; SLOPE B 11.1928; VARIANCE B 3.30064; VARAINCE A 20.0015; M 2.45552; ANTILOG 2.85443; LC_{50} 2.9.

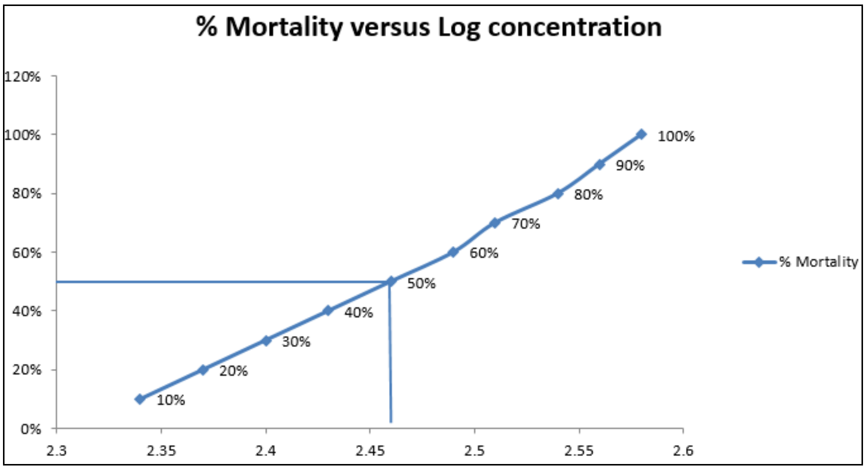

Fig 2. Relationship between the Log conc. of Cupper sulfate and percent mortality of *Labeo rohita* for 96 hrs.

Table 3: Observed per cent mortality, probit mortaliy and LC_{50} value of the fish *Labeo rohita* exposed to combine synergism (Azoxystrobin 23% SC + Copper sulfate) for static 96 hrs.

S.no.	Dose in mg/ml	Mortality	Probit value(Y)	Log(100*Dose) =X	X*X	Y*Y	XY
1	1.39	10%	3.7184	2.14301	4.59251	13.8265	7.96859
2	1.57	30%	4.4756	2.1959	4.82198	20.031	9.82797
3	1.75	50%	5	2.24304	5.03122	25	11.2152
4	1.93	70%	5.5244	2.28556	5.22377	30.519	12.6263
5	2.11	90%	6.2816	2.32428	5.40229	39.4585	14.6002
			25	11.1918	25.0718	128.835	56.2383

X 2.23836; Y 5; SXX 0.02053; SYY 3.83499; SXY 0.27933; SLOPE B 13.6087; VARIANCE B 4.87193; VARAINCE A 24.5096; M 2.23836 ANTILOG 1.73125; LC_{50} 1.73.

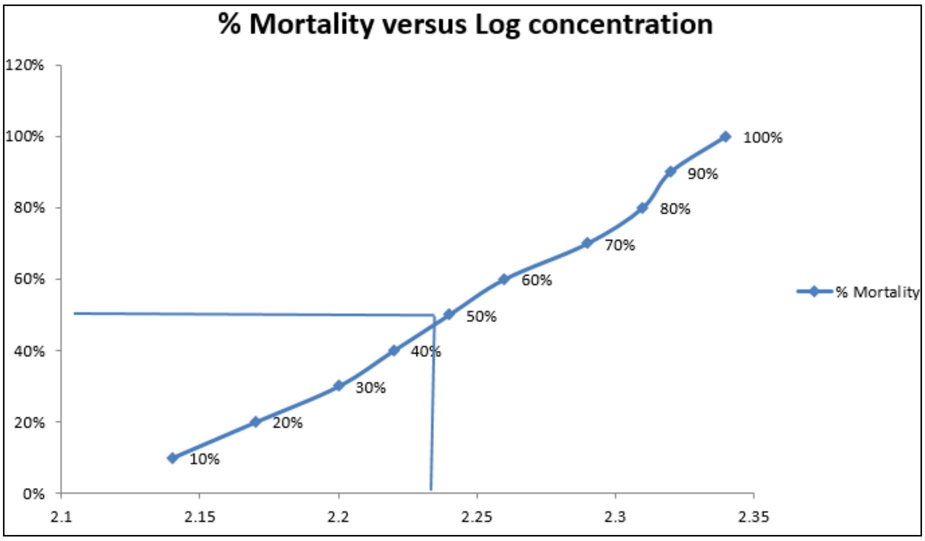

Fig 3: Relationship between the Log conc. of combine synergism (Azoxystrobin 23% SC + Copper sulfate) and percent mortality of *Labeo rohita* for 96 hrs.

SR (synergistic ratio) model

The toxicity of synergistic action is superior to that of components, while the antagonistic act has lower toxicity than that of the components. Considering the Loewe additivity equation, when the sum is higher than 1 (>1) then this suggests that a higher total concentration is required to produce the same effect which assumes an antagonistic effect (infra-additive). If the value is lower than 1 (<1), then it is a synergistic effect (supra-additive) (Altenburger, R *et al.*, 2003) [4]. SR1 was calculated using LC_{50} of a Azoxystrobin acting alone/ LC_{50} of mixture, while SR 2 was calculated using LC_{50} of a copper sulfate acting alone/ LC_{50} of mixture followed by Kanu C. Kingsley 2019[18].

Table 4: Type of interactions between Azoxystrobin and Copper sulfate.

		Period of exposure			
S. No	Parameters	24hrs	48hrs	72hrs	96hrs
1	SR1	2.18	2.13	2.03	1.89
2	SR2	1.91	1.87	1.78	1.66
3	Interpretation	Synergism	synergism	synergism	Synergism

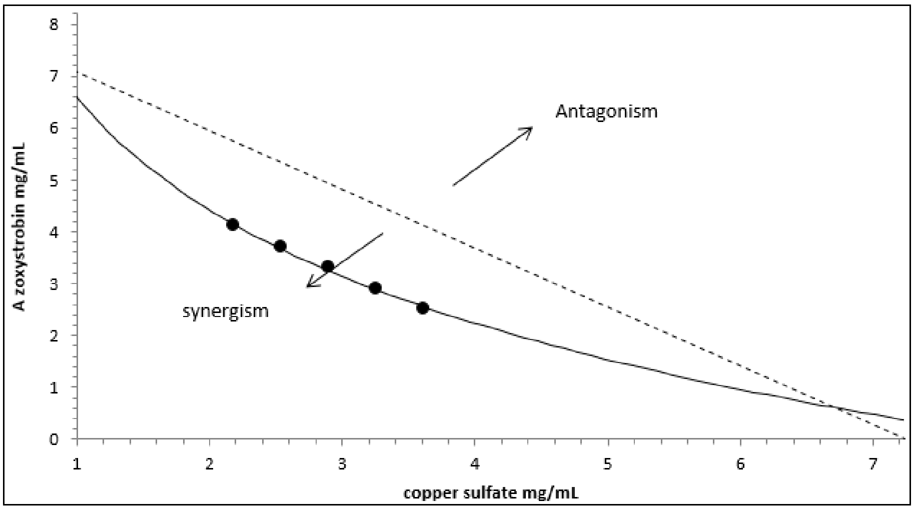

Fig 4: Isobologram illustrating the type of interaction between Azoxystrobin and Copper sulfate.

Table 5: Confidence Levels of 95% and Regression values for combine synergism (Azoxystrobin 23% SC + Copper sulfate) exposed to fish *Labeo rohita* at different exposed periods.

.S.No	Exposure time in hrs	LC50 in % Concentration	Lower and Upper confidence limits	Regression equation Y=(y-bx)+bx and R^2	Toxicity factor
Azoxystrobin					
1	24	5.70	4.915±6.485	Y=20.12x-50.40, R^2=0.9929	1.00
2	48	4.90	4.115±5.685	Y=17.25x-41.35, R^2=0.9924	1.16
3	72	4.10	3.315±4.885	Y=14.37x-32.49, R^2=0.9915	1.39
4	96	3.30	2.515±4.085	Y=11.48x-23.83, R^2=0.9900	1.73
Copper sulfate					
5	24	5.00	4.2932±5.7068	Y=19.60x-47.85, R^2=0.9928	1.00
6	48	4.30	3.5932±5.0068	Y=16.81x-39.21, R^2=0.9923	1.16
7	72	3.60	2.8932±4.3068	Y=14.01x-30.75, R^2=0.9914	1.39
8	96	2.90	2.1932±4.6068	Y=11.19x-22.48, R^2=0.9897	1.72
Combine mixture					
9	24	2.62	2.2666 to 2.9734	Y=20.55x-44.66, R^2=0.9930	1.00
10	48	2.30	1.9466 to 2.6534	Y=18.01x-37.48, R^2=0.9926	1.14
11	72	2.02	1.6666 to 2.3734	Y=15.77x-31.30, R^2=0.9920	1.30
12	96	1.75	1.3966 to 2.1034	Y=13.61x-25.46, R^2=0.9912	1.50

The confidence interval values obtained in this study are important statistical output that can be used to evaluate whether one is more significantly sensitive to another result. The results of regression analysis indicated that the mortality rate (Y) is positively correlated the concentration (X). The toxicity factor indicates that toxicity of the single pesticides and the combine mixture increased as the duration of exposure increased. The toxicity factor (TF) revealed that Combine mixture was 1.73 and 1.72 times at 96 hrs more toxic than Azoxystrobin and Copper sulfate respectively. This is summarized in terms of the degree of toxicity as thus; Combined mixture > Copper sulfate > Azoxystrobin. It shows combine mixture was more toxicity than Azoxystrobin and copper sulfate.

Discussion

The current study evaluated that the independent and combine lethal toxicity of Azoxystrobin and copper sulfate in *Labeo rohita*. All the physico-chemical parameters showed optimal water quality for the survival of the fingerlings during the 96 h acute toxicity tests. Experimentally derived LC_{50} values are used to classify the toxicity of a chemical as highly toxic, moderately toxic and slightly toxic. According to Choudhury N, 2018[10], if the LC_{50} value of fungicide between the ranges of 1.1 to 10mg/L are considered moderately toxic. In general, acute toxicology studies (using the TGAI) indicate that Azoxystrobin is practically nontoxic to birds, mammals and bees but highly toxic to freshwater fish. In the present study, the 24, 48, 72, and 96 LC_{50} indicate that formulated Azoxystrobin and copper sulfate were toxic to *Labeo rohita* fingerlings as the duration of exposure increased. The LC_{50} of combine synergism reported in the current study for *Labeo rohita* is lower than the LC_{50} of individual toxicants that is 1.75 mg/L reported for the same test fish.

The LC_{50} values differ from genus to genus and species to species for the same or different pesticides because of different mode of action and physiology of organism. In this study, the combine mixture of Azoxystrobin and copper sulfate was slightly more toxic to *Labeo rohita* fingerlings than each pesticides acting alone as well as the 24, 48, 72, and 96 h LC_{50} values of the pesticide mixture were lower than those of the pesticides independently. It is provide there was synergistic interaction between Azoxystrobin and copper sulfate. But the underlying mechanisms of the combined action is not clear, it is likely that some fluctuations while exposed to fish. The inhibition of biotransformation enzymes by copper might decreased the metabolism of Azoxystrobin leading to increase accumulation to Lethal Concentration in the exposed fish. The risk of individual chemicals is less than the risk of a mixture of chemicals, thus understanding the effects of pesticide mixture is critical to the ecological risk assessment of pesticides [Kortenkamp *et al.*, 2009] [19].

Regarding azoxystrobin, data on aquatic toxicity are scarce (Rodrigues *et al.*, 2013c), 96hrs LC_{50} value for *Oncorhynchus mykiss* is 470 (400-5,800) μg L-1 highly toxic (US-EPA, 1997) [29], for *Lepomis macrochirus* is 1,100 (900-1,700) μg L-1 moderately toxic (EPA, 1997), and the euryhaline species *Cyprinodon variegatus* is 670 (560-800) μg L-1 (US-EPA, 2012a) [330]. Choudhury, *et al.*, (2017) [9] reported that due to long term exposure of *Channa punctatus* and O*reochromis mossambicus* to Hexaconazole 5%SC and Azoxystrobin 23% SC caused over expression of some lower molecular protein as compared to control and LC_{50} was determined. LC_{50} for Amister was detected to be 0.008 ml/lit and that for Contaf plus was found to be 0.025 ml/lit. The 48hrs LC_{50} value for *Ctenopharyngodon idella* larvae (<10 days) is 549 (419-771) μg L-1 (Liu *et al.*, 2013) [21].

Nowadays, different forms of copper have distinctive and specific usages, e.g., copper sulfate is a well-established pesticide which is used to control the growth of algae in lakes and ponds. The copper sulphate is worldwide used as an algaecide and a fungicide in aquaculture and agriculture (Lasiene *et al.*, 2016) [20]. Deactivating the fungi enzymes prevents fungal spores from germinating. This process of deactivation is achieved via the free cupric ions, which are established as the most toxic forms of copper. Complexes of copper with other ligands may or may not be bioavailable for use in aquatic organisms (Nemi Malhotra *et al.*, 2020) [22]. Exposure to copper sulfate at 1.5 mg/L and then CuO-NPs at 200 mg/L showed higher influences on growth indices, survival, and pathological signs of the gills of grass carp fingerlings after 30 days of exposure (Abdollahzadeh *et al.*, 2018) [1].

The metal toxicity trend for *R. sumatrana* and *P. reticulata* from most to least toxic was Cu > Cd > Zn > Pb > Ni > Al > Fe > Mn and Cu > Cd > Zn > Fe > Pb > Al > Ni > Mn, respectively (Mohammad *et al.*, 2015). The median LC_{50} value of $CuSO_4$ for *C.fusca* was found to be 6.928 mg/L by EPA method and estimated 6.787 mg/L with SPSS statistical software (Iman zarei *et al.*, 2013) [17] and also stated that two factors, water hardness and pH levels, could affect the acute toxicity of copper sulphate on the *C. fusca*. According to Pandari Reddy *et al.*, 2016 [25] The LC_{50} value for Cuso4 at 96 hrs was found to be 58 mg/L to *Sarotherodon mossambica*. Asim Ullah *et al.*, 2016 reported that the 96-hr LC_{50} of $CuSO_4$ for the fish, *Oreochromis niloticus* was found to 30 mg/l. The 96h LC_{50} value for ($CuSO_4.5H_2O$) was 3.15 mg/L (Abdul Latif *et al.*, 2014) [2]. The 96h LC_{50} values for copper sulphate ($CuSO_4.5H_2O$) and lead nitrate [$Pb(NO_3)_2$] were 3.15 mg L-1 and 6.80 mg L-1 respectively(Abdul Latif *et al.*, 2013).

Interaction describes the combined effect of two or more chemical components as stronger (synergistic) or weaker (antagonistic/ inhibitive) on the basis of dose and response addition. Interactions may vary at dose levels, duration of exposure, biological target animals and environmental conditions. Usually the complex effects at the level of population and ecosystem are unknown. The mode of action of pesticides is not the same for all types of organisms like microorganisms, plants, invertebrates and vertebrates. The same chemical components of a mixture might have a similar mode of action on a same group of organisms and different modes of action on another group. Fig.III.4 indicates that the interaction between Azoxystrobin and Copper sulfate may be synergistic given that the SR1 and SR2 > 1 (Table 4) and the isobole of the mixture was below the additivity line. Figure 2 indicates a synergistic interaction between Azoxystrobin and Copper sulfate in *Labeo rohita*, synergism occurred at 96 h. The confidence interval value is useful and significant as it can be utilized for comparison of another chemical or treatment to the same test organism species (Basirun *et al.*, 2018) [8]. The 95% confidence interval significant value is at the $p<0.05$ level.

Cadmium considerably enhanced the detoxifying processes of permethrin in the midges, which largely explained the observed antagonistic interaction between permethrin and cadmium exposed to the insect *Chironomus dilutus* (chen *et al.*, 2015) [35]. W Tyler Mehler *et al.*, 2011[31] stated an antagonistic toxic response was observed when the benthic invertebrate *Chironomus dilutus* was simultaneously exposed to the two contaminants cypermethrin and lead in both water and sediment exposures. Toxicants which influence the amount of biotransformation enzymes can influence on the toxicity of other chemicals (Nielsen E *et al.*, 2008) [23]. According to Kanu C. Kingsley, 2019[18], the estimated 24 hrs, 48 hrs, 72 hrs and 96 hrs LC_{50} for copper hydroxide were 198.66 mg/L, 167.51 mg/L, 138.64 mg/L, and 104.82 mg/L; glyphosate were 162.92 mg/L, 103.88 mg/L, 61.95 mg/L, and 52.6l mg/L; while the mixtures were 63.18 mg/L, 59.06 mg/L, 56.42 mg/L, and 50.67 mg/L, respectively. Glyphosate was 2 times more toxic than copper hydroxide to *C. gariepinus* when acting singly. Wu S *et al.*, 2018[34] stated that binary mixtures of fenvalerate and triadimefon could simultaneously induce endocrine disruption and oxidative stress in a synergistic manner during rare minnow embryo development and TDF enhanced the acute toxicity of FEN.

The information from the article Singh *et al.*, 2017[24] may be useful for different environment protection agencies and policy makers to consider the combined effects of heavy metals and pesticides on humans while designing strategies toward environmental protection and safety regulations about human health. There is a need to conduct toxicity studies on pesticide mixtures to assess its suitability for detecting toxicity, as well as experiments involving a complex mixture of pollutants, in order to determine aquatic ecosystems monitoring program and it can help to improve the quality of life because the evaluation of single chemical toxicity for specific species and environmental compartments may not show the real toxicity data in real life. Mixtures of chemicals present in aquatic environments may elicit toxicity due to additive or synergistic effects among the constituents or, vice versa, the adverse outcome may be reduced by antagonistic interactions. Deviations from additively should be explained either by the perturbations of toxicokinetic parameters and/or chemical toxicodynamics (Dondero, F *et al.*, 2011) [11]. This study showed that the both fungicide Azoxystrobin and copper sulfate were toxic to *Labeo rohita* and the mixture of both pesticide produced a synergistic effect.

Conclusion

The study of exploration of toxicity of chemical mixtures are important because independent chemical toxicity in the environment is a myth as most of the time chemical exposure occurs as a response to a mixture rather than independent chemical. The evaluation of single chemical toxicity for specific species and environmental compartments may not show the real toxicity in real life. The components in a mixture may show ideal additive behavior of response or effects or may induce either synergistic or antagonistic effects. Hence; an attempt has been made to study the toxicity of Azoxystrobin fungicide, copper sulfate and their combine synergism induced toxicity in the freshwater fish *Labeo rohita* (Hamilton). Pesticide can be an important crop production tool to maximize the yield but heavy and indiscriminate use of chemicals also exposes farmers to serious health risks, result in negative consequences on non-target organisms. Pesticide must be lethal to the targeted organisms only not to the non-targeted organisms because they alter immuno and neurotoxicological function by effecting or trigger natural hormones in the body of exposures. Authors suggest the farmers should be take careful precautions while using these pesticides to minimize their health problems and to maintain ecological balance.

Acknowledgment
The authors are thankful to the Department of zoology and Aquaculture, Acharya Nagarjuna University for providing necessary Laboratory facilities. Lalitha Vinnakota is also grateful to the UGC-RGNF for providing the financial assistance for conducting research work.

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