Research Article

Behavioral and Hematological Alterations in Grass Carp (*Ctenopharyngodon idella*) Exposed to Bifenthrin

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Abstract | Insecticides are used across the world as it is evaluated that poses a risk to aquatic life especially fish, which are very sensitive in response to change in environmental conditions. The current study evaluated the effects of bifenthrin on *Ctenopharyngodon idella's* behavioral and hematology alterations. The acute toxicity 96-hr LC_{50} of bifenthrin for grass carp was determined as 6.5µg/L. Fish were divided into four groups i.e., one control and three experimental groups having eight fish in each group. Control group was not exposed to bifenthrin. Experimental fish were exposed to sublethal (1/3rd of LC_{50}) doses of bifenthrin i.e., 2.16 µg/L for 30 days. Behavioral parameters of *C. idella* were observed at acute as well as sub-lethal concentrations. It was observed that behavioral parameters of *C. idella* such as opercular movement, somersaulting activity, convulsions rate, air gulfing was significantly ($p<0.05$) increased by increasing the bifenthrin concentration. Fin movements and swimming rate was also increased with increase in bifenthrin concentration but at later stage fish became motionless and sluggish. Body color was changed from grey to pale and gills from bright to light red color as bifenthrin concentration increased. Blood parameters such as red blood cell counts (RBCs), White blood cell counts (WBCs), Hematocrit (Hct), Hemoglobin (Hb), Mean corpuscular hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) were measured at sub lethal exposure of bifenthrin after 15 and 30 days. The findings revealed that RBCs, Hb, Hct, PCV and MCHC were decreased significantly ($p<0.05$) and WBCs, MCV and MCH were increased significantly ($p<0.05$) after bifenthrin exposure. It was concluded that bifenthrin has potential to disturb the behavior and to alter the hematological profile of *C. idella*. The aim of present study is to explore the toxicity of insecticides.

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Introduction

All toxicants are not pollutants, but all pollutants are toxicants. Toxicants cause death at lethal concentrations, but they render animals unfit to live in sub lethal concentrations. The rapid modifications in the characters of fish are indicators for pesticide contamination (Ullah *et al*., 2015).

It was discovered that these pyrethroids are used to preserve agricultural product and food from harmful insects and to manage animal ectoparasites (Laskowski, 2002). In the last two decades, their popularity has exploded (Wardhaugh, 2005). Though they have made a major contribution to human wellbeing, they have considerable negative impacts on non-target species (John, 2007). These pesticides, even when used in confined regions, are carried away and transported by rain and flooding and end up in rivers, lakes and ponds showed to be very disastrous to aquatic life that is vital for human consumption due to its high nutritional value (Mahboob *et al*., 2014; Arjmandi *et al*., 2010).

Pesticide is used estimated to be over 5.6 billion pounds per year globally, with consumption rising unexpectedly (Alavanja, 2009), with 45.0 %, 25.0 %, and 25.0 % in Europe, the United States, and the rest of the globe, respectively (Bourguet and Guillemaud, 2016). China and the United States are two of the world's leading pesticide manufacturers. Pakistan is the second-highest consumer of pesticides among South Asian countries (Waheed *et al*., 2017). In Pakistan, over 108 different insecticides are currently in use (Mehmood *et al*., 2017).

FMC Corporation, whose corporate headquarters are presently situated in Philadelphia, PA, was the first to produce bifenthrin. In Dec. 9, 1980 BF distinct from other synthetic pesticides, has a greater lethalness having negative impacts on aquatic organisms (Manzoor and Pervez, 2017). Bifenthrin is an insecticide used to restrict varied kinds of pests that harm to plants. The use of bifenthrin for insect pest management has been studied by the Forest Service (Fettig *et al*., 2013).

Previous studies evaluated different toxicological endpoints to assess bifenthrin induced toxicities including behavioral, biochemical and hematological abnormalities in *C. idella* (Ullah *et al*., 2022). The toxicity of bifenthrin also studies in fathead minnow (Beggel *et al*., 2010), trout (Velisek *et al*., 2009b) and zebra fish (Jin *et al*., 2013; Bertotto *et al*., 2017). Hematological parameters are significant tools have been used by many fish biologists to analyze fish health, pathological and physiological adaptability (Seriani *et al*., 2012; Gabriel *et al*., 2011).

Ctenopharyngodon idella was chosen for these tests from a variety of fish species because it plays an essential role in Pakistan's carp polyculture system (Khan *et al*., 2004; Chilton and Magnelia, 2008) and has attracted the attention of fish culturists due to the high quality of its flesh and its flavor (FAO, 2007). Due to limited studies of bifenthrin toxicity on Chinese carps the present study was conducted to assess the behavioral and hematological abnormalities in *C. idella.* This study will help to understand the toxicity of different pyrethroids to non-target animals especially fish.

Materials and Methods

Collection and acclimatization of fish

Fingerlings of *Ctenopharyngodon idella* were brought from Wazirabad fish farming seeds hatchery. After collection they were securely shifted to Zoology lab of GC Women University Sialkot and placed in glass aquariums with proper aeration. For about ten days, they were acclimatized under laboratory conditions and fed with palletized supplemental meal twice a day. Physico-chemical parameters were determined throughout the study.

Preparation of bifenthrin solution

The analytical grade bifenthrin was bought from market. Every week standard solution was prepared by adding the necessary amount of methanol which was used to make concentrations for acute toxicity studies.

Phase -I

Acute toxicity test: To find out the LC_{50} and lethal dosage of bifenthrin for *C. idella* acute toxicity test was performed for 96 h. Fish had been exposed to different concentrations of bifenthrin individually and result on fish mortality was obtained. LC_{50} of bifenthrin to fish were determined by using Probit Analysis method (Finney, 1971). Control group was managed in aquarium without any insecticide exposure.

Physio-chemical parameter's determination: Physico-chemical parameters i.e. water temperature, pH, hardness, carbon dioxide, dissolved oxygen, ammonia, calcium and magnesium were measured on daily basis (APHA, 2005).

Phase- II

Effects of sublethal (1/3rd of LC_{50}) exposure of bifenthrin to *C. Idella*: Fish *C. idella* were given sub-lethal bifenthrin dosages for 30 days after acute toxicological tests. Eight fish were kept in both experimental and control group. In the second phase, 1/3rd sub lethal dose of bifenthrin was given to the *C. idella* . The exposing media was generally changed every week. The required bifenthrin dose had been maintained throughout the study.

Monitoring the behavior of *C. idella* under sublethal (1/3rd of LC_{50}) exposure of bifenthrin: During exposure to bifenthrin at sub-lethal concentration, behavioral patterns of *C. idella* including equilibrium status, convulsions, somersaulting activity, swimming rate, fin movements, body color, gills color of control as well as experimental group were monitored on daily basis during the whole research period. Behavioral responses were monitored throughout the experiment for 30 days by using the method of Ullah *et al*. (2021).

Hematological analysis

Hematological variations in *C. idella* were determined when exposed to 1/3rd sub-lethal concentration of bifenthrin. Effect of this chemical on blood parameters including Erythrocyte count (RBC), White blood cell count (WBC), hemoglobin (Hb), hematocrit (PCV), mean corpuscular volume (MCV), Mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), were measured according to the method of Velisek *et al*. (2009a).

Statistical analysis

Throughout the experiment, the data of different variables were obtained and statistically analyzed by applying the method of Steel *et al*. (1996). ANOVA was used to find out the relationship between various parameters which were under study. To find out the statistical relationship between variables with 3 replicates for each test, correlation analysis was also conducted. LC_{50} of bifenthrin to *C. idella* was determined by using Probit Analysis (Finney, 1971).

Results and Discussion

Acute toxicity test to determine LC_{50} of bifenthrin in C. idella under 96 h exposure period

The acute toxicity test was performed to find out LC_{50} of bifenthrin to *C. idella* during 96 h. *C. idella* was exposed to various concentrations of bifenthrin during 96 h trial. Experiment was performed in three trials. Control group showed no mortality during the whole period. Aquariums were checked out on regular basis to remove dead fish. Final value of LC_{50} of bifenthrin against grass carp was 6.5 µg/L measured by using probit analysis method. 100 % mortality after 96 hours was seen at 10.50 µg/L concentration of bifenthrin. Mortality rate of *C. idella* at different concentrations of using probit analysis bifenthrin were shown in Figure 1.

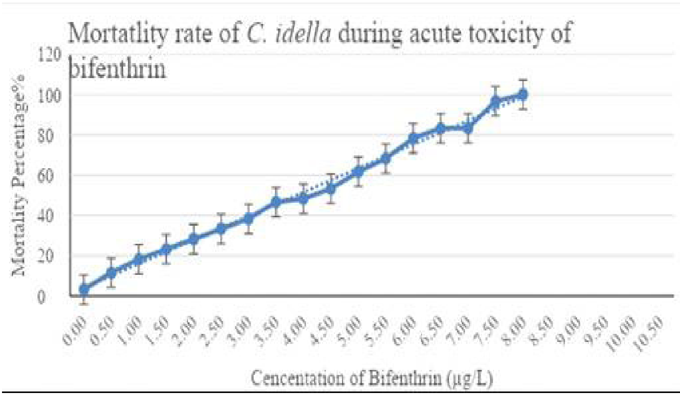

Figure 1: *Mortality rate of C. idella to bifenthrin concentrations exposure during 96 h acute toxicity.*

Physico-chemistry of water during 96 h exposure period under different concentrations of bifenthrin to C. idella

Fish were divided into two groups, control and experimental. *C. idella* were exposed to 1/3rd sub lethal of bifenthrin LC_{50} concentration which was 2.16 µg/L for 30 days. Physical parameters of water including pH (7.5), temperature (29 °C), total hardness (300 mg/L), carbon dioxide (1.04-2.02 mg/L), calcium (21-23 mg/L), total ammonia (1.24-1.60 mg/L), dissolved oxygen (6.50-5.85 mg/L), and magnesium (60.81-59.87 mg/L) were noted.

Alteration in behavioral patterns of C. idella under sublethal (1/3rd of LC_{50}) exposure of bifenthrin for 30 days

Calculated value of sublethal (1/3rd 0f LC_{50}) of bifenthrin was 2.16µg/L. Experimental fish were exposed to this dose for 30 days. Variations in behavior of *C. idella* were noticed on daily and weekly basis.

Variation in body color, gills color, equilibrium status and convulsion rate in C. idella under sublethal (1/3rd of LC_{50}) exposure of bifenthrin

C. idella was exposed to sublethal (1/3rd of LC_{50}) of bifenthrin. Body color of *C. idella* changed from grey color to light yellow color. Highly significant variations in body color, gills color, equilibrium status and convulsion rate in *C. idella* were seen. Body color changed from grey to pale yellow color and gills color changed from bright red to light red color. Fish adapt vertical position in last week hence equilibrium status was disturbed. In last two weeks convulsion rate in *C. idella* was seen. Graphical representation of results is shown in Figure 2.

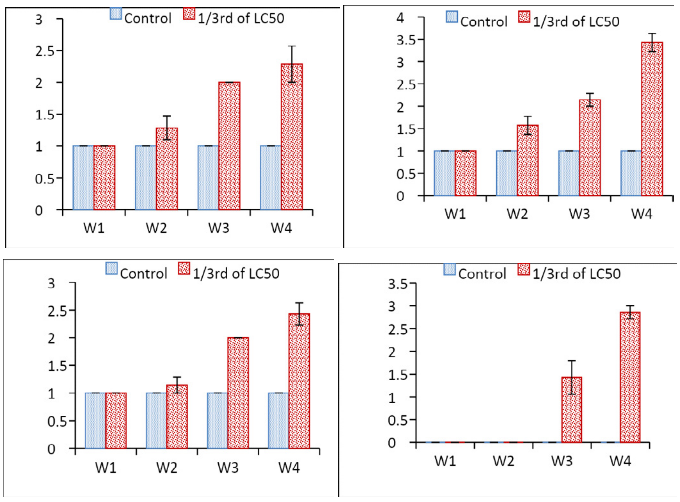

Figure 2: *Rate of variation in body color, gills color, equilibrium status and convulsions rate of C. idella under sublethal (1/3rd of LC_{50}) exposure of bifenthrin.*

Effect of sublethal (1/3rd of LC_{50}) exposure of bifenthrin on descaling, swimming rate and fin movements of C. idella

Highly significant alterations in descaling, swimming rate and fin movements were observed under bifenthrin exposure. Descaling and fin movements were increased in last two weeks. However, swimming rate was decreased in last week. Fish became motionless might be due to muscles weakness. Rate of alterations in these parameters were shown in Figure 3.

Effect on opercular movements, somersaulting activity and air gulfing in C. idella under sublethal (1/3rd of LC_{50}) exposure of bifenthrin:

After exposure to bifenthrin, maximum significant variations in opercular movements, somersaulting activity and air gulfing in *C. idella* were observed. Opercular movements were enhanced gradually might be due to suffocation. Air gulfing and somersaulting activity was also extreme in last two weeks and this might be due to oxygen deficiency. Graphical plot for these variations are seen in Figure 4.

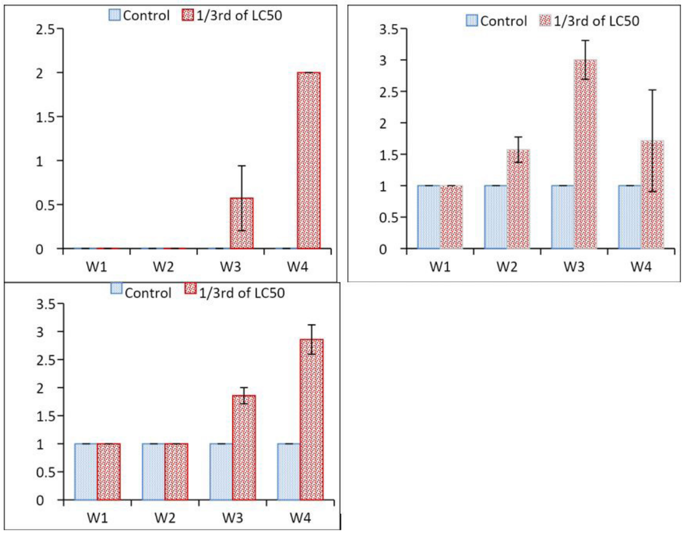

Figure 3: *Rate of variation on descaling, swimming rate and fin movements of C. idella under sublethal (1/3rd of LC_{50}) exposure of bifenthrin.*

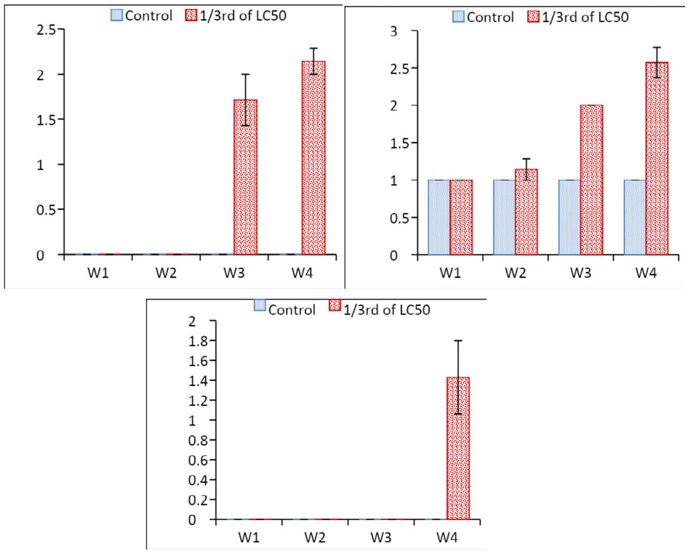

Figure 4: *Rate of variation on air gulfing, opercular movements and somersaulting activity of C. idella under sublethal (1/3rd of LC_{50}) exposure of bifenthrin.*

Determination of blood parameters of Ctenopharyngodon idella under sublethal (1/3rd of LC_{50}) exposure of bifenthrin after 15 and 30 days

Blood parameters were measured and showed significant results ($p<0.05$). Hematological profile in grass carp was disturbed due to bifenthrin exposure. There are significant ($p<0.05$) decreased in the values of RBCs, Hct, Hb and MCHC as compared to control group. Whereas WBCs, MCH and MCV values were increased after exposure of bifenthrin for 15 and 30 days. These results were compared with control group. Alteration rate of these parameters are shown in Table 1.

Table 1: *Hematological studies of C. idella in control and sub lethal toxicity (1/3rd of LC_{50}) of bifenthrin after 15 and 30 days.*

Hematological parameters	Control	1/3rd bifenthrin exposure	
		After 15 days	After 30 days
RBC's ($x10^6/\mu L$)	1.19 ± 0.02^a	0.83 ± 0.01^b	0.47 ± 0.01^c
Hb (g/dL)	6.1 ± 0.02^a	4.9 ± 0.01^b	4.51 ± 0.01^c
Hct %	17.3 ± 0.02^a	11.9 ± 0.01^b	8.06 ± 0.01^c
WBCsC ($10^3/\mu L$)	119.61 ± 0.02^c	141.5 ± 0.01^b	150.75 ± 0.01^a
MCV (fL)	66.9 ± 0.02^a	43.4 ± 0.01^b	58.32 ± 0.01^c
MCH (pg)	47.0 ± 0.02^c	59.0 ± 0.01^b	67.81 ± 0.01^a
MCHC (g/dL)	45.06 ± 0.02^a	41.2 ± 0.01^b	20.07 ± 0.01^c

Present research was conducted to determine the toxicity of bifenthrin on behavioral and blood parameters of *C. idella*. For this purpose, fingerlings of *C. idella* were brought to laboratory and experiment was performed for 96h. Calculated LC_{50} and sublethal (1/3rd of LC_{50}) value of bifenthrin to *C. idella* was 6.5 µg/L and 2.16 µg/L, respectively.

Previous investigations on various fish revealed different LC_{50} concentrations of bifenthrin. Liu *et al*. (2005) determined the LC_{50} of bifenthrin against Tilapia specie (0.80mg/L) and *Cyprinus carpio* (2.08mg/L). LC_{50} concentration of bifenthrin against *Cyprinus carpio* was 57.5 mg/L (Velisek *et al*., 2009a). Farag *et al*. (2021) has determined the 96h LC_{50} of bifenthrin against *Oreochromis niloticus* and it was 6.8µg/L which is much closer to present research result. Whereas in sheep's head minnow it was 17.5 µg/L and in rainbow trout it was 0.1 5µg/L (Yang *et al*., 2018).

Acute and sub lethal effect of bifenthrin to adult and larva of grass shrimp were observed. LC_{50} of bifenthrin for adult shrimp was 0.2 0µg/L and for larva it was 0.013 µg/L. LC_{50} for adult and larval shrimp was 0.339 µg/L (Harper *et al*., 2008). it revealed that bifenthrin is more toxic to above mentioned fish species as compared to grass carp.

The LC_{50} value of bifenthrin in present research work was double than the 3.464 µg/L LC_{50} value to *Clarias batrachus* (Saha *et al*., 2021). The results show that bifenthrin is more toxic to *C. idella* as compared to *Clarias batrachus*. Similarly, Ullah *et al*. (2021) has determined the 6 µg/L LC_{50} value against *C. idella* which was almost similar to current research work.

The variations in behavioral parameters of *C. idella* were observed at acute toxicity test and at sublethal (1/3rd of LC_{50}) exposure of bifentrhrin. In present research work it was observed that during acute toxicity test by increasing the concentration of bifenthrin variations in hyperactivity, somersaulting activity, opercular movements, air gulfing and convulsions rate were noticed. Fin movements and swimming rate was also enhanced due to increase in bifenthrin doses. Loss in equilibrium was also observed fish adapt the vertical body position. Descaling was also noticed. At the end fish become motionless and sluggish might be due to muscles weakness. No such changes were seen in control group. These results were much similar to many studies, Mundy *et al*. (2020) has used the behavioral examine to find out the bifenthrin exposure effects and they observed the high hyperactivity in larva during 96h exposure of bifenthrin. Saha *et al*. (2018) studied the behavior of *C. idella* against exposure to mixture of pesticide. Abnormal behavior such as variation in opercular movements, jumping, jerky movements, erratic swimming was noticed. Equilibrium status and opercular movement increases with increasing exposure and decline by increasing duration exposure. Somersaulting in fish was observed at high dose. Acute exposure of bifenthrin to fish results in behavioral abnormality. Hyperactivity, disruption in schooling response, balance loss, disturb swimming rate and loss of equilibrium were noticed. At late phase of bifenthrin exposure, the fish adapts vertical poster and also turned into motionless state and sluggishness (David *et al*., 2013).

Grass carp treated with bifenthrin turned into light yellow color was observed. Repeated visits at surface to gain fresh oxygen were increased. Other scientists also observed the same behavioral responses to exposure of various insecticides in different fish i.e., bifenthrin exposure to *Cyprinus carpio* (Velisek *et al*., 2009a), deltamethrin exposure to *Hypophthalmichthys molitrix* (Ullah *et al*., 2018) and Putitora exposed to cypermethrin (Ullah *et al*., 2014).

Somersaulting in fish was observed at high dose. Actually, this was the avoidance response due to change in surrounding environment due to chemical exposure (Saha *et al*., 2018). In addition, Ullah *et al*. (2022) had observed the loss of equilibrium, Jumping, rapid swimming and increase in hyperactivity in *C. idella* exposed to acute concentrations of bifenthrin.

In present research work, blood parameters of *C. idella* including RBCs, WBCs, Hb, Hct, MCV, MCH and MCHC were studied under sublethal (1/3rd of LC_{50}) exposure of bifenthrin. Results showed increased in WBCs, MCV, MCH and MCHC values gradually after 15 and 30 days whereas decreased in values of RBCs, Hb, and PCV

Ullah *et al*. (2021) described the bifenthrin exposure impacts on hematological parameters of *C. idella*. Their results showed decrease in hemoglobin and RBCs values. Hence bifenthrin exposure resulted in anemia in fish. Likewise, WBCs count increased by test chemical exposure. Increased in platelets count was also measured. Decrease in hemoglobin and RBCs count due to bifenthrin exposure to fish was studied by Bloom *et al*. (2008).

Velisek *et al*. (2009a) revealed that the fish with LC_{50} concentration of bifenthrin for 96h and examined their hematological, histopathological and biochemical profiles. The result showed that glucose plasma, aspartate aminotransferase, ammonia, and absolute monocyte counts, as well as creatine kinase were considerably higher in the experimental group as compared to the control.

Valisek *et al*. (2009b) studied the influence of bifenthrin at acute exposure on some biochemical, hematological and parameters of *Oncorhynchus mykiss*, and it was observed that fish displayed hematological significant decreases in erythrocyte hemoglobin and mean erythrocyte volume, and band neurophil granulocytes as compared to control. Hepatocytes degeneration was detected histologically, hence bifenthrin was therefore categorized as a substance highly toxic to fish. Impact of pesticides bifenthrin and chlorpyrifos in erythrocytes count of *Labeo rohita* was studied by Bano *et al*. (2021). They concluded that up to 56 days gradual increase in damage was observed and in the next fourteen days minor decrease was observed. Present research results were almost similar with the work of other scientist work. Limited studies were available on blood parameter change due to bifenthrin exposure. However, it was concluded that bifenthrin is highly vulnerable to *C. idella*.

Conclusions and Recommendations

C. idella is highly sensitive to bifenthrin. Acute exposure of bifenthrin induced many changes in behavior of fish. These findings suggest that bifenthrin is strongly vulnerable pyrethroid and can cause serious adverse effect on aquatic life. So, there is need to monitor the extensive and unnecessary use of bifenthrin on regular basis. Use of bifenthrin should be according to environmental law to avoid any danger to public health as well as to aquatic life.

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Novelty Statement

The current study highlighting the insecticide contamination as major aquatic environmental issue which poses a significant threat to aquatic organisms.

Author's Contribution

Moazama Batool: Planning research, supervision of study and provide experimental setup, data analysis, interpretation, manuscript write up.
Syeda Ansa Fatima: Performed the experiment and prepared initial draft.
Saima Naz and Qurat-Ul-Ain: Helped in analyze the experimental data and writing research paper.
Sheeza Bano: Reanalyze the data, edited and finalize the manuscript writing.
Ghulam Abbas: Reviewed the final version of manuscript.
Ahmad Manan Mustafa Chatha: helped in data compilation and manuscript writing.

Conflict of interest
The authors have declared no conflict of interest.

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