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Address for correspondence:
Mushtakh R. Hashmi
Research Scholar, Department of Zoology and Fishery Science, Rajarshi Shahu Mahavidyalaya [Autonomous] Latur Maharashtra.
Email: hmushtakh7@gmail.com.

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Efficiency of Methylene Blue in Treating Ichthyophthirius multifiliis in Siamese Fighting Fish (*Betta splendens*)

Mushtakh R. Hashmi[1], Dr. Amjatkhan V. Pathan[2]
[1]Research Scholar, Department of Zoology and Fishery Science, Rajarshi Shahu Mahavidyalaya [Autonomous] Latur Maharashtra.
[2]Associate Professor, Corresponding Author & Head, Department of Zoology and fishery Science Azad Mahavidyalaya, tq. Ausa Dist. Latur Maharashtra.
Email: hmushtakh7@gmail.com.

Abstract

The Siamese fighting fish (Betta splendens) and other freshwater fish are susceptible to the common protozoan parasite Ichthyophthirius multifiliis, also referred to as "Ich" or white spot disease. The effectiveness of methylene blue, a common antiparasitic and antifungal medication, in treating multifiliis infections in Betta splendens is assessed in this study. White spot disease, caused by the protozoan parasite Ichthyophthirius multifiliis, is a common and highly contagious infection in betta fish (Betta splendens), characterized by small white cysts on the skin, fins, and gills The results indicate that, especially at doses between 2 and 3 ppm, methylene blue is a practical and affordable treatment option for Ichthyophthirius multifiliis in Betta splendens. Methylene blue (C16H18ClN3S), a synthetic dye with antiparasitic and antifungal properties, functions by disrupting cellular respiration in pathogens and has been widely used in aquaculture for treating external infections However, because of the risk of toxicity or stress, higher dosages need to be closely monitored. To investigate long-term effects, ideal dose intervals, and synergistic effects with different treatments, more research is advised. This study backs up the safe application of methylene blue as a good substitute for more aggressive chemical treatments in the maintenance of ornamental fish health.

Keywords: *Betta Splendens, Ichthyophthirius multifiliis, White spot disease, Methylene blue (MB)*

Introduction:

Aquaculture has become a vital industry in the world's food supply and ornamental fish trade, making be a major issue among aquaculturists' many problems, frequently resulting in significant financial losses and jeopardising fish welfare. White spot disease, or ichthyophthiriasis as it is scientifically named, is one such widespread parasitic condition brought on by the protozoan Ichthyophthirius multifiliis. The presence of tiny white cysts on the skin, fins, and gills of afflicted fish is a hallmark of white spot disease. These cysts are the encysted trophonts of I. multifiliis, which cause respiratory distress, inflammation, and increased mucus production by burrowing into the epithelial tissues. In aquaculture systems with a high population density, I. multifiliis is extremely contagious due to its free-swimming theront stage, which actively seeks out hosts. White spot disease requires quick and efficient treatment methods due to its high fatality rates and quick dissemination. Chemicals like formalin and malachite green have been used in traditional treatments; however, worries about their toxicity and potential effects on the environment have prompted research into alternative therapies.

Methylthioninium chloride, also referred to as methylene blue (MB), is a heterocyclic aromatic chemical molecule that has long been used in aquaculture and medicine. Because of its antibacterial, antifungal, and antiparasitic qualities, MB was first synthesised in the late 19th century. MB has been used in aquaculture to treat protozoan infestations, fungal diseases, and fish toxicity from cyanide and nitrite. The chemical inhibits the metabolic activity of bacteria by interfering with their redox mechanisms. MB has established itself as a useful instrument in the control of fish health because to its effectiveness against a wide range of infections and its generally positive safety profile. MB mainly uses its redox characteristics to produce its antiparasitic actions. By taking up electrons, MB interferes with the protozoan mitochondria's electron transport chain, which impairs ATP generation and ultimately results in cell death. Furthermore, when exposed to light, MB has been shown to produce reactive oxygen species (ROS), which enhances its antibacterial properties. The capacity of MB to pierce the cyst wall and target the trophont stage is especially advantageous in the case of I. multifiliis.

According to studies, MB can successfully lower the parasite burden in diseased fish, increasing their chances of recovery and survival. Siamese fighting

fish, or *Betta splendens*, are well-known for their vivid colours and combative tendencies. They are widely bred in aquaculture settings and are a popular choice among aficionados for ornamental fish. However, both breeders and hobbyists face serious difficulties because to their vulnerability to parasite illnesses, such as white spot disease. Because of its effectiveness and simplicity of use, MB has attracted attention for its use in treating I. multifiliis infections in B. splendens. Direct contact with the parasite can be achieved by administering MB through bath treatments. In order to guarantee efficacy and reduce any possible adverse effects, optimal dosage is essential. According to the severity of the infection, doses between 2 and 3 ppm have often been used, and treatment periods have ranged from a few days to several days. The effectiveness and safety profile of MB in aquaculture have been the subject of recent studies. Soltanian et al. (2021) looked into how MB affected goldfish (Carassius auratus) and discovered that although MB had antiparasitic benefits, long-term exposure changed oxidative stress markers and haematological parameters. These results highlight how crucial it is to follow prescribed dosages and treatment schedules in order to minimise any potential side effects. Chronic exposure to MB impacted reproduction and physiological processes, according to another study on the toxicity of MB to the freshwater zooplankton species Daphnia magna (PubMed, 2023). Despite the fact that D. magna is not a target species in aquaculture, our results draw attention to the ecological issues surrounding the use of MB and stress the necessity of appropriate application and disposal procedures.

The protozoan parasite Ichthyophthirius multifiliis is the cause of white spot illness, which has a major negative influence on the behaviour and general health of betta fish (*Betta splendens*). The parasite creates visible white cysts that resemble salt grains after invading the skin, fins, and gill tissues. The parasite damages tissue, irritates the skin, and produces too much mucus while feeding on epithelial cells. Abnormal behaviours include flashing (rubbing against surfaces), lethargy, clamped fins, and laboured breathing due to gill involvement are common in infected bettas. High mortality rates, respiratory failure, and subsequent bacterial infections can result from the illness if treatment is not received. Because of the parasite's quick life cycle and contagious nature, early detection and timely treatment are essential to avoiding widespread infection in aquarium settings. In shared aquatic habitats, white spot disease spreads quickly and is extremely contagious. The parasite Ichthyophthirius multifiliis develops beneath the fish's skin, then leaves the host and encysts on the tank substrate, where it splits into hundreds of free-swimming theronts. The infectious stage, these theronts actively look for new hosts. This life cycle spreads swiftly in overcrowded or badly managed aquariums, infecting several fish in a short amount of time. A full-tank outbreak can be caused by a single infected fish because theronts can live in the water for several hours while looking for a host. Fish immunity is weakened by things like stress, bad water quality, and temperature fluctuations, which increases their susceptibility to illness.

Material Methodology:

1. Study Species:

The study was conducted using Two adult male *Betta splendens* (Siamese fighting fish), aged between 6 to 8 months, which is Red Coloured Rose Tail Betta fish and obtained from a reputable ornamental fish breeder shop Located in Latur city Market Maharashtra. Only healthy, with (standard length: 5.5 ± 0.5 cm) were selected.

2. Experimental Setup:

2.1 Aquarium Condition:

A total of 2 glass mini-aquaria with partition and cover shelter especially made and sell in the market for Betta fish which of (18x12x12 cm) were used. Each tank was filled with 2.25 litres, of dechlorinated fresh tap water and maintained at a temperature of 28 ± 1°C. pH levels were kept between 6.8 and 7.2, with regular monitoring. A 12:12 hour light-dark photoperiod was maintained throughout the study. Filtration was minimal to avoid stress; water was changed every day to maintain optimal conditions. Protein rich similar feed provided to both the betta fish on 24-hour time duration.

2.1. Image of Transparent Mini-aquarium tank with the used to monitor the effect of Methylene Blue on White Spot Disease in *Betta splendens*.

2.2 Treatment Protocol:
Treatment began 24 hours after confirmation of infection (appearance of white spots on skin and fins). Methylene blue (1% solution) that is 0.5 ml per 2 litres of water that is (2.5 PPM) was added to treatment tanks at the assigned concentrations. A 100% daily water change was performed prior to each re-dosing to maintain water quality and consistency in dosage. Treatment was administered for **7** consecutive days. No feeding was provided during treatment to reduce organic waste and enhance water quality.

2.3 Water Quality Monitoring:
Water parameters (temperature, pH, ammonia, nitrite, and dissolved oxygen) were monitored daily using API freshwater test kits to ensure a stable environment throughout the study. All tanks were maintained under 12:12 hour light-dark cycles.

3. Clinical Observation and Data Collection:
Fish were observed twice daily for clinical signs of infection, behavioural changes, mortality, and external lesions. Parasite load was assessed by counting visible white spots on the body and fins under a stereomicroscope before treatment (Day 0), mid-treatment (Day 4), and at the end (Day 7). recovery rates were recorded. Fish were observed twice daily for clinical signs of infection, including the appearance of white spots, behavioural changes, and overall health. The size and location of the white spots were recorded to assess the progression of *I. multifiliis* infection with a white spot approximately 1.3 cm in diameter on the operculum, which was used as a reference to gauge the severity of infection. The presence and size of white spots were measured and documented under a stereomicroscope before treatment (Day 0), midway through treatment (Day 4), and at the end of the treatment period (Day 7). Additionally, any changes in the fish's behaviour (e.g., scratching, lethargy, or clamped fins) were noted, along with survival and recovery rates at the end of the trial.

4. Methylene Blue:
Methylene blue (C16H18ClN3S) is a synthetic thiazine dye originally developed for textile use but has since found wide applications in medicine and aquaculture due to its antimicrobial and antiparasitic properties. In aquaculture, it is commonly used as a treatment for external parasites, fungal infections, and egg disinfection. Methylene blue works by interfering with the respiratory enzymes of pathogens, effectively disrupting their metabolism and killing them, especially in their free-swimming stages.

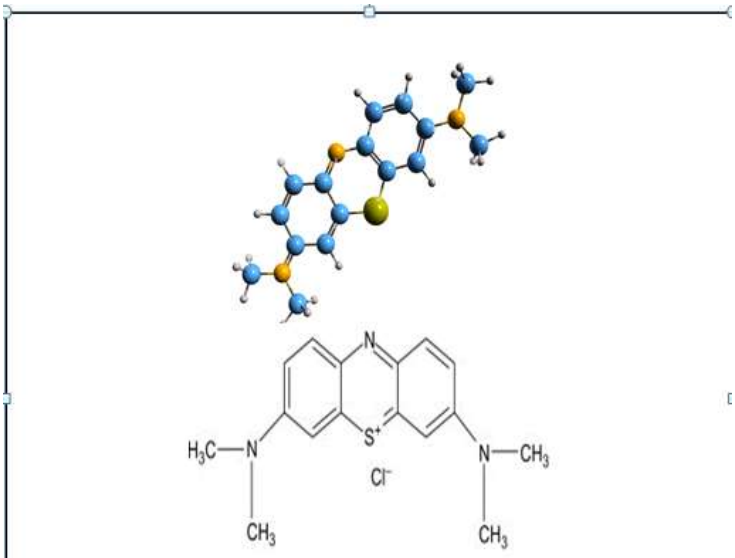


Figure. Methylene Blue (C16H18ClN3S) with Structural Formaula.

Observation:
During the course of the infection with *Ichthyophthirius multifiliis*, several distinct behavioral abnormalities were observed in *Betta splendens*, indicating stress and declining health. The behaviors were consistent across multiple individuals and served as non-invasive indicators of disease severity. Fish were observed twice daily for clinical signs of infection, including the appearance of white spots, behavioural changes, and overall health. Infected bettas exhibited several distinct behavioural changes, which were indicative of the stress and discomfort caused by *I. multifiliis* infection. Common symptoms included and Below is a detailed account of the observations:

A. Behavioural Observation:

1. **Loss of Appetite:**Infected bettas displayed a noticeable decline in feeding response. Fish that previously exhibited aggressive feeding behaviour completely ignored food during the infection period. Pellets remained

uneaten, and in some cases, fish showed disinterest even when food was placed directly in their line of sight. This anorexia persisted until the mid-stages of treatment.

2. **Stress Indicators:**Elevated stress was evident in multiple ways. The fish showed increased sensitivity to movement around the tank and external stimuli. Gills appeared to move more rapidly than usual, indicating laboured breathing. Mucus production was also slightly elevated.
3. **Abnormal Swimming Behaviour (Upside-Down Movement):**Several fish exhibited erratic swimming behaviour, including floating upside down or on their sides for short intervals. While not constant, this behaviour occurred intermittently, particularly when the fish attempted to move suddenly. This may indicate compromised buoyancy control, a symptom linked to systemic stress or parasitic interference with the swim bladder or musculature.
4. **Paused or Hesitant Movement:**Infected fish often paused mid-swim and remained motionless in the water column for several seconds to minutes. These pauses did not resemble resting behavior but seemed involuntary, possibly due to fatigue or neurological irritation from the parasite.
5. **Reduced Activity:**One of the most prominent behavioural changes was a marked reduction in overall activity. Healthy bettas are naturally active and curious, but infected individuals became withdrawn and showed minimal interest in exploring their environment. Fins appeared clamped, and their usually flowing movement was diminished.
6. **Corner-Settling Behaviour:**Most affected fish tended to settle down in the corners or bottom of the tank, especially in shaded or hidden areas. This behaviour was observed consistently, with fish avoiding open spaces and exhibiting a hunched posture, possibly as a self-protective response to discomfort or weakness.

   These behavioral changes were observed in correlation with the physical signs of *I. multifiliis* infection, particularly the presence of white cysts on the body, including a notable 1.3 cm white lesion on the operculum in one specimen. All behaviours gradually returned to normal as treatment with methylene blue progressed and parasite load decreased.

B. Clinical Observation:

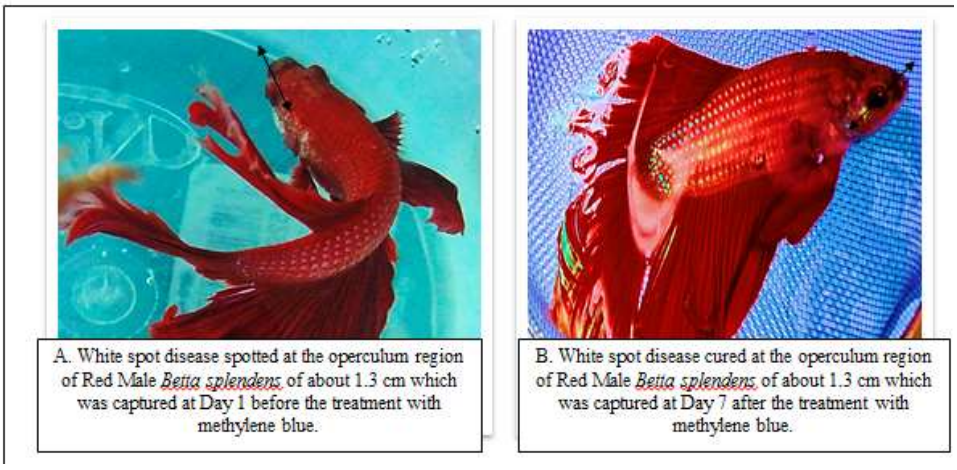
Over the 7-day treatment period, methylene blue demonstrated noticeable efficacy in controlling and curing *Ichthyophthirius multifiliis* infections in *Betta splendens*. Infected fish were treated with a 0.5 mL dose of 1% methylene blue per 2 litres of DE chlorinated water, and daily water changes with re-dosing were maintained consistently throughout the treatment.

By **Day 2**, the intensity of clinical signs began to decrease. Fish showed slight improvement in behavior, including occasional interest in food and more regular swimming patterns. The number of white cysts (visible spots) began to reduce in both size and number. Fish that initially displayed severe symptoms such as upside-down swimming, clamped fins, and corner-settling behavior began to exhibit longer periods of normal upright swimming and reduced surface gasping.

By **Day 4**, white spots on the body and fins had visibly diminished, and fish became significantly more active. The prominent 1.3 cm lesion previously observed on the operculum appeared less raised and began to fade in colour. Behavioral abnormalities such as lethargy and loss of appetite continued to improve, with most fish beginning to feed normally.

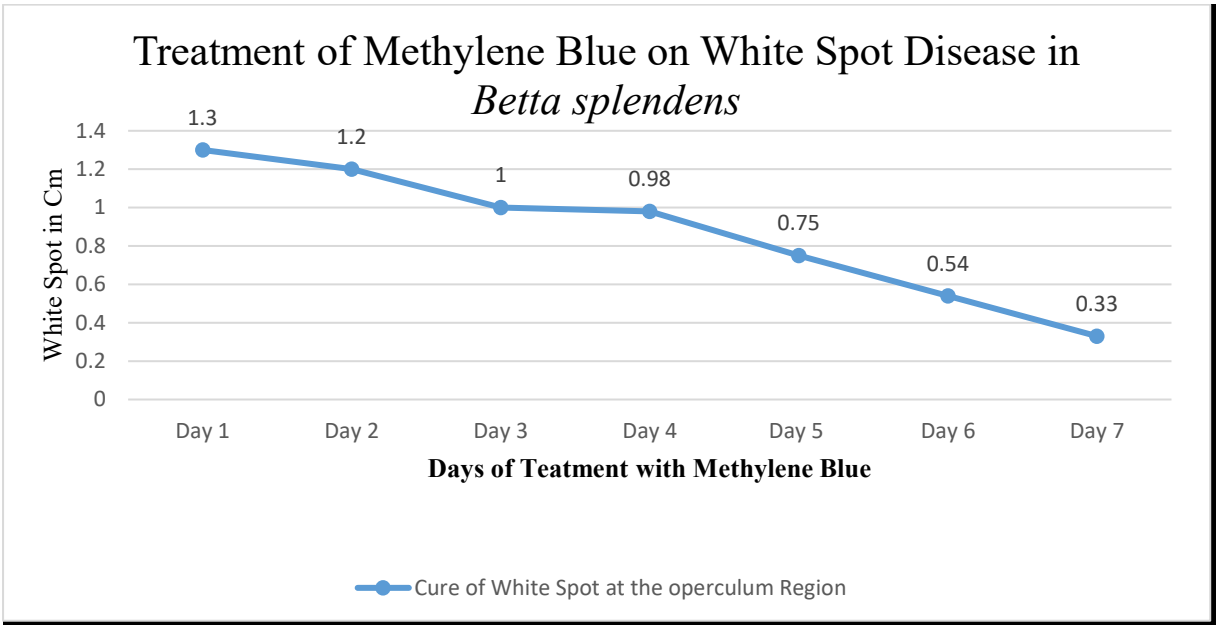
By **Day 7**, nearly all white spots had disappeared from the external body surface, and all treated fish exhibited normal behavior — active swimming, proper response to feeding, and typical territorial display. The mucous layer on the skin appeared healthy, and fins were no longer clamped. No mortality was recorded in the treated group during the trial period.

Methylene blue is believed to act by interfering with the cellular respiration of the parasite, especially in the free-swimming theront stage. Additionally, its antifungal and antiseptic properties help prevent secondary infections during the healing process. The consistent reduction in external cysts and restoration of normal behavior strongly indicate the compound's therapeutic value in managing *I. multifiliis* infections in ornamental fish like *Betta splendens*.



A. White spot disease spotted at the operculum region of Red Male *Betta splendens* of about 1.3 cm which was captured at Day 1 before the treatment with methylene blue.

B. White spot disease cured at the operculum region of Red Male *Betta splendens* of about 1.3 cm which was captured at Day 7 after the treatment with methylene blue.



Result and Discussion:

The application of methylene blue (MB) demonstrated significant therapeutic effectiveness in treating *Ichthyophthirius multifiliis* infection in *Betta splendens*. Fish that initially exhibited classic symptoms of white spot disease such as visible white cysts (including a notable 1.3 cm lesion on the operculum), lethargy, clamped fins, loss of appetite, upside-down swimming, and corner-hiding behaviour showed progressive improvement over a 7-day treatment course.

Visible white spots began to diminish by the second day of treatment, and behavioural changes also started to reverse. By Day 4, white lesions had shrunk noticeably in both number and size, with several fish returning to normal upright swimming and beginning to accept food. By Day 7, nearly all physical signs of *I. multifiliis* had disappeared, and the behaviour of the treated fish had normalized, including active swimming, responsive feeding, and typical territorial displays. No mortality was observed in the treated fish, and water parameters remained within optimal range throughout the experiment.

Conclusion:

The present study demonstrates that methylene blue is an effective and practical treatment for white spot disease (*Ichthyophthirius multifiliis*) in *Betta splendens*. A dosage of 0.5 mL of 1% methylene blue in 2 litres of water, administered over a 7-day period with daily water changes, successfully eliminated visible white cysts and restored normal behavioral patterns in infected fish. The therapeutic action of methylene blue, which targets the parasite's respiratory system during its free-swimming theront stage, significantly reduced parasite load without causing adverse effects to the fish.

In addition to its antiparasitic properties, methylene blue also exhibited secondary benefits such as preventing opportunistic infections and supporting tissue recovery. Treated fish showed clear signs of recovery including regained appetite, increased activity, and disappearance of abnormal swimming behaviors. No mortality was observed throughout the treatment, affirming the safety of methylene blue at the applied concentration.

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