International Journal of Zoology Studies
ISSN: 2455-7269; Impact Factor: RJIF 5.14
Received: 07-03-2021, Accepted: 08-04-2021, Published: 21-05-2021
www.zoologyjournals.com
Volume 6; Issue 3; 2021; Page No. 26-29

Effect of *Allium sativum* on the aggressive behavior of male *Betta splendens*

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Abstract
Allium sativum (Garlic) extract was administered to aquarium fish *Betta splendens* (Siamese fighting fish) and the effect of the extract on the aggressive behavior of male *Betta splendens* was assessed based on the following characteristics like duration of avoidance, duration of lateral display, duration of extreme fight and number of opercular flaring. The fish had shown significant reduction in the aggressive behavior after exposing them in *Allium sativum* extract of different concentrations (1mL, 2mL, 3mL and 4mL) for 3 hours prior to the experimental observations. The characters were observed for 20 minutes and the duration of avoidance was increased from 0mL concentration of *Allium sativum* extract (10.28±0.04mins) to 4mL concentration (20.00±0.00). Whereas, duration of lateral display showed significant reduction from 0mL concentration of *Allum sativum* (8.32±0.01mins) to 4mL concentration (0.00±0.00). Similarly duration of extreme fight was also reduced from 0mL concentration (1.41±0.01mins) to 4mL concentration (0.00±0.00). Number of opercular flaring, which is the most distinctive character to measure the aggression of *Betta splendens* was also significantly reduced from 0mL concentration (21.75±0.96) to 4mL concentration (4.25±0.96). *Allium sativum* has a stimulatory effect on the brain serotonin level of male *Betta splendens* which reduced the aggression of the fish.

Keywords: *Allium sativum*, *Betta splendens*, duration of avoidance, duration of lateral display, duration of extreme fight, number of opercular level, brain serotonin

Introduction
Animals compete aggressively with individuals of the same species over limited resources like territory and mates (Parker, 1974). Successful displays can increase access to mates, mating success and territory size (Alton *et al*., 2013) [3].*B. splendens* are a facultatively air-breathing freshwater Anabantoid fish, which inhabit hypoxic waters where air-breathing is essential (Graham, 1997) [17]. The wild Siamese fighting fish are often found swimming amongst the inland waters of the Orient. They are native to Thailand but are found worldwide in pet stores as a domesticated fish (Hargrove, 1999). Domestic breeds have long been considered model organisms for behavioural studies because of their aggression, and stereotypic and conspicuous displays (Tate *et al*., 2017). Male *B. splendens* have multiple visual displays, including fin flaring and opercular flaring, which are designed to intimidate opponents by increasing their perceived body size (Simpson, 1968). Examining social interactions in *B. splendens* has suggested that opercular flaring (raising of the operculae) and lateral displays (fin flaring while showing their broadside) are considered to be the most common aggressive displays in male-male and male-female interactions (Forsatkar *et al*., 2016) [15]. Low level of serotonin is said to be the reason for the aggressiveness of Siamese fighting fish (Simpson, 1968).
Garlic or *Allium sativum* is regarded as both food and medicinal herb. Increasing attention has focused on the biological functions and health benefits of garlic as a potentially major dietary component. Chronic garlic administration has been shown to reinforce memory function. Evidence also shows that garlic administration in rats affects brain serotonin (5-hydroxytryptamine [5-HT]) levels (Heider, 2014) [18].
Antimicrobial and therapeutic properties of *Allium sativum* on fishes have been known and studied, but its effect on aggressive behaviour of fishes was not yet documented. Therefore an attempt has been made to study the effect of *Allium sativum* extract on the aggressive behaviour of male *Betta splendens.*

Materials and methods
Collection and maintenance of *Betta splendens*
Adult male *B. splendens* (N=8) were housed individually in visually isolated 5 inch bowls and are kept in room temperature. Males were kept in isolation in their home bowls for 7 days prior to the behavioral observations to reduce the effects of prior social experience on their responses. Individuals were fed once a day, 7 days a week, on a mix of *Daphnia* spp., *Artemia* spp., and Tetra *Betta* flake food.

Collection of *Allium sativum*
Fresh bulbs of *Allium sativum* were purchased from the local market in Angadikadavu, Kerala (India).

Preparation of *Allium sativum* extract
About 50 g of chopped *Allium sativum* slices were imbibed in water at ambient room temperature (35.9 ± 0.96˚ C) and is made into garlic paste by crushing them in a grinder and extract was obtained.

Experimental set-up
For all experiments, the small fish tanks (20 x 10 x 12cm) containing 2L of well water aged for 18 h overnight at room temperature of 35.9 ± 0.96˚ C and 100mL of previously exposed fish water were used. All tanks were covered to remove external stimuli, except the aggression trail. The

behavior was recorded using a mobile video and stopwatch. To study the aggressive behaviour in male *Betta splendens* after exposing it to *Allium sativum* extract for 3 hours in each group (group 1; 1mL, group 2; 2mL, group 3; 3ml, group 4; 4ml). Males of same colour, body size, and pattern were placed together in a small fish tank (20 x 10 x 12cm). They were given 10 minutes to acclimatize before the observations and their behavior was recorded for 20 minutes. Duration of avoidance, Duration of lateral Display, Duration of extreme fight, Number of opercular flaring were recorded. Each individual were observed once per day and repeat encounters were allowed to occur.

Statistical analysis

The results of duration of avoidance, duration of lateral display, duration of extreme fight and number of opercular flaring were analyzed using SPSS Software developed by IBM (version 25). The results were mean ± S.D of triplicate analysis.

Results

Exposure of male *Betta splendens* in *Allium sativum* extract has shown significant reduction in the aggressive behavior of *Betta splendens* and the results were confirmed by observing the following characteristics of the fish.

Duration of avoidance

Duration of avoidance, of fishes increases as the concentration of *Allium sativum* extract in 500mL of water increases from 0mL, control (10.28±0.04) to 4mL (20.00±0.00). Data was collected by observing the character for 20 minutes and data collected is represented in the form of mean and standard deviation in Table 1 and a line graph (figure 1) is plotted to show the change using the mean values.

Table 1: Duration of avoidance shown by male *Betta splendens* fish for 20 minutes. Data obtained as a result of triplicate analysis expressed as mean± S.D. All values are significant at p≤0.05.

Concentration of garlic Juice (mL)	Duration of avoidance (minutes)
0mL (control)	10.28 ±0.04
1mL	12.12 ±0.00
2mL	13.33 ±0.02
3mL	19.19 ±0.50
4mL	20.00 ±0.00

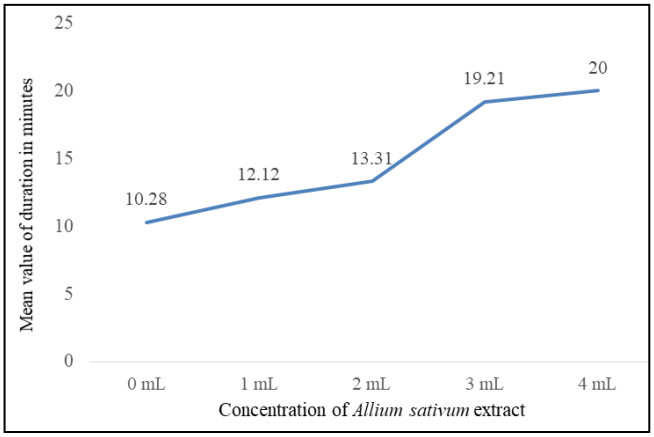

Fig 1: Graph representing duration of avoidance exhibited by *Betta splendens* for 20 minutes after exposing to *Allium sativum* extract for 3 hours

Duration of lateral display

Duration of lateral display decreases as the concentration of *Allium sativum* extract increases from 0mL, control (8.33±0.01) to 4mL (0.00±0.00). Data was collected by observing the character for 20 minutes and data collected is represented in the form of mean and standard deviation in Table 2 and a line graph (figure 2) is plotted to show the change using the mean values.

Table 2: Duration of lateral display shown by male *Betta splendens* fish for 20 minutes. Data obtained as a result of triplicate analysis expressed as mean± S.D. All values are significant at p≤0.05.

Concentration of garlic Juice (mL)	Duration of Lateral Display (minutes)
0mL (control)	8.32±0.01
1mL	7.17±0.01
2mL	6.44±0.01
3mL	1.04±0.01
4mL	0.00±0.00

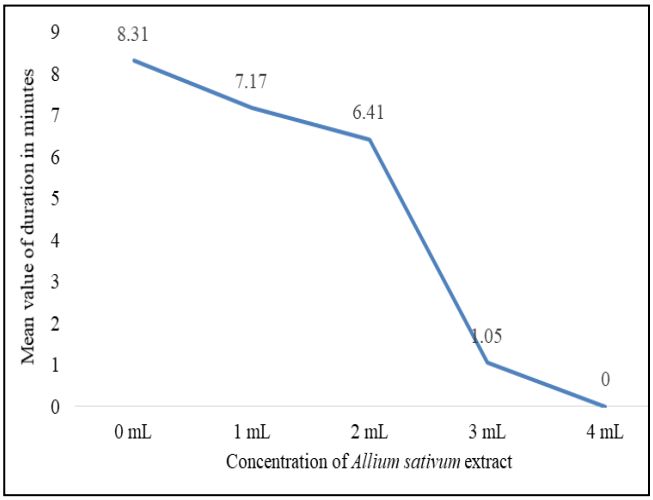

Fig 2: Graph representing duration of lateral display exhibited by *Betta splendens* for 20 minutes after exposing to *Allium sativum* extract for 3 hours.

Duration of extreme fight

Duration of extreme fight decreases as the concentration of *Allium sativum* extract increases from 0mL, control (1.41±0.01) to 4mL (0.00±0.00). Data was collected by observing the character for 20 minutes and data collected is represented in the form of mean and standard deviation in Table 3 and a line graph (figure 3) is plotted to show the change using the mean values.

Table 3: Duration of extreme fight shown by male *Betta splendens* fish for 20 minutes**.** Data obtained as a result of triplicate analysis expressed as mean± S.D. All values are significant at p≤0.05.

Concentration of garlic Juice (mL)	Duration of Extreme Fight (minutes)
0mL (control)	1.41±0.01
1mL	0.72±0.01
2mL	0.28±0.01
3mL	0.00±0.00
4mL	0.00±0.00

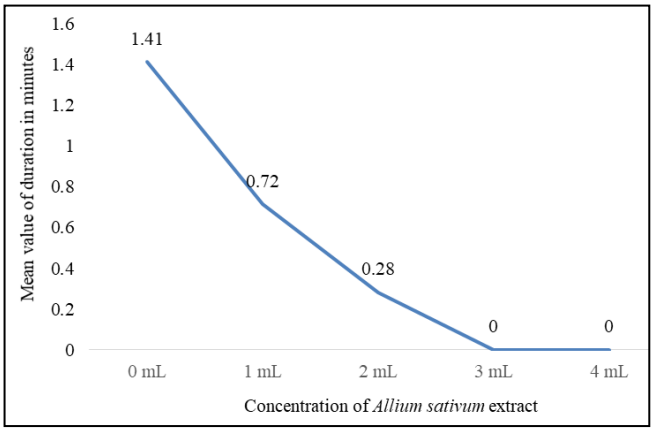

Fig 3: Graph representing duration of extreme fight exhibited by *Betta splendens* for 20 minutes after exposing to *Allium sativum* extract for 3 hours.

Number of opercular flaring

Number of opercular flaring decrease as the concentration of *Allium sativum* extract increases from 0mL, control (21.75±0.96) to 4mL (4.25±0.956). Data was collected by observing the character for 20 minutes and data collected is represented in the form of mean and standard deviation in Table 4 and a line graph (figure 4) is plotted to show the change using the mean values.

Table 4: Number of opercular flaring shown by male *Betta splendens* fish for 20 minutes. Data obtained as a result of triplicate analysis expressed as mean± S.D. All values are significant at p≤0.05.

Concentration of garlic Juice (mL)	Number of Opercular Flaring
0mL (control)	21.75±0.96
1mL	19.00±0.82
2mL	14.25±0.96
3mL	7.75±0.96
4mL	4.25±0.96

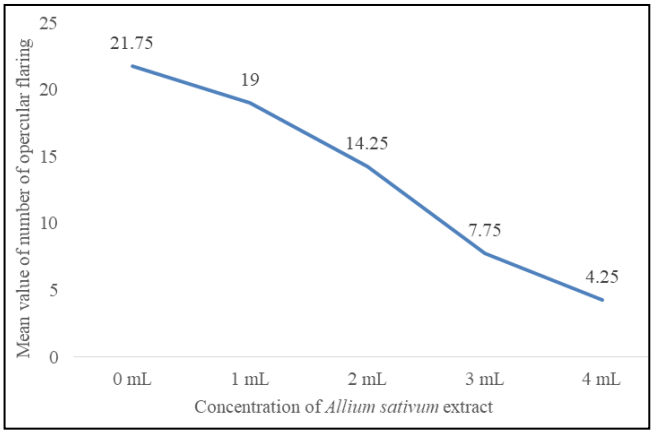

Fig 4: Graph representing number of opercular flaring exhibited by *Betta splendens* for 20 minutes after exposing to *Allium sativum* extract for 3 hours

Discussion

The current study was aimed to evaluate the change in aggressive behavior of *Betta splendens* in *Allium sativum* extract. This study shows that realistic environmental concentrations of *Allium sativum* could be affecting the aggressiveness of the adult male *Betta* fish that is aggressiveness of the fish is significantly reduced and shows decrease in the aggressive behavior as the concentration of *Allium sativum* is being increased. During the experimental period, the male *Betta* fishes had shown aggressive nature and lateral displays when placed together in bowls which are transparent. These observations were similar to the reports of Forsatkar *et al*., 2016 [15], where they mentioned that displays often start with low-level aggressive behaviors, that is, fin flaring, which can develop into more aggressive actions, that is, opercular flaring and biting. The observations also confirmed that opercular flaring and lateral displays are the most common aggressive displays in male-male interactions.

When *Betta* fishes were first placed together in the tank they avoid each other for some time before fighting each other and showing the lateral display. The duration of avoidance was having least value in 0mL, which is the control (10.28±0.04mins), where there was no *Allium sativum* extract. The value of duration of avoidance which is measured in minutes increases significantly as the concentration of *Allium sativum* in 500mL of water for the exposure of fish for 3 hours was increased from 1mL (12.12±0.00) to 2mL (13.33±0.02) and to 3mL (19.19±0.51) and finally the highest value for 4mL (20.00±0.00). This shows that when the concentration of *Allium sativum* increases the duration of avoidance in *Betta splendens* also increases.

Lateral displays are warnings signs which include flaring their gills and spreading out their fins; this is in an attempt to make them look like a more serious threat and to give the other *Betta* a chance to leave before a fight ensues. Lateral displays are highly visual and males typically display near their opponent (Simpson, 1968). The duration of lateral display, measured in minutes, was having highest value in 0mL, which is the control (8.32±0.01mins), where there was no *Allium sativum* extract. The value of duration of lateral display decreases significantly as the concentration of *Allium sativum* in 500mL of water for the exposure of fish for 3 hours was increased from 1mL (7.17±0.01mins) to 2mL (6.44±0.01mins) and to 3mL (1.04±0.01mins) and finally the least value for 4mL (0.00±0.00). *Betta* fishes show no lateral display or no aggressive character at 4mL. This shows that as the concentration of *Allium sativum* increases the duration of lateral display of fish decreases.

After giving the warning signs that is the later displays, *Betta splendens* fight each other. They will start to chase each other and bite the fins and tails. This will continue until one fish die. As the observation was for 20mins, fish were taken out before dying. Duration of extreme fight decreases simultaneously as the concentration of *Allium sativum* extract was increased in 500mL of water, where the fish has been exposed for 3 hours. The duration of extreme fight, measured in minutes, was having highest value in 0mL, which is the control (1.41±0.01mins), where there was no *Allium sativum* extract. The value of duration of extreme fight decreases significantly as the concentration of *Allium sativum* in 500mL of water for the exposure of fish for 3 hours was increased from 1mL (0.72±0.01mins) to 2mL (0.28±0.01mins) and finally the least value for 3mL (0.00±0.00mins) and 4mL (0.00±0.00). *Betta* fishes show no extreme fights or no highest aggressive character at 3mL and 4mL. This shows that as the concentration of *Allium sativum* increases the duration of extreme fight of fish decreases.

The duration of opercular flaring relates to a condition of

individual and has been demonstrated to be a reliable predictor of the winner of a male-male aggressive interaction (Simpson, 1968). Number of opercular flaring decreases simultaneously as the concentration of *Allium sativum* extract was increased in 500mL of water, where the fish has been exposed for 3 hours. Number of opercular flaring was having highest value in 0mL, which is the control (21.75±0.96), where there was no *Allium sativum* extract. The value of opercular flaring decreases significantly as the concentration of *Allium sativum* in 500mL of water for the exposure of fish for 3 hours was decreased from 1mL (19.00±0.82) to 2mL (14.25±0.96) and to 3mL (7.75±0.96) and finally the least value for 4mL (4.25±0.96). This shows that as the concentration of garlic increases the duration of extreme fight of fish decreases.

In the present study it is observed as that blue-finned males being most aggressive, while red-finned males were the least aggressive. Similar results were made by Simpson (1968) also demonstrated that blue males displayed more readily and attacked more frequently than different colored males when shown a mirror or puppet. Also it is reported that, fishes of different fin colour when put together show no aggressive behavior. During the study, it was also observed that males with large body size are more aggressive than the others. This is agreeable to the record that larger and heavier males tend to be stronger and more aggressive in many species leading to dominance (Arnott and Elwood, 2009: Portugal *et al*., 2017) [4, 3].

Conclusion

The present study demonstrated that exposure of *B. splendens* to the *Allium sativum* extract has significantly reduced the aggressive behavior of the fish as *Allium sativum* has stimulatory effect on the brain serotonin level of *Betta splendens.* The conclusion was made by analyzing the characters like duration of avoidance, which was increased by increase in the concentration of *Allium sativum* extract and the duration of lateral display, duration of extreme fight and number of opercular flaring which were decreased by the increase in concentration of *Allium sativum* extract.

Acknowledgement

Author's sincere thanks to all teaching and non-teaching staffs of Department of Zoology (PG/UA), PSG College of Arts and Science, Coimbatore, Tamil Nadu, India.

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