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PERFORMANCE AND FEEDING RESPONSE OF *TOR PUTITORA* TO FORMULATED DIET IN RELATION TO TEMPERATURE UNDER CAPTIVE CONDITIONS

Abstract.- Mahaseer, *Tor putitora* collected from Nala Gumraa, near Bara Kahu, Islamabad and fed on feed used in Nepal for commercial fish farming, showed positive growth response. However, fish did not feed when the temperature was below 16°C. Fish lost body weight during this period. The survival rate was 81%, which showed that fish can be reared in fish farm.

Key words: Mahaseer, *Tor putitora*, feeding response, formulated diet.

Aquaculture is an efficient mean of animal protein production through extensive and intensive systems (Edward, *Aquaculture Asia,* **2**: 4-7, 1997). Fish farming is gaining popularity in private and public sector in Pakistan. There are many species which are potential candidates for use in fish farming. One such species is Mahaseer, *Tor putitora*. This fish is inhabitant of clear shallow streams with the gravel bed all along the Himalayan foothills. Mahaseer grows to a large size and ranks very high as food and considered game fish for anglers (Subhan and Hafeez, *Proc. 1st Symp. Fish Fish. Pakistan*, pp. 1-13, 1991). In Pakistan mahaseer is not presently cultured and is under stress to the level of extinction in the endemic natural waters like rivers Harro, Indus and Hub in their upper reaches (Akhtar, *Sectoral study on cold water fishes in Pakistan,* FAO, Rome, 1999).

Conservation strategies and culture of a fish require information about the bahvioural response of fish such as survival at various temperature in conjunction with growth under captive conditions, acceptability of artificial diet and feed conversion and susceptibility levels to handling stress. Supplementary feeding is known to increase the carrying capacity of culture system and can enhance fish production by many folds (Hepher, *Proc. 9th Int. Congr. Nutr.* (Mexico 1972), vol. 3, pp. 183-198, 1976; Devaraj, *Proc. 9th Int. Congr. Nutr.* (Mexico 1972), vol. 3, pp. 131-135, 1976). However, in Pakistan the use of supplementary feed is very limited.

The fish diets are usually rich in protein and vitamins and hence are expensive. The cost of feed in culture system may comprise upto 50% or more of total cost depending on the level of farming (Collius and Delmondo, In: *Advances in aquaculture* (eds. T.V.R. Rillay and W.A. Dill). Fishing News Books, Farnham, Surrey, England, pp. 472-477, 1979). Therefore, the quality and quantity of fish feed is the major factor determining profitability of fish farming because feed itself represent single largest expenditure in farming (Devaraj and Seenappa, *Optimum dietary protein and protein to energy ratio for* Catla *studies on the nutritional requirements and feed formulation for cultivable carps*. University of Agricultural Sciences Hable, Banglore, India, pp. 8-69, 1991). Protein requirement of the fish also varies from species to species, even in the same species at different stages of life. Protein is obtained either from animal or from plants sources. The protein requirement in the diet for maximum growth of fish lies between 35-50% depending on the genetic characteristic of the fish (Tocan and Jackson, In: *Nutrition and feeding in fish* (eds. C.B. Cowey, A.M. Mackie and J.G. Bell), Academic Press, London, UK, pp. 119-145, 1985).

Mahaseer (*Tor putitora*) is being cultured commercially in Nepal (Pantha, In: *Farm made aquafeed* (eds. M.B. New, A.G.J. Tacon and I. Csavas), Regional Expert Consultation on Farm Made Aquafeed (Thailand 14-18 December, 1992), pp. 297-316, 1993) and attempts have been made in India and Bangladesh to improve the culture practices for sustainable yield (Haque *et al*., *J. Asiat. Soc. Bangladesh Sci.*, **22**: 279-282, 1995). The present study is the first attempt to collect young fish from wild and study the survival, growth and acceptability of artificial diet under captive conditions. The present study was conducted to (i) determine the survival, adaptability and maintenance of *T. putitora,* collected from wild,

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during transportation, (ii) explore the availability of feed ingredients for preparing *Tor putitora* diet and feed formulation with desired protein level, and (iii) determine the feeding behaviour of the fish to test diet particularly in relation to temperature.

Materials and methods

The present study was conducted at NARC in Aquaculture and Fisheries Research Institute (AFRI) during September to December, 1999.

Fish for the present study were collected from the Nala Gumraa, a tributary of Korang River, a small river originating from hilly areas of Murree and Islamabad. Five sampling trips were made to collect the specimen by using cast net of mesh size 2.5 cm^2. The fish were collected from a stretch of about 10 Km in the stream starting from villages Bara Kahu, moving toward Pind Bagwal and culminating at Shah Nara. The fish were transported to the study site in thermopore fish holds. One hundred and twenty fish were caught during 5 trips. Thirty five fish died during transportation. The fishes hauling was done at temperature 25-27°C and the hauling distance was about 30 Km.

The fish were kept in five flow-through concrete raceways tank, each of size 23.3 x 2.7 x 2.4 cubic feet for 30 days for acclimation. The water quality was studied and water was partially changed after every 3 days. Fish were fed on the artificial diet to habituate the fish for formulated diet. Each fish were tagged by using anchor floy-tag. Fishes were weighed by using electric balance Model No. EP-12 Ka (Yagami International Japan) and four groups in accordance with the body size were identified: Group A, 30-40 g; Group B, 40-50 g; Group C, 50-60 g and Group D, 60-70 g.

During this period the temperature, pH and conductivity were measured with the help of digital thermometer, pH meter and conductivity meter, respectively. The water of each raceway was changed twice a week.

A feed used in Nepal for Mahaseer culture was selected as test diet (Pantha, In: *Farm made aquafeed* (eds. M.B. New, A.G.J. Tacon and I. Csavas), Regional Expert Consultation on Farm Made Aquafeed (Thailand, 14-18 December, 1992), pp. 297-316, 1993). The feed was prepared by mixing soybean meal 35%, mustard oil cake 20%, fish meal 12%, wheat flour, 12%, vitamin premix 10%, maize flour 10%, rice polish 1%. This feed is generally used in Nepal for commercial fish farming. The feed ingredients were grinded, by using the electric grinder, weighed separately and mixed together in required quantity to get the desired level of protein. The feed was pelleted using the ordinary electric mixer and feed was dried. Proximate analysis of test diet was done by Feed Testing Laboratory of Poultry Research Institute Government of Punjab, Rawalpindi. Fish were fed on the test diet for thirty days. Fortnightly result were recorded.

ANOVA was used to determine the significance of the result.

Results and discussion

The transportation of fish from wild conditions to field was studied and it was found that in five different haults, the average survival rate during all five trips was about 73%. *T. putitora* was found in waters of transitional temperature and was sensitive to handling stress (Akhtar, *Sectoral study on cold water fishes in Pakistan,* FAO, Rome, 1999). The present result confirm that the fish requires proper post-harvest handling and transportation to avoid mass scale mortality. The adaptability of fish under captive conditions during the study indicated that the survival rate of the fish was independent of body size.

During the experimental period temperature ranged from 8-16°C, while pH ranged between 8 and 9 which is suitable for fish growth.

The result of proximate analysis were, dry matter 90%, moisture 10%, crude protein 27%, crude fat 9.5%, crude fibre 4.5%, ash 10% and aflatoxin 19 ppb. According to Pantha's formula, the percentage of protein was 30% (Pantha, In: *Farm made aquafeed* (eds. M.B. New, A.G.J. Tacon and I. Csavas), Regional Expert Consultation on farm made aqufeed (Thailand 14-18 December, 1992): 297-316, 1993).

Figure 1 sows growth pattern in four weight groups: A, 30-40 g, B, 40-50 g; C, 50-60 g and D, 60-70 g. A and B group showed no mortality whereas one mortality in Group C and two in Group D were recorded.

During the feeding trials mortality rate was 8%. There was maximum mortality in high weight group. It was noted that there is positive relationship between the size and mortality. No study has been done earlier to corroborate or negate these findings.

Fig. 1. Growth pattern of four groups of *Tor putitora* after 30 days of feeding special diet.

Fig. 2. Comparison in weight loss among 4 groups of *Tor putitora*.

There was continuous decrease in weight in each group, which showed the negative growth of the fish at temperatures below 16°C. The average weight loss in each group is shown in Figure 1. The average weight loss in group A, B, C and D after 15 days was 1.89 g, 3.69 g, 2.57 g and 2.86 g, respectively. The average weight loss after 30 days in-group A, B, C and D was 3.77 g, 4.67 g, 6.05 g and 6.52 g, respectively (Fig. 2).

The fish in their normal environment usually undergo protected periods of starvation. The starvation in fish generally known as warm water species, corroborate with low temperature (Javaid, *Some factors affecting the temperature selection of three species of Salmonids*. Ph.D. thesis, Carleton, Univ. Ottawa, Canada, pp. 2-7, 1967). During this period various physiological parameters of the animals undergo pronounced changes. In carps as the temperature reached below 10°C, activity level decreased, feeding stopped (Bailey, *Water quality chemistry*; Fish Farming Manual for Pakistan. Agricultural Development Bank of Pakistan, pp. 69-72, 1985; Ali, *Feed recommended for important culturable species.* Freshwater Fishery Biology. Nasim Book Depot, Hyderabad, pp. 277-279, 1999). For routine metabolic activity that is sustainability of the fish, body resources start breaking (particularly fats starts disintegrating) which cause loss of weight. This implication is also evident in fish farming in Pakistan where low temperature causes cessation of feeding activity in warm water fish. *T. putitora* is also a semi-cold water carp which prefers a range of temperature between 18-30°C (Akhtar, *Aquatic biodiversity.* Pakistan National Report on the Implementation of the Convention on Biological Diversity, Ministry of Environment, Local Govt. and Rural Development, Government of Pakistan, pp. 51-59, 1991).

It is concluded that *T. putitora* does not feed at low temperature (below 16°C), though the it accepts formulated feed at higher temperature.

Department of Biological Sciences, Quaid-i-Azam University, Islamabad (AN) And Aquaculture and Fisheries Research Institute, National Agricultural Research Centre, Islamabad (MZ, NA, AR, MA), Pakistan

A. NAZIR
N. AKHTAR
M. ZAFAR
M. AFZAL
A. RAB

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ALTERED SENSITIVITY OF *STAPHYLOCOCCUS AUREUS* TO STREPTOMYCIN AFTER CONTINUOUS EXPOSURE TO A HOMEOPATHIC DRUG ECHINACEA

Abstract.- The growth of *Staphylococcus aureus,* exposed to homeopathic drug Echinacea at concentrations of 45.45, 4.45, 0.45 µg/ml was inhibited 60%, 46% and 25%, respectively. Echinacea in 6, 30, 200 and 1000 potencies produced almost negligible inhibition, whereas Streptomycin at 45.45 µg/ml concentration produced an inhibition of 93%. When *Staphylococcus aureus* was exposed to Echinacea at a concentration of 454 µg/ml for 24, 48 and 72 hours, growth inhibition of 82, 70 and 75% was observed, respectively, whereas streptomycin produced 83.4% inhibition.

Keywords: Sensitivity, *Staphylococcus aureus*, Echinacea, antibiotic resistance, streptomycin.

Staphylococcus aureus causes boils, abscess, wound infection, food poisoning in man and mastitis in cattle (Burows, *Text book of microbiology,* W.B. Saunders, London, 1963; Pelczar *et al*., *Microbiology concepts and applications,* McGraw Hill, New York, 1993). Certain *S. aureus* strains become resistant to a number of antibiotics (Sanjeev and Iyer, *Fish Technol*., **25**: 139-141, 1988) and these resistant strains cause severe health problems in general and in hospitals in particular (Ohta *et al*., *Bot. Mar*., **37**: 561-566, 1994). Thus it becomes essential to search for such agents which show antibacterial action against these resistant bacteria. Decano *et al*. (*J. Appl. Phycol.,* **2**: 79-81, 1990) reported antibacterial action of a compound extracted from *Nostoc*. Choi *et al*. (*Bull. Korean Fish Soc.,* **233**: 297-302, 1990) reported inhibition in growth of a number of bacteria using 50 ppm grape fruit seed extracts. Hanafy and Hatem (*J. Ethnopharm*., **34**: 275-278, 1991) and Mirza *et al*. (*Biologia*, **45**: 61-67, 1999) reported the inhibition of *S. aureus* by ethanol extract of *Nigella sativa*.

Echinacea a homeopathic drug, is used for treating low typhoid conditions, diphtheria, malignant scarlatina, carbuncles, boils and snake bites (Clarke, *A dictionary of practical materia medica*, vol. 1, Medical Book Center, Urdu Bazar, Lahore, 1990). It is a popular herbal supplement with anti-inflammatory and antibiotic properties, it is also effective against flu and cold viruses (Rembert, *Herbs to the rescue,* the Environmental (Jan./Feb.), 1998). Polysaccharide extracted from plant cell culture of *Echinacea purpurae* enhances the resistance of immunosuppressed mice against systemic infections with *Candida albican* and *Listeria monocytogenes* (Steinmeuller *et al*., *Immunopharmacol.,* **7**: 37-40, 1993). Aqueous extracts of the roots of *E. augustifolia* showed anti-inflammatory activity (Tragni *et al*., *Pharmacol. Res. Commun.,* **20**: 87-90, 1989). In the present study efforts were made to determine whether *S. aureus* showed any resistance on continuous exposure to Echinacea Q or not.

Materials and methods

Staphylococcus aureus obtained from King Edward Medical College, Lahore were maintained on Staphylococcus selective agar no. 110 at 37°C (Collins *et al*., *Collins and Lyne's microbiological methods,* Butterworths, London, 1989).

For studying the effect of Echinacea Q in concentrations of 45.40, 4.54, 0.45 µg/ml, and potencies 6, 30, 200 and 1000 on *S. aureus* under aseptic conditions, 5 ml of 18 hours old inoculum, with OD adjusted at 0.005 at 600 nm by adding sterilized nutrient broth, was added in sterilized test tube and then 0.5 ml of each concentration of Echinacea Q (45.40 µg/ml, 4.54 µg/ml, 0.45 µg/ml) and potencies 6, 30, 200 and 1000 was added separately and the optical density and viable count were determined at an interval of one hour upto a period of five hours (Seeley and Van Demark, *Microbes in action, laboratory manual of microbiology*, W.H. Freeman, San Francisco, 1981). All the samples were taken in triplicate.

For studying the continuous effect of Echinacea Q (454.5 µg/ml), 0.5 ml of inoculum and 0.5 ml of Echinacea were added to three sets of test tubes each having 10 ml of sterilized nutrient broth, at 37°C for 24, 48 and 72 hours, respectively, in duplicate. The drug-exposed bacteria were streaked

Table I.- Effect of Echinacea and streptomycin on the growth of *Staphylococcus aureus,* expressed as optical density and viable count (Mean±SEM)

Drug	Initial O.D.	Final O.D. after 5 hours	*Initial counts*	Final count after 5 hours
Normal	0.007±0.0001	0.101±0.0032	$1.5x10^7$±0.064	$7.5x10^7$±0.068
Control (70% ethanol treated)	0.006±0.0002	0.054±0.0028	$1.4x10^7$±0.160	$6.3x10^7$±0.491
Echinacea Q (45.45 µg/ml)	0.005±0.0004	0.021±0.0016	$1.3x10^7$±0.080	$2.5x10^7$±1.120
Echinacea Q (4.54 µg/ml)	0.008±0.0001	0.029±0.0016	$1.1x10^7$±0.329	$3.4x10^7$±0.040
Echinacea Q (0.45 µg/ml)	0.006±0.0005	0.042±0.0001	$1.4x10^7$±0.800	$4.7x10^7$±0.410
Echinacea 6	0.006±0.0008	0.049±0.0002	$1.4x10^7$±0.027	$5.8x10^7$±0.224
Echinacea 30	0.007±0.0003	0.051±0.0002	$1.3x10^7$±0.080	$6.2x10^7$±0.482
Echinacea 200	0.007±0.0001	0.053±0.0004	$1.4x10^7$±0.632	$6.0x10^7$±0.081
Echinacea 1000	0.007±0.0002	0.053±0.0004	$1.3x10^7$±0.329	$6.3x10^7$±1.408
Streptomycin (45.4 µg/ml)	0.006±0.0001	0.009±0.0048	$1.7x10^7$±0.239	$0.4x10^7$±0.248

Table II.- Effect of Echinacea Q and streptomycin on the growth of previously exposed *Staphylococcus aureus* to Echinacea Q for 24, 48 and 72 hours, expressed as optical density and viable count (Mean±SEM).

Drug treatment	Initial O.D.	Final O.D. after 5 hours	Initial count	Final count after 5 hours
Normal	0.007±0.004	0.110±0.004	$1.5x10^7$±0.012	$9.0x10^7$±0.163
Control (70% ethanol treated)	0.006±0.001	0.055±0.004	$1.4x10^7$±0.081	$8.0x10^7$±0.209
Echinacea Q (454 µg/ml) 24 hrs exposure	0.005±0.003	0.010±0.003	$1.3x10^7$±0.020	$2.1x10^7$±1.800
Echinacea Q (454 µg/ml) 48 hrs exposure	0.005±0.001	0.013±0.008	$1.1x10^7$±0.012	$2.5x10^7$±0.081
Echinacea Q (454 µg/ml) 72 hrs exposure	0.006±0.002	0.014±0.008	$1.4x10^7$±0.013	$2.8x10^7$±0.1.22
Streptomycin (45.4 µg/ml)	0.006±0.002	0.008±0.008	$1.7x10^7$±0.013	$1.3x10^7$±0.150

onto the nutrient agar for making subcultures of the resistant bacteria if any. A colony of *S. aureus* exposed to Echinacea Q (454.5 µg/ml) was taken from these subcultures respectively and transferred to the sterilized nutrient broth and incubated for 18 hours at 37°C. Sterilized nutrient broth was added in 18 hours old inoculum to adjust the optical density to 0.005 at 600 nm. Then 5 ml of this sample was taken and 0.5 ml of Echinacea Q (454.5 µg/ml) was added then optical densities and were determined (Seeley and VanDenmark, *Microbes in action, Laboratory manual of microbiology*, Freeman, San Francisco, 1981). For comparative studies the normal (without any treatment) and the control (treated with 70% alcohol, as homeopathic drugs are prepared in 70% ethanol) and Streptomycin treated samples were also studied. All samples were taken in triplicate.

The results were processed for statistical analysis to work out mean, standard deviation, standard error of mean and Student's t test (Saunders and Fleming, *Mathematics and statistics*, William Clows, London, 1971).

Results

The inhibitory action of the Echinacea observed as optical density produced 61, 46.2, 22, 9, 6, 1.85 and 1.85% in 45.40, 4.54, 0.45 µg/ml of Echinacea Q and potencies 6, 30, 200 and 1000, respectively. However, Streptomycin produced 83% inhibition in 45.40 µg/ml concentration when compared with control (Table I).

Considering viable count Echinacea Q at above concentrations and potencies 6, 30, 200 and 1000 produced respectively 60, 46, 25, 8, 1.6, 4.8 and 3% inhibition. Streptomycin however, produced 92.6% inhibition (Table I).

S. aureus when exposed to Echinacea Q (454 µg/ml) for 24 hours and later was exposed to the same concentration and the O.D. reduced 82%,

while the 48 and 72 hours cultures showed 76 and 75% reduction, respectively. Streptomycin (45.40 µg/ml) showed 83% reduction in growth (Table II). The viable count were reduced 75, 69 and 65% in 24, 48 and 72 hours exposed *S. aureus*. Streptomycin (45.40 µg/ml), on the other hand, showed 84% reduction (Table II).

Discussion

The present study indicates that Echinacea is a strong antimicrobial agent for *S. aureus* and the most effective potency of Echinacea is Q concentration of 45.40 µg/ml. However, this concentration of Echinacea is less effective than Streptomycin used at the same concentration.

Longer exposure of bacteria to the drug Echinacea Q showed that *S. aureus* developed resistance after exposure to drug for at least 48 hours. On the other hand *S. aureus* showed no resistance to Streptomycin. *E. purpurae* and *E. augustifolai* contains glycine betaine which is a quaternary ammonium compound and were found to be effective against Gram positive bacteria (Soicke *et al*., *Fitoterapia*, **59**: 3-5, 1988) but Ross (*Introductory microbiology,* Howell, London, 1983) was of the view that the quaternary ammonium compounds are the surface-active agents and do not mix well and may not be able to penetrate the clusters of the cells to kill those inside. Rembert (Herbs to the rescue. *The Environmental* (Jan./Feb.), 1998) reported that Echinacea fights infection by increasing phagocytosis. Egert and Beuschar (*Planta Med*., **58**: 163-165, 1992) reported an immunoreactive antigen from its extracts while Elsasser *et al*. (*J. Clin. Lab. Anal*., **10**: 441-445, 1996) reported the production of cytokine from leukocytes culture after Echinacea extract treatment.

Thus it may be assumed that the inhibition shown by Echinacea may be due to the presence of quaternary ammonium compound. It can also be assumed that this compound may have a bacteriostatic action as the bacteria start developing again resulting in an increased growth rate.

The present studies showed that Echinacea can be used as an effective anti-microbial agent but its continued use can produce resistance like that of antibiotics. However, this resistance can be removed by using different potencies of the drug.

Department of Zoology,
Government College University
Lahore

FAUZIA TANVIR
SHAZIA TANVIR
M. ASHRAF MIRZA
AZIZULLAH

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RESISTANCE OF *AEROMONAS* SPP. IN THE FISH, *CATLA CATLA*, AGAINST SOME ANTIBACTERIAL AGENTS

Abstract.- Studies were carried out to determine the present status of *Aeromonas* spp. in kidney and slime of *Catla catla* in pond water and their sensitivity patterns against various antibacterial agents. *Aeromonas* Agar Base supplemented with C.F.C.SR 103 (Oxoid) was used as a selective medium for the isolation of *Aeromonas* spp. A number of antibacterial agents including some common antibiotics were tested against the isolated *Aeromonas* spp in the pond water; kidney and slime, *Catla catla*. The number of *Aeromonas* spp varied from $4.3x10^2$ to $9.5x10^1$ CFU/ml. $4.5x10^2$ to $8.9x10^1$ CFU/g and $5.7x\ 10^2$ to $9.9x\ 10^1$ CFU/ml in pond water, kidney and slime, respectively, whereas total load of bacteria varied $2.9x10^6$ to $1.7x\ 10^4$ CFU/ml, $3.8x10^4$ to $2.7x10^3$ CFU/g and $6.1x10^7$ to $3.1x10^6$ CFU/g in pond water, kidney and slime, respectively. The antibacterial agents, used to find .out the resistance patterns were oxolinic acid (2 µg/disc), potentiated sulphonamides (25 µg/disc), chloramphenicol (10 µg/disc), streptomycin (10 µg/disc), oxytetracycline (30 µg/disc) and erythromycin (10 µg/disc). The highest resistance was found for oxytetracycline and erythromycin.

Key words: Antibiotic resistance, *Aeromonas* sp., *Catla catla*.

Fish is the most important export item of Bangladesh which contributes about 9.12% of the foreign earning and shares 4.12% of GDP and 80% of animal protein intake. Moreover, fishing is an important economic activity both in monetary and nutritional point of view. More than eleven million people live directly or indirectly on fishery. Because

of increasing fish demand for human consumption and decreasing production of fish in natural water, fish farming and the fish production from culture fisheries has become popular and has already increased from 1.23 MT (1984-85) to 3.90 MT (1995-96) (Nuruzzaman, *Prospective on fisheries development in Bangladesh.* Bangladesh Agricultural Research Council (BARC), 198 pp., 1990).

However, recently fish disease, especially microbial fish disease outbreak has been one of the major barriers for the production of fish in the country. Chowdhury (Research priorities for microbial fish disease and its control in Bangladesh. In: *Proceedings of the workshop on research priorities in Bangladesh for fish health, disease prevention and pathology*, 17th May, edited by Alan Tollervey, 811, 1993) in an investigation of fish farm by spot observation suspected that many farmed fishes were suffering from diseases like Septicemia, Columnaris etc. caused by bacterial pathogens. But research on bacterial diseases in our country is in a rudimentary stage. As a result the farmers do not take any action against diseases. Sometimes they use different chemicals and antibiotics to control these diseases without any recognized doses and thus making bacteria/ pathogens resistant.

In case of fish farming, species selection is an important factor influencing sustainable yield. There are many indigenous commercial species in our country, among them catla *(Catla catla)* is very popular cultivable fish for its taste, fast growth and market value. This species is known to be more susceptible to various bacterial diseases. So attention has been paid to the investigation for the resistance pattern of this fish.

Materials and methods

Samples for the study

Five carp polyculture ponds were selected under Dhaka Fisheries Ltd. in Gazipur district for the sampling of pond water and the fish *Catla catla.* The fish was 50g to 100 g in weight. The ponds under study were designated Pond-I, Pond-2, Pond-3, Pond-4 and Pond-5. Samples were brought to the Fish Disease Laboratory under the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh.

Culture media

A selective medium, Aeromonas Agar Base supplemented with C-F-C SR 103 (Oxoid) was found suitable for the isolation of *Aeromonas sp.* both from fish organs and pond water (by preliminary test) and used for the present study. However, for other purpose such as total bacterial counting, transfer of bacteria, Tryptone Soya Agar (TSA, Oxoid) was used. For antibiotic sensitivity test, Isosensitest Agar (Oxoid) was used.

Antibacterial agents

In the present study six antibacterial agents were used. They were oxolinic acid (2 µg/disc), potential sulphonamides (25 µg/disc), chloramphenicol (10 µg/disc), streptomycin (10 µg/disc), oxytetracycline (30 µg/disc) and erythromycin (10 µg/disc). All the antibacterial agents were prepared by Oxoid Ltd.

Determination of bacterial content

The number of bacteria in pond water, kidney and slime of the fish were determined by standard plate count (spreading) method. Total number of bacteria was determined on TSA. *Aeromonas* spp. were determined on Aeromonas Agar Base.

Sensitivity test of Aeromonas *sp. against various antibacterial agents*

From the Aeromonas Agar Base medium, a number of well developed colonies were randomly selected to observe the sensitivity pattern of various antibacterial agents of *Aeromonas* spp. using separate vials. Suspensions were prepared for each of the isolates in physiological saline and 0.1 ml of the solution was transferred to the ISO-Sensi-Test agar plates and spread with the sterile "L" shaped glass rod. Six antibacterial discs were dispensed on the inoculated plates by placing the Oxoid Unipath Disc Dispenser Mark-II and incubated at a temperature of 25°C for 24 hours. Sensitivity was recorded according to the normal growth response. Resistant (R) zone was recognized when the growth was normal and when the clear zone was observed surrounding the disc, was recognized as a sensitive one by measuring the zone of inhibition in mm. However, zone of confusing growth around the discs were noted as ±.

Table I.- Total bacterial content and total number of *Aeromonas* spp.in the pond water, kidney and slime of sampled *Catla catla*

Fish Pond	Water (CFU/ml)		Kidney (CFU/g)		Slime (CFU/g)	
	Total number of bacteria	Number of *Aeromonas* spp.	Total number of bacteria	Number of *Aeromonas* spp.	Total number of bacteria	Number of *Aeromonas* spp.
Pond-l	$3.5x10^5$	$9.lx10^2$	$1.6x10^4$	$3.7x10^2$	$9.5x10^6$	$3.lx10^4$
Pond –2	$2.6x10^6$	$3.4xl0^3$	$2.7x10^3$	$6.lx10^2$	$6.lx10^7$	$3.2x10^3$
Pond –3	$3.lxl0^5$	$4.lxl0^4$	$2.lx10^4$	$3.5xl0^3$	$3.lxl0^6$	$5.9x10^2$
Pond –4	$1.7xl0^4$	$3.lxl0^3$	$3.8xI0^4$	$l.lxl0^2$	$5.lx10^7$	$3.lx10^3$
Pond-5	$2.9xl0^6$	$7.4xl0^4$	$5.lx10^3$	$2.9x\ 10^2$	$3.8xl0^6$	$4.2x10^3$

Table II. Sensitivity patterns of *Aeromonas* spp. against various antibacterial agents.

Isolates from	Response to various antibacterial agents (drug disc) with their zone of inhibition (mm)					
	OA	OT	SXT	S	C	E
Pond Water						
Pond-l	+16	R	±10.5	±08	R	+09
Pond -2	±18	R	+12	R	R	R
Pond -3	R	R	R	±17.2	+15	R
Pond -4	R	+10	R	R	R	R
Pond-5	+10	R	R	R	R	R
Kidney						
Pond-l	R	R	R	R	+16	+14
Pond -2	R	+13	R	R	R	R
Pond -3	+11	R	+15.1	+15.1	R	R
Pond -4	+15.5	R	R	R	R	R
Pond-5	R	R	+19	+17.2	+06	R
Slime						
Pond-l	R	R	+16.5	R	+12.4	R
Pond -2	+15	R	±17	±08	R	R
Pond -3	±09	R	R	±11	±13	R
Pond -4	R	R	R	R	R	R
Pond-5	R	R	+12	+14.3	R	+10

±, Confusing zone; C, Chloramphenical; E, Erythromycin; OA, Oxolonic acid; OT, Oxytetracycline; R, Resistant; S, Streptomycin; SXT, Potentiated sulphonamides.

Results and discussion

Total bacterial content in the sampled pond water, fish kidney, and fish slime varied in number from $2.9x10^6$ to $1.7x\ 10^4$ CFU/ml $3.8x10^4$ to $2.7x10^3$ CFU/g and $6.1x10^7$ to $3.1x10^6$ CFU/g in pond water; kidney and slime, respectively (Table I). *Aeromonas* spp. was found in both water and fish organs (slime and kidney) of all ponds with some variation in numbers (Table I). The numbers of *Aeromonas* spp in the water of different pond water varied from $4.3x10^2$ to $9.5x10^1$ CFU/ml and those in kidney and slime $4.5x10^1$ to $8.9x10^1$ CFU/g and $5.7x10^2$ to $9.5x10^1$ CFU/g, respectively.

Sensitivity patterns of *Aeromonas* spp against various antibacterial agents were found to be highly diversified (Table II). The results of the present study revealed that pond water and fish organs carried a large number of *Aeromonas* spp., though their numbers were different in different pond water and different organs of same fish and same organ of different fish, which is similar with the finding of Chowdhury *et al.* (In: *Proc. Int. Congr. Qual. Vet. Serv. 21st Century*, pp. 8-83, 1994). A high percentage of resistant *Aeromonas* spp isolates were

detected against erythromycin and oxytetracycline. The present results agree with those of Dixon and Issvoran (*J. Wld. aquat.. Soc.,* **24**: 102-104, 1993), who studied antibacterial drug resistance in *Aeromonas* spp isolated from domestic gold fish and koi and found that more than 60% of *Aeromonas* spp were resistant to tetracycline.

The bacterial isolates showed drug resistance in multiple patterns. The results were similar with other reports (Watanabe *et al., Ann. N. Y. Acad. Sci.,* **182:** 383-410, 1971; Toranzo *et al., Appl. environ. Microbiol.,* **48**: 872-877, 1984; Aoki *et al., Fish Path.,* **20**: 199-208, 1985).

Technical School and College **M.A.R. MRIDHA**
Kushtia, Bangladesh (MARH, NTN) **N.T. NAREJO***
Department of Aquaculture, **M.S. UDDIN**
Bangladesh Agricultural University, **M.S. KABIR**
Mymensingh (MBRC, MSU, MSK), **M. KARIM**
Institute of Aquaculture, **M.B.R. CHOWDHURY**
University of Stirling, FK94LA,
Scotland, UK (MK)

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* Corresponding author: Present address: Department of Fresh Water Biology and Fisheries, University of Sindh, Jamshoro, Pakistan.

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A NEW SPECIES OF FRUIT FLIES OF GENUS *BACTROCERA* (DACINAE, TEPHRITIDAE: DIPTERA) FROM PAKISTAN

Abstract.- Identification of fruit flies has an important role in formulating IPM tools, quarantine decisions, protecting environment and conservation of biodiversity. In the present article, a new species of fruit flies of genus *Bacterocera* (Tephritidae: Diptera) from Pakistan is described.

Key Words: Fruit flies, Dacines, Tephritidae, Diptera, Pakistan

In the present study a new species of fruit flies in genus *Bactrocera* is described. The specimens were collected from Pakistan using cue lure and deposited in the Natural History Museum, London. Specimens were examined using 'Wild Herrbrugg' microscope. The classifications of Drew (*Mem. Qd. Mus*., **26**: 1-521, 1989) and terminology of McAlpine (Morphology and terminology – adults. In: *Manual of Nearctic Diptera–1* (eds. J.F. McAlpine, B.V. Peterson, G.E. Shewell, H.J. Teskey, J.R. Vockeroth and D.M. Wood), 27:9-63 Monograph of the Biosystematics Research Institute, Ottawa, 1981), White and Elson-Harris (*Fruit flies of economic significance: their identification and bionomics.* CAB International, UK pp. 1-601, 1992) was followed.

Bactrocera (Zeugodacus) kaghanae, new species (Fig.1)

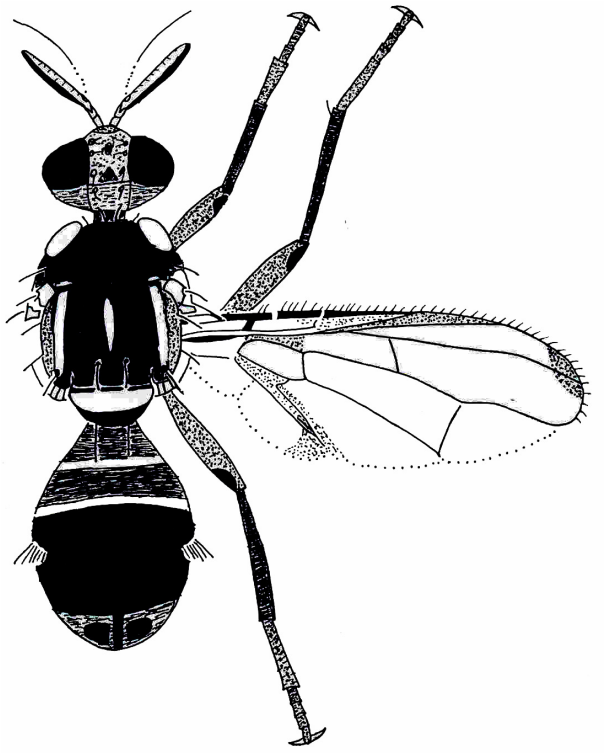

Fig. 1. *Bactrocera (Zeugodacus) kaghanae,* new species

Description

Head

First flagellomere shorter than ptilinal fissure. Face with a black spot in each antennal furrow. Frontal setae three pairs.

Thorax

Tomentum pattern with wide longitudinal gap in the middle of prescutum. Scutum colour (other than vittae) black. Postpronotal lob yellow. Notopleuron variable from yellow black to black. Notopleural vitta absent. Lateral vitta of scutum present, yellow, ending in front of intra alar seta. Medial vitta of scutum present, yellow. Katatergite yellow. Anatergite yellow Scutellum yellow with black mark on apex. Postpronotal seta absent. Anterior supra-alar seta present. Prescutellar acrostichal seta present. Scutellar setae one pair. Anepisternal stripe extended forward to anterior notopleural seta.

Legs

All tibiae brown black. All femora yellow brown with apical black spot sometimes faded.

Wing

Wing with out any pattern (except costal band). Costal band width from vein Sc to slightly below vein R_{4+5} at wing apex. (cell sc and r_1 light brown) and a spot at apex of vein R_{4+5}. Cell bc without microtrichae. Cell c with microtrichae in the anterior apex only. Cell br with microtrichae at the base. Wing length: 6.2 – 7.0mm.

Abdomen

Abdominal tergites not fused (except I and II). Tergites markings: all tergites brown black, tergite I and II each apical transverse yellow brown band. Tergite I not wasp-waisted. Tergite III (males) with pecten. Sternite V (male) without V- shaped notch. Posterior surstylus lobe long. Male attracted to cue lure.

Material examined

Holotype 1 male Pakistan, Kaghan, 5.viii.1993. Paratypes 8 male, same data as holotype, Murree, 2 male, 3.viii.1993.

Comments

This species is different from *Bactrocera (Zeugodacus) scutellaris* Bezzi in having extended anepisternal lobe.

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Pakistan Museum Natural History **K. MAHMOOD**
Islamabad, 44000, Pakistan
Email. dkmahmood@msn.com

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THE FIRST REPORT OF THE POST-CRANIAL SKELETAL MEASUREMENTS OF WOOLLY FLY SQUIRREL, *EUPETAURUS CINERIUS*, WITH OBSERVATIONS ON ITS BEHAVIOUR

Abstract.- This paper gives the first ever record of the post cranial skeletal measurements of woolly flying squirrel *Eupetaurus cinereus* collected in June, 2002. Observations on its ecology, state of habitat, food and behaviour are given. Unpublished external measurements of a specimen collected earlier in 1962 are also given.

Key words: Head and body length, behaviour in captivity.

A young male Woolly Flying Squirrel, *Eupetaurus cinereus*, was collected and brought alive to the second author by a shepherd, in Gilgit city, from about 2800 m elevation above sea level, near village Shani Harai [Latitude (N): 35°-52' and Longitude (E): 74°-18'], upper reaches of Jutial stream. This small stream flows measurements of post cranial bones in literature. The specimen of which measurements are given below, was not fully mature. This was obvious when compared with external measurements of six specimens collected by Zahler and Woods (In: *Biodiversity of Pakistan* (eds. S.A. Mufti, C.W. Woods and S.A. Hassan),

Pakistan Museum of Natural History, Islamabad and Florida Museum of Natural History, Gainesville, FL, USA, 1997). After taking the measurement in millimeters, the specimen was mounted and its skeleton was articulated for display.

Live weight

1.5 kg (soon after it was taken from the shepherd on 19th June 2002).

Head and body

360, tail; 500, ear; 50, hind foot; 85, hair length; 60 on back and 80 on tail.

Skull

Total length 74.6; zygomatic width 45.4; nasal length 25.4; diastema 15.6; palate length 27.0; tympanic bulla 114.0; maxillary tooth-row 20.1; width at post orbital processes of frontal 30.9; frontal width at superorbital notches 16.5; pterygoid processes are long and reach the one fourth of tympanic bulla in front side; hyoid bones: anterior cornua has ceratohyal 10.0 and stylohyal 16.0; posterior cornua flat and curved; basihyal also flat.

Skeleton

Vertebrae: Cervical 39.0; thoracic 88.0; lumbar 131.0; sacrum 40.0; caudal 469.0; pelvis length 80.0; pelvis width at anterior end 37.0; pelvis width at posterior end 38.0; clavical length 3.0; scapula length 53.0; scapula breadth 25.0; sternal length 65.0; humerus 87.0; radius 83.0; ulna 93.0; metacarpals; i. 2.0; ii. 12.0; iii. IS.0; iv. 16.0; v. 15.0; femur 106.0; tibia 115.0; fibula 111.0; calcareum 22.0; metatarsal: i. 17.0; ii. 26.0; iii. 27.0; iv. 29.0; v. 27.0. 5th metatarsal with outward projection at rear end, which is 9.2 mm long.

Food

It was observed to eat buds of Blue Pine (*Pinus wallichiana*), Juniper (*Juniperus excelsa*), Chilgoza pine (*Pinus gerardiana*), Spruce (*Picea smithiana*), Oaks (*Quercus smithiana*) and (*Q. baloot*) in the wild, usually throughout the year. However, in August and September it may invade the walnut (*Juglans regia*) trees, if growing within its usual gliding range (pers. comm. Local communities). In captivity in Gilgit city, it ate buds of Blue Pine, Spruce, Deodar (*Cedrus deodara*), Chilghoza Pine, and tender leaves of Juniper (*Juniperus*). It also ate mango (all parts of the fruit except the stone), and water melon soft parts when brought to Islamabad. Faecal pellets were round and blackish. Colour of the urine was umber- brown.

Behaviour in captivity

During the daytime it searched for dark places of the room, under the furniture or corners. It slept on its side, with upper forelimb stretched at about right angle of the body and the fingers kept open with planter pads clearly visible. Upper incisors were visible and the eyes were closed. Some times it slept on its back. When its sleeping place was made wet, it curled its tail forward under its body. Soon it started licking its wet fur. In the presence of plenty of food in the room it woke up several times to eat and slept again. While eating, it reacted to any domestic animal if came closer to few feet by erecting its fur and adopting a ball- like posture with tail erected over the back.

In Islamabad, it was kept in a soft-board carton placed in a bathroom with tiled floor. Whenever carton flaps were opened, it ran out, about six ft. away and urinated and defaecated. It strayed in the bathroom for about ten minutes and entered back in the carton. It did not urinate or defaecate inside the carton. After coming out of the carton it uttered low sound: 'Kus kus kus kus kus'. It also moved towards the observer and touched the legs with its mouth, licked for a short time and did not bite. If picked up gently it remained quiet and docile. On a grassy lawn it moved with crouched back in typical squirrel fashion.

Discussion

In 1962, the first author had collected a specimen of woolly flying squirrel from Sai Nullah. This was shot when it came out of a hole in a trunk of an old Oak tree at around 8500ft elevation. An old hunter had guided the author to this tree. The belief that this flying squirrel is rigidly adapted to caves in vertical rocks and that it has some association with salajit' was perhaps developed due to its frequent occurrence in caves in rocks. These caves are explored by 'Salajit' hunters. The second reason could be lesser number of trees with holes in

the trunks. The author found very few woodpeckers in these dry temperate forests as compared to moist temperate forests. This specimen when collected in 1962 could not be identified by the first author with the available literature at that time. He, however, knew that it was not *Petaurista* or *Hylopetes.* That specimen was larger than the present specimen. Its external measurements (in millimeters) recorded in the field notebook of the first author are: Head and body 400t; tail 555; ear 48: hind foot 92; sex: male; location: a forest patch at around 8,500 ft. in the valley of Sai Nullah, shot by the first author on the night of 18th September, 1962, The study Skin and skull remained with him, until in 1965, when he joined the Punjab University and gave these along with several other study skins of large red and Kashmir flying squirrels to the Zoology museum for storage. Its information and measurements remained unpublished.

The first author (*Mammals of West Pakistan*, Central Urdu Board, Lahore, 1969) reported its distribution based on the information published in literature at that time (Ellerman and Morrison-Scott, *Checklist of Palaearctic and Indian Mammals,* 1758 to 1946, pp. 810. British Museum (Natural History), London, 1951; Walker *et al., Mammals of the world*, vol. 2, The John Hopkins Press, Baltimore, 1964). Roberts (*Mammals of Pakistan*. Earnest Benn Limited, London, 1977; *Mammals of Pakistan* (second revised edition) Oxford University Press, Karachi, 1997), however, erroneously wrote about the first author's record of the woolly flying squirrel from Hazara district and Chilas and its misidentification by considering a 'melanic version of 'large red Flying Squirrel' *Petaurista petaurista* as woolly flying squirrel. The first author never wrote that, rather he had actually mentioned about the distribution of woolly flying squirrel in Hunza, Gilgit and Baltistan (Mirza, *Mammals of West Pakistan*. Central Urdu Board, Lahore, 1969). The first author had collected black flying Squirrels from Hazara, about which he wrote, that "among large red flying squirrels, some times, melanic individuals also appear. The young ones of these black flying squirrels are normal, like any other large red flying squirrel." The mistake in the *Mammals of Pakistan* (Roberts, *Mammals of Pakistan*. Earnest Benn Limited, London, 1977) was personally pointed out to its author. Unfortunately, this error was again repeated in the second edition of the book (Roberts, *Mammals of Pakistan* (second revised edition) Oxford University Press, Karachi, 1997).

Zahler (*J. Mammals*, **77**: 54-57, 1996) published the rediscovery of woolly flying squirrel in the absence of unpublished information about specimen collected by the first author in 1962. Rasool (*Woolly Flying Squirrel-Extinct or Alive*. Tigerpaper, 1996) published about the threat of extinction being faced by the woolly flying squirrel mainly based on the information from locals gathered during his wildlife service. Zahler and Woods (In: *Biodiversity of Pakistan* (eds. S.A. Mufti, C.W. Woods and S.A. Hassan), Pakistan Museum of Natural History, Islamabad and Florida Museum of Natural History, Gainesville, FL, USA, 1997) discussed its status in Northern Areas and wrote about the known specimens in the museums. Rasool (`Jungle ke Basi' (Urdu language) *Wildlife of Northern Areas,* 1997) summarized the available information about this species in his book. Mirza (*Pakistan J. Ornith*., **1**: 53-58, 1997) studied birds found in the habitat of Woolly Flying Squirrel, in Sai Nullah, Gilgit, Northern Areas. Fast deforestation of its habitat was photographed which resulted in the sanction of a conservation project by UNDP for this species. Mirza (*Illustrated handbook of animal biodiversity of Pakistan*. Centre for Environmental Research and Conservation, Islamabad, 1998) gave its distribution based on the latest fieldwork.

Centre for Environmental Research and Conservation, 229-B, Street 4, F-10/3, Islamabad (ZBM) and Belour Advisory and Social Development Organization, Gilgit, Pakistan (GR)

Z.B. MIRZA
GHULAM RASOOL

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