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ARTICLE

Development and Evaluation of IPM Modules for the Management of Guava Fruit Fly

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ABSTRACT

Field studies were carried out during kharif 2016 and summer 2017 at Udyanagiri, UHS, Bagalkot, Karnataka, India to evaluate IPM modules against fruit fly in an already established guava orchard of variety Sardar (L-49). Among four modules, the mean fruit damage was significantly the lowest in M3 (0.68%) followed by M2 (1.19%) and M1 (2.21%) and were on par with each other during kharif 2016. During summer 2017, M3 recorded significantly lowest damage (0.59%) followed by M2 (0.92%) and M1 (2.41%) but were on with each other. The highest per cent protection was afforded by M3 (95.76 and 96.76, respectively) during 2016 and 2017. The average fruit yield over the years of experimentation revealed significantly the highest fruit yield (8.13 t/ha) from M3 followed by M2 (7.32 t/ha) and M1 (5.31 t/ha). Among the four modules, highest B:C was from M3 (7.65) followed by M2 (6.67) and M1 (4.91).

1. Introduction

The guava, botanically known as Psidium guajava L. belongs to the family of Myrtaceae. It is one of the most common fruits grown in India. The fruits are very rich in vitamin C (100-260 mg/100 g pulp). Fruits are also rich in minerals like Calcium, Phosphorous, acidity (2.4%), carbohydrates (9-10%), total soluble sugars (13%), Vitamin A, B2 pantothenic acid, riboflavin, thiamin, niacin and pectin. In India, the total area under guava cultivation is 2.60 lakh hectares with an annual production of 38.26 lakh metric tonnes [1]. About 80 species of insects have been recorded on guava [2] and [3] affecting yield and quality of fruits. Spiraling whitefly, guava kajji bug and fruit fly are the major constraints. Among the fruit flies the Oriental fruit fly, Bactrocera dorsalis Hendel (Diptera: Tephritidae) is the most important and destructive pest associated with guava [4] and [5].

In general, fruit flies are very difficult to manage as they are polyphagous, multivoltine and adults having high mobility and fecundity. Conventionally farmers are applying various types of chemical insecticides to control. Eggs and maggots remain protected in the host tissues, pupae in the soil and thus most insecticidal applications are ineffective. The unscientific use of synthetic insecticides, besides leading to the residual problems, also results in resistance development in fruit flies, outbreak of secondary pests, undesirable effect on non target organisms and

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serious environment pollution. To manage these fruit flies, it is important to look for different eco friendly options particularly because high export potential for guava which demands production of residue free fruit this has prompted to take up this study with an objective of developing alternate methods for management of fruit flies.

2. Materials and Methods

Field studies were carried out during kharif 2016 and summer 2017 at Udyanagiri, UHS, Bagalkot to evaluate three IPM modules against fruit fly in an already established guava orchard of variety Sardar (L-49) and 6 year old over an area of one acre. Three different IPM modules along with untreated control were evaluated viz., Module I- Organics, Module II- Integrated, Module III- Recommended package of practices of [6] and Module IV- Untreated control (Table 1). The crop spacing was 6 m x 6 m. Total 15 plants were selected for each module and were replicated five times. Area for each treatment was 10 guntas. The spray was done with the help of knapsack sprayer. The insecticides were applied at flowering and fruit initiation stage, based on the ETL.

Table 1. Treatment details of IPM Modules against guava fruit fly

Modules	Treatments
M_1: Organic module	(a) Application of neem cake to soil @ 250 kg/ac (b) Spraying of neem oil @ 1% (c) Installation of fruit fly traps- methyl eugenol @ 2% and malathion 50 EC @ 1.0 ml/l (10 traps/ac) (d) Collection and destruction of affected fruits
M_2: Integrated module	(a) Raking of soil around the tree and drenching with chlorpyriphos @ 4.0 ml/l (b) Alternative bait spray (malathion 50 EC @ 2 ml + 10 g jaggery per l and Azadiracthin 10000 ppm @ 1.0 ml/l during fruiting stage (c) Installation of methyl eugenol bottle traps @ 10 traps/ac (d) Collection destruction of affected fruits
M_3: RPP-recommended POP	Spraying with dimethoate 30 EC @ 1.70 ml along with 10 g jaggery per l (UHS, POP)
M_4	Untreated control

Note: POP=Package of practices

3. Statistical Analysis

Observations on fruit damage and oviposition punctures were recorded on 7th and 14th days after treatment starting from fruit initiation stage from five plants Fruit infestation was recorded by selecting 25 fruits from each plants randomly from each treatment at each harvest based on oviposition punctures made by the fruit flies. The yield of fruit per plant was taken from all the harvests of guava. Treatment wise yield of healthy fruits was recorded at each harvest and extrapolated into t per ha. The data thus obtained were statistically analysed. Economics of treatment was worked out based on yield data, cost of treatments, net profit and Cost Benefit Ratio.

4. Results

The data obtained on efficacy of treatments in IPM modules against fruit fly in the field trial during kharif 2016 are given in Table 2 and Figure1. On 7th day after first treatment, M3 [(a. Spraying of dimethoate 30 EC @ 1.70 ml/l along with 10 g jaggery (UHS, POP)] recorded no fruit damage (0.00%). The next best treatment was M2 [a) Raking of soil around the tree and drenching with chlorpyriphos @ 4.0 ml/l b. Alternative bait spray (malathion 50 EC @ 2 ml/l + 10 g jaggery) and Azadiracthin 10000 ppm @ 1.0 ml/l during fruiting stage c. Installation of methyl eugenol water bottle traps @ 10 traps/acre d. Collection destruction of affected fruits)] which recorded significantly lowest fruit damage (0.10%) followed by M1 [(a. Application of neem

Table 2. Effect of IPM modules against guava fruit fly (2015-16)

Modules	Fruit infestation (%)								Over all mean fruit infestation (%)	Protection over control (%)
	1st treatment		2nd treatment		3rd treatment		4th treatment			
	7 DAT	14 DAT	7 DAT	14 DAT	7 DAT	14 DAT	7 DAT	14 DAT		
M_1: Organic module	0.23 (1.47)^b	1.09 (3.75)^b	0.47 (2.67)^b	0.60 (1.37)^b	1.68 (7.39)^b	2.82 (9.58)^b	4.48 (12.20)^b	6.34 (14.22)^b	2.21 (7.61)^b	86.24
M_2: Integrated module	0.10 (1.04)^b	0.40 (1.82)^b	0.51 (1.87)^b	0.58 (2.19)^b	1.32 (6.49)^b	1.50 (6.95)^c	3.82 (11.24)^b	1.32 (4.93)^c	1.19 (5.67)^b	92.59
M_3: RPP-recommended POP	0.00 (0.28)^b	0.00 (0.28)^b	0.00 (0.28)^b	0.38 (1.81)^b	0.99 (5.66)^b	1.07 (5.91)^c	1.14 (6.11)^c	1.86 (5.98)^c	0.68 (3.75)^b	95.76
M_4: Untreated control	4.24 (10.27)^a	4.02 (11.52)^a	6.86 (13.01)^a	7.66 (15.08)^a	11.20 (19.48)^a	13.50 (21.52)^a	36.20 (36.97)^a	44.93 (42.05)^a	16.07 (22.36)^a	-
S. Em ±	1.77	1.46	2.09	2.08	0.70	0.43	0.46	1.63	1.36	-
CD at 5 %	5.54	4.50	6.42	0.94	2.15	1.32	1.44	5.01	4.05	-

Notes: Means followed by same alphabet do not differ significantly (0.05) by DMRT (p=0.05)

Figures in the parenthesis are arc sine transformed values

DAT- Days after treatment

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oil @ 1% c. Installation of fruit fly traps- methyl eugenol @ 1 ml and malathion 50 EC @ 1.0 (10 traps/acre), d. Collection and destruction of affected fruits)] recording fruit damage of 0.23 per cent being on par with each other. M4 (untreated control) recorded significantly the highest fruit damage (4.24%).

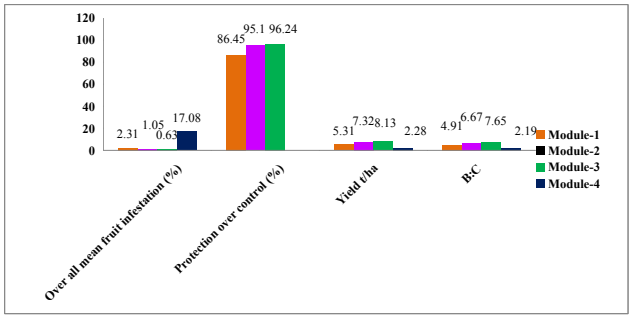

Figure 1. Effect of IPM modules against guava fruit fly 2015-16 and 2016-17

On the 14th day after first treatment, M3 recorded no fruit damage (0.00%). The next best treatment was M2 which recorded significantly lowest fruit damage (0.40%) followed by M1 (1.09%) and were on par with each other. M4 (untreated control) recorded significantly highest fruit damage (4.02%). On the 7th day after second treatment, M3 recorded no fruit damage (0.00%). The next best treatment was M2 which recorded significantly the lowest fruit damage (0.51%) followed by M1 recording fruit damage of 0.47 per cent and these two modules were on par with M3. M4 (untreated control) recorded significantly highest fruit damage (6.86%). On the 14th day after second treatment, M3 recorded significantly the lowest fruit damage (0.38%). The next best treatment was M2 that recorded significantly the lowest fruit damage (0.58%) followed by M1 (0.60%) and these two modules were on par with M3. M4 (untreated control) recorded significantly highest fruit damage (7.66%).

On the 7th day after third treatment, M3 recorded significantly the lowest fruit damage (0.99%). The next best treatment was M2 which recorded significantly lowest fruit damage (1.32%) followed by M1 (1.68%) and these two modules were on par with M3. M4 (untreated control) recorded significantly highest fruit damage (11.20%). On the 14th day after third treatment, M3 recorded significantly the lowest fruit damage (1.07%). On the 7th day after fourth treatment, also M3 recorded significantly the lowest fruit damage (1.14%). The next best treatments were M2 (3.82%) and M1 (4.48%) and these two modules were on par with each other. M4 (untreated control) recorded significantly highest fruit damage (36.20%). On the 14th day after fourth treatment, M2 recorded significantly the lowest fruit damage (1.32%) followed by M3 with fruit damage of 1.86 per cent and were on par. The next best treatment was M1 (6.34%). M4 (untreated control) recorded significantly highest fruit damage (44.93%). Until 7th day of 3rd treatment there was no influence of treatments, though appeared better than control. After that organic module could not compete with the IPM and POP. However, overall efficacy indicated no significant difference among treatments. Among four modules, the mean fruit damage was the lowest in M3 (0.68%) followed by M2 (1.19%) and M1 (2.21%) and were on par with each other, and superior over M4 (untreated control) which recorded significantly highest fruit damage (16.07%).

The highest per cent protection was noticed in M3 (95.76%). M2 and M1 also recorded significantly higher protection over control (92.59 and 86.24%, respectively). Until 7th day of 3rd treatment, there was no difference between treatments though looked better than control. After that organic module could not compete with the IPM and POP. However, overall efficacy indicated no difference among treatments.

The data obtained on efficacy of IPM modules against fruit fly in the field trial of summer 2017 are presented in Table 3. At 7th day after first treatment, M1 recorded significantly lowest fruit damage (0.10%) followed by M2 (0.20%) and M3 (0.48%) and these modules were on par with each other. M4 (untreated control) recorded significantly highest fruit damage (6.62%). On the 14th day after first treatment, M3 recorded significantly lowest fruit damage (0.54%) followed by M2 (0.66%) both being on par. M1 also recorded significantly lowest fruit damage (2.55%) than M4 (untreated control) which recorded significantly highest fruit damage (8.72%). On the 7th day after second treatment, M2 was free from fruit damage (0.00%). The next best treatments were M1 (0.20%) and M3 (0.33%) and were on par with each other. M4 (untreated control) recorded significantly highest fruit damage (13.85%). On the 14th day after second treatment, M2 and M3 suffered no fruit damage (0.00%). M1 also recorded significantly lowest fruit damage (1.68%) than M4, which recorded significantly highest fruit damage (15.92%). On the 7th day after third treatment, M3 recorded significantly lowest fruit damage (0.26%) on par with M2 (0.29%) and M1 (0.76%). M4 (untreated control) recorded significantly highest fruit damage (20.53%). On the 14th day after third treatment, M3 recorded significantly lowest fruit damage (1.07%) on par with M2 (1.64%) and M1 (2.62%). M4 (untreated control) recorded significantly highest fruit damage (15.14%).

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Table 3. Effects of IPM modules against guava fruit fly (2016-17)

Modules	Fruit infestation (%)								Over all mean fruit infestation (%)	Protection over control (%)
	1st treatment		2nd treatment		3rd treatment		4th treatment			
	7 DAT	14 DAT	7 DAT	14 DAT	7 DAT	14 DAT	7 DAT	14 DAT		
M_1: Organic module	0.10 (1.04)^b	2.55 (9.04)^b	0.20 (1.37)^b	1.68 (7.37)^b	0.76 (3.31)^b	2.62 (8.30)^b	5.50 (11.82)^b	5.94 (14.05)^b	2.41 (7.87)^b	86.67
M_2: Integrated module	0.20 (1.37)^b	0.66 (4.52)^c	0.00 (0.28)^b	0.00 (0.28)c	0.29 (1.62)^b	1.64 (4.68)^b	2.46 (10.54)^b	2.12 (8.24)^c	0.92 (4.45)^b	94.91
M_3: RPP-recommended POP	0.48 (0.28)^b	0.54 (3.27)^c	0.33 (1.70)^b	0.00 (0.28)^c	0.26 (1.55)^b	1.07 (5.25)^b	0.58 (5.20)^b	1.46 (6.83)^c	0.59 (3.99)^b	96.73
M_4: Untreated control	6.62 (14.51)^a	8.72 (17.12)^a	13.85 (21.68)^a	15.92 (23.11) ^{a}	20.53 (26.67)^a	15.14 (22.10)^a	17.06 (24.89)^a	46.81 (43.17)^a	18.08 (24.35)^a	-
S. Em ±	1.13	0.97	1.12	1.34	1.94	2.74	2.25	0.58	1.41	-
CD at 5 %	3.50	2.99	3.46	4.13	5.97	8.43	6.92	1.81	4.16	-

Notes: Means followed by same alphabet do not differ significantly (0.05) by DMRT (p=0.05)

Figures in the parenthesis are arc sine transformed values

DAT- Days after treatment

On the 7th day after fourth treatment also M3 showed significantly lowest fruit damage (0.58%) but on par with M2 (2.46%) and M1 (5.50%). The M4 (untreated control) recorded significantly highest fruit damage (17.06%). On the 14th day after fourth treatment, M3 recorded significantly lowest fruit damage (1.46%) followed by M2 (2.12%) and both being on par. The next best treatment was M1 that recorded fruit damage of 5.94 per cent. M4 (untreated control) recorded significantly highest fruit damage (46.81%).

Among the four modules tested, the over all mean fruit damage in M3 was significantly lowest (0.59%) followed by M2 (0.92%) and M1 (2.41%) and were on with M3. M4 (untreated control) recorded significantly highest fruit damage (18.08%). The highest per cent protection was afforded by M3 (96.73%). M2 and M1 (94.91 and 86.67%, respectively) were the next best modules and significant over untreated control. In the Second year, performance was similar to the previous year in general, however the organic module showed superiority over IPM and POP modules. The average fruit yield over the years of experimentation was 8.13 t per ha from M3 followed by M2 (7.32 t/ha) and M1 (5.31 t/ha). Significantly lowest fruit yield was recorded in M4 (untreated control) (2.28 t/ha).

The economics of each module was worked out based on yield of marketable fruits obtained in experiment during the year 2015-16 and 2016-17 as presented in Table 4. Among different modules, maximum gross income was obtained from M3 fetching Rs. 4,06,500/ha, followed by M2 with Rs. 3,66,000 per ha. The next best treatment was M1 with Rs. 2,65,500 per ha. M4 (untreated control) recorded grass returns of Rs. 1,14,000 per ha. M3 provided maximum net profit Rs. 3,53,377 per ha, followed by M2 with Rs. 3,11,146 per ha. The next best treatment in obtaining high net return was M1 recording Rs. 2,11,498 per ha. M4 (untreated control) recorded low net returns (Rs. 62,000). Cost benefit ratio (B:C) was worked out for each module. Among the four modules, highest B:C was from M3 (7.65) followed by M2 (6.67) and M1 (4.91). Relatively low B:C of 2.19 was seen in M4 (untreated control).

Table 4. Economics of IPM modules against guava fruit fly (2015-16 and 2016-17)

Modules	Yield (t/ha)	Grass return (Rs/ha)	Total cost (Rs/ha)	Treatment cost (Rs/ha)	Net returns (Rs/ha)	B:C
M_1: Organic module	5.31	265500	54002	2002	211498	4.91
M_2: Integrated module	7.32	366000	54854	2854	311146	6.67
M_3: RPP-recommended POP	8.13	406500	53123	1123	353377	7.65
M_4: Untreated control	2.28	114000	52000	-	62000	2.19

Notes: Market price: Rs 50 Rs/ Kg

Gross return = Yield x Market price

Net Returns = Gross return-Total Cost of cultivation

B:C ratio = Gross Returns/Total Cost of cultivation

5. Discussion

Though repeated application of bait spray proved effective, the residual effects of dimethoate which is having a systemic nature cannot be ignored. So, bait spray during initial period followed by neem based commercial insecticides or relying upon neem or spray integrated with methyl eugenol pheromone traps could be good option to reduce residue problem and high pest pressure situation. [7] also assessed the effectiveness of a locally recommended IPM package that comprised of weekly removal of fall-

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en fruits tri weekly inter-trees ploughing and raking and three fortnightly cover sprays of insecticide. Cost- benefit returns were dependent on the level of pest pressure, and in years of low pressure the package may not recover its costs, necessitating a threshold approach. According to [8] field sanitation (weeding and pruning of dead branches), use of methyl eugenol (sex pheromone traps), and bagging of fruits increased the yield of fresh fruits of guava. Clean culture, orchard sanitation or removal and destruction of the insect infested fruits either by burning or deep burying and ploughing around the trees have been proved to be an effective tool in the management of fruit flies. M3 has given higher protection might be attributed to reduction in pupae in the soil and reduction in fruit fly population by methyl eugenol traps and chemicals used in the components. [9] indicated that IPM module consisting of raking of soil under the tree, collection and destruction of fallen fruits (Sanitation), MAT and BAT resulted in high yield of fruits (24.5 t/ha). In the present study M3 has produced higher yield which might be attributed to lower infestation by the fruit fly and repeated timely application.

According to the reports of [10], hoeing under the tree canopy at 15 days interval along with collection of fallen fruits and burying deep in the soil and spray of spinosad was found most effective in reducing the fruit fly infestation (6% and 6.3% for the year 2013 and 2014, respectively) with cost benefit ratio of 1: 14.7, followed by the treatment comprising of hoeing and sanitation along with the spray Diptrex 80% WP @ 150 gm/100 liter of water (CBR= 1: 14.85). Hoeing under tree canopy alone proved to be least effective with average fruit fly infestation 16.67 and 15.85 per cent for the year 2013 and 2014 respectively with lowest CBR. The highest net returns and B:C in M3 might be attributed to lower cost of insecticide and higher yield compared to other modules. This particular module could be the choice by the farmer. However, M2 with integrated approach reduces dependency on the insecticide based bait which would be detrimental by way of residues especially when applied at delayed pest build-up situations. Hence, M2 appears to be the most viable option with respect to harvesting of yield on par with M3 and off course with slightly lower B:C ratio.

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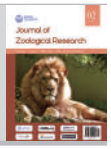

REVIEW

Review on Using of Macro Algae (seaweeds) in Fish Nutrition

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ABSTRACT

Currently, the search is on for alternative sources of feed ingredients, the main reasons being the increasing cost and uncertainty about the continuous supply of common feed ingredients especially fishmeal and soybean meal. The importance of macro algae or seaweeds as a potential substitute protein source for fish nutrition cultured has been documented in recent years. Macro algae are receiving consideration for their essential amino acid content and high protein value, trace metals and vitamins in fish nutrition. In addition, macro algae or seaweeds could be a potential low cost source of protein for fishes. Furthermore, the economic comparison of feed cost revealed that the increase in the level of dried and fresh seaweeds in alternative feeding treatments, and commercial diets used for fish growth have decreased which led to a significant decrease in the cost of feed. From the results of previous studies, using of macro algae (seaweeds) in fish diets may improves growth performance, feed efficiency, physiological activity, carcass quality, disease resistance and reduced stress response. This review describes effects of using of macro algae (seaweeds) in diets on growth performance of fish.

1. Introduction

Aquafeed accounts for about 50-80 percent of aquaculture production cost and therefore, its use has to be carefully considered and managed. Nutritionally balanced fish diets generally contain fish meal, soybean meal, wheat bran and yellow corn. Currently, the search is on for alternative sources of feed ingredients, the main reasons being the increasing cost and uncertainty about the continuous supply of common feed ingredients. The importance of macro algae or seaweeds as a potential substitute protein source for fish nutrition cultured has been documented in recent years [1]. The annual global aquaculture production of macro algae or seaweeds was 145 tonnes (including; brown, green and red seaweeds and different aquatic plants) in 2007 [2]. Global production has been dominated by marine macro algae or seaweeds, grown in both marine water and brackish water. Macro algae are receiving consideration for their essential amino acid content and high protein value, trace metals and vitamins in fish nutrition [1]. In addition, macro algae or seaweeds could be a potential low cost source of protein for fishes [3]. Furthermore, the economic comparison of feed cost revealed that the increase in the level of dried and fresh seaweeds in alternative feeding treatments, and commercial diets used for fish growth have decreased which led to a significant

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decrease in the cost of feed.

Macro algae (seaweeds) are plantlike organisms that generally live attached to rocks or other solid substrata in coastal areas. Seaweeds belong to three different groups, empirically distinguished on the basis of thallus color: green algae (phylum; Chlorophyta, classes; Chlorophyceae, Bryopsidophyceae, Prasinophyceae, Dasycladophyceae and Ulvophyceae), brown algae (phylum; Heterokontophyta (also known as the Ochrophyta) class; Phaeophyceae) and red algae (phylum; Rhodophyta). About 8000 species of macro algae (seaweeds) along the world's coast live and they may extend as deep as 270 m [4]. A total of 350 species of red seaweeds, 90 species of brown seaweeds and 25 species of green seaweeds are found in the world sea area that are commercially important because of their protein, amino acids and mineral contents [5].

The aim of this review was, to evaluate the overall effects of using of macro algae (seaweeds) in fish diets on growth rates, survival rate, feed efficiency, body chemical composition and blood indices of fishes.

2. Importance of Macro Algae (Seaweeds) in Fish Nutrition

Marine macro algae (seaweeds) have been used for healthy feed supplement providing necessary amino acids, fatty acids, beneficial polysaccharides, antioxidants, minerals and vitamins [7,8]. They prefer as food by herbivorous fishes since their stomach have low pH levels and specialize guts required for the digestion of plant materials [9]. Moreover, they improve the immune system, antiviral, antimicrobial, improved gut function and stress resistance serves as an alternative for fish meal, and they would help to take the pressure off wild fish stocks [10]. There is limited evidence that herbivorous and omnivorous fish were more effective at digesting and utilizing seaweed in diet.

Macro algal polysaccharides play vital role in feeding process since they have direct impact on the efficiency of nutrient assimilation in fish gut since polysaccharide can affect digestibility [11]. Alginate extracted from Ascophyllum nodosum etimulated lysozyme activity of Salmo salar [12]. Besides the nutritional value, seaweed contain bioactive compounds which exhibited antimicrobial, antiviral, antioxidative, anti-inflammatory, and neuroprotective so improved the immune response and stress resistance and act as scavenger to reactive oxygen species "ROS" [13]. Fucoidan from Sargassum wightii increased immunological parameters such as phagocytic activity, total leucocyte count and respiratory burst activity of Pangasianodon hypophthalmus [14].

Inclusion of agar from red seaweed enhanced the survival rate of *Aeromonas hydrophila*. Interestingly, seaweed act as the major market for astaxanthin so act as pigmentation source in aquaculture [15]. Astaxanthin, a carotenoid equipped with two asymmetric carbon located at the 3 and 3´ position of the benzenoid rings on either end of the molecule. In 1987, the United States Food and Drug Administration approved the use of astaxanthin as a feed additive for aquaculture and subsequently in 1999 astaxanthin where be approved as a nutraceutical. It was the most important carotenoid in rainbow trouts and salmons [16].

3. Chemical Composition of Macro Algae (seaweeds)

The protein content of macro algae or seaweeds varies with different species and seasonal period. In general, the protein content of brown seaweeds is low (3 - 5% of the dry weight (DW)) compared to that of the red or green seaweeds (10 - 47% DW). The content of crude protein, crud lipid, fiber and ash in green seaweeds meals from 7 - 29%, 0.5 - 4%, 3 - 6% and from 13 - 36%, respectively [17, 18]. Macro algae contain low amounts of lipids (1 to 3%), medium/high amounts of proteins (10 to 47%) and high amounts of carbohydrates (up to 60%) with a variable content of mineral ash (7–38%) [19]. The high carbohydrate content includes a large variety of easily-soluble polysaccharides, such as mannitol, laminarin, fucoidan or alginate in brown types; mannans, starch and sulphated galactans in red types and Ulvan in green types [20]. Other non carbohydrate products obtained from macro algae include proteins, lipids, terpenoids, and phenols and minerals such as phosphorus, potash and iodine useful for animal nutrition and human [21]. Chemical composition of some macro algae (seaweeds) are shown in Table 1.

Table 1. Chemical composition of some macro algae or seaweed (moisture (M), crude protein (CP), ether extract (EE), crude fiber (CF) and nitrogen free extract (NFE)) (%, on dry matter basis)

Species	M	CP	EE	CF	Ash	NFE	References
Green algae							
M. genuflexa	88.57	17.63	1.71	--	36.83	43.83	[22]
M. genuflexa	88.80	11.78	1.48	--	53.58	33.16	[22]
E. intestinalis	85.19	15.81	1.35	--	48.48	34.35	[22]
E. flaxusa	77.83	25.64	2.16	4.59	28.75	38.86	[23]
E. flaxusa	78.60	25.03	1.74	4.61	30.19	38.43	[24]
U. lactuca	--	20.33	3.21	9.87	17.98	48.34	[25]

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U. fasciata	76.10	27	0.57	9.81	20.06	42.56	[24]
U. fasciata	88.77	20.66	2.44	--	45.09	31.82	[22]
C. glomerata	89.57	8.81	3.09	--	21.42	66.67	[22]
C. laetevirens	90.44	14.63	3.64	--	12.82	68.92	[22]
Red algae							
P. capillacea	--	18.92	2.74	12.02	20.95	44.99	[25]
H. cornuta	87.31	11.69	3.61	--	38.79	45.91	[22]
G. corticata	94.58	16.41	2.14	--	15.93	65.52	[22]
G. corticata	84.08	11.72	1.71	--	10.32	76.26	[22]

Notes: M., Mougeotia; E., Enteromorpha; U., Ulva; C., Cladophora; P., Petrocladia; H., Hypnea; G., Gracilaria.

4. Effect of Using of Macro Algae or Seaweeds in Diets on Growth Performance of Fish

Recently, there are many researches have been carried out on the use of seaweeds as ingredient for aquafeed for different fish species. For example but not limited to.

Khalafalla and El-Hais [25] studied the effect of red algae (*Pterocladia capillacea*) and green algae (*Ulva lactuca*) at three levels (0.0, 2.5 and 5%) on blood indices, growth rates, feed efficiency and carcass composition of *Oreochromis niloticus* fingerlings. The results showed that, no significant effect were obtained for liver enzymes activity and serum total protein, globulin and albumin. Values of the growth rates and feed efficiency were significantly higher with Nile tilapia fed on diets (2.5 and 5%) for both red and green algae supplementation. Fish fed diet (5%) of green algae (*Ulva lactuca*) had acceptable growth rates compared to other diets. Nile tilapia fed supplemented diets had insignificant effect with slight increases and decreases for carcass lipids and protein. Also, Garcia-Casal *et al*. [26] reported that the better growth and nutrient utilization were using 5% *U. rigida* dietary supplementation for Nile tilapia (*Oreochromis niloticus*). In addition, Guroy *et al*. [27] observed that the weight gain value was higher for *Oreochromis niloticus* fed on diets supplemented with different levels of *Ulva* meal (5 to 10%). The incorporation 5% of green seaweed (*Ulva lactuca*) in Nile tilapia (*Oreochromis niloticus*) feeds promoted growth, diet utilization, immune response [28]. Siddik *et al*. [29] found that, Nile tilapia (*Oreochromis niloticus*) fed alternative 1 day commercial diet and 1 consecutive day dried or fresh seaweeds (*Enteromorpha sp*.) showed similar feed efficiency to fish feed the commercial diet. These results revealed that seaweeds can be used 1 day after using 1 day commercial diet without affecting feed efficiency of Nile tilapia.

Saleh [23] studied the effect of use fresh macro algae or seaweeds (*Enteromorpha flaxuse*) with or without artificial diet on growth rates, survival percentage and feed efficiency of hybrid red tilapia juvenile. Red tilapia juvenile were fed on three feeds (artificial feed only, fresh macro algae only and 50% artificial feed with 50% fresh macro algae. The highest final weight and specific growth rate of fish were recorded with fed on artificial feed alone and fresh algae alone. But, fish fed on artificial feed alone led to higher final length, total weight gain and daily growth rate. The best feed conversion ratio was recorded with red tilapia fed on artificial feed alone. Red tilapia juvenile were not acceptance of feeding on fresh algae with feeding on artificial feed, this may be the reason for the lower growth in this treatment. Survival percentage was within the range 86–90%, with insignificant differences among treatments. Also, El-Tawil [30] reported that the specific growth rate improved significantly with increasing green seaweeds (*Ulva sp.*) level in the diet up to 15% of red tilapia (*Oreochromis sp.*). And increasing green seaweeds (*Ulva sp.*) level beyond 15% had insignificant differences on growth. Supplementation of *Ulva sp.* to the prepared red tilapia (*Oreochromis sp.*) diet had a positive effect on feed conversion ratio except red tilapia fed the diet containing 25% *Ulva sp.* level with the poorest feed conversion ratio value [30]. Moreover, Costa *et al*. [31] observed that the dried and fresh brown seaweed can be used as a feed to substitute commercial diets for fish juveniles such as red tilapia (*Oreochromis sp.*), spotted scat (*Scatophagus argus*) and giant gourami (*Osphronemus goramy*).

In the other studies, Yousif *et al*. [32] showed that, rabbitfish (*Siganus canaliculatus*) were fed a diet with addition of a known weight of fresh green algae (*Enteromorpha sp.*) placed in plastic baskets at the bottom of the rearing tanks was the best in feed conversion ratio than the other treatments. Moreover, Abdel-Aziz and Ragab [24] who reported that, the green seaweed (*Ulva* and *Enteromorpha*) exhibited a positive effect on growth parameters of rabbitfish (*Siganus rivulatus*) fry and reduce of the feed cost as half of the feeding rate with artificial diet, but replacement of artificial diet with fresh seaweeds had negative consequences on growth parameters of *Siganus rivulatus* fry. Moreover, Shude *et al*. [33] indicated that incorporation of dried seaweeds (*Gracilaria lemaneiformis*) in rabbitfish (*Siganus canaliculatus*) juvenile diet is feasible. In addition, Xu *et al*. [34] recommend a level of less than 33% dried (*Gracilaria lemaneiformis*) in the *Siganus canaliculatus* diet.

In the study of Kotnala *et al*. [35] investigated the growth performance of Indian major carp (*Catla catla*)

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over a period through formulated feeds consisting of three seaweeds, namely *Padina tetrastomatica*, *Chlorodesmis fastigiata* and *Stoechospermum marginatum*. The results demonstrated that seaweeds, such as *P. tetrastomatica* and *C. fastigiata,* could be used in commercially formulated feed to get better growth of the fingerlings of major carps. Also, Diler *et al*. [36] reported that the inclusion of *Ulva rigida* meal at 5 - 15% replacing wheat meal in fish diets improved the growth performance of common carp.

Valente *et al*. [37] studied the inclusion of three seaweeds *Ulva rigida* (UR), *Gracilaria bursapastoris* (GP) and *Gracilaria cornea* (GC) in diets of European sea bass (*Dicentrarchus labrax*) juveniles on the growth performance, feed utilisation and body composition. Six diets were formulated to replace 5% (UR-5, GP-5 and GC-5 diets) and 10% (UR-10, GP-10 and GC-10 diets) fish protein hydrolysate by each of the three seaweeds. The results showed that inclusion of UR and GP up to 10%, can be considered as new ingredients in sea bass diets, as no negative effect on growth rates, feed efficiency and body composition. But, the inclusion of GC should be limited to 5% of the diet. Also, Wassef *et al*. [38] reported that the feeding sea bass at low level (5%) of *Ulva sp.* or *Ptercladia capillacea* meal had the better growth, survival rates and feed efficiency among all the dietary groups. The inclusion of 5% red seaweed (*Pterocladia capillacea*) enhanced some growth performance parameters of European seabass (*Dicentrarchus labrax*) fry, with an increase in body weight, and weight gain [38].

Yildirim *et al*. [2] studied the growth performance of rainbow trout fed with diets containing seaweeds (*Enteromorpha linza* and *Ulva lactuca*). Fishes were fed on three diets containing (0.0 as control group, 10% *Enteromorpha linza* meal and 10% *Ulva lactuca* meal) The results found that a diet with seaweeds (*Enteromorpha linza* and *Ulva lactuca*) inclusion at 10% levels resulted in weaker growth and feed efficiency compared to the control group of rainbow trout. Elmorshedy [39] observed that the final body weight and specific growth rate of gray mullet (*Liza ramada*) were higher significantly with increasing macro algae level (*Ulva sp.*) up to 28% in the gray mullet diet. Patel *et al*. [1] studied the three experimental diets consisting of seaweed *Ulva lactuca* at 10%, 20% and 30% with control diet without seaweed on growth and survival of *Labeo rohita* fry. Fish fed with 10% *Ulva sp.* meal observed an increased survival and growth rates and also a significant increase was found in specific growth rate, feed conversion ratio and protein efficiency ratio.

Ulva rigida low-level dietary incorporation has improved growth, feed efficiency, carcass quality, disease resistance, physiological activity and reduced stress response [37, 40]. Siddik *et al*. [29] found that similar survival rates of Nile tilapia juvenile fed diets with seaweeds and without seaweeds. Also, Rahman and Meyer [41] showed that similar survival rates of Common carp fed diet with seaweeds and without seaweeds.

On the other hand, Siddik *et al*. [29] reported that lowest final body weight and specific growth rate were showed in treatments feeding dried and fresh seaweeds (*Enteromorpha sp.*) as single feeds in Nile tilapia (*Oreochromis niloticus*). Also, the inclusion of 20-30% different macro algae (*Gracilaria cornea*, *Ulva lactuca*, *U. rigida* and *Cystoseira barbata*) in different species of fish meals decreased all growth performance and feed utilization parameters [42].

5. Conclusion

These findings in this review confirm the positive effects reported on promoted growth rates and survival percentage of fishes with the addition of macro algae or seaweeds in fish diets. Using of macro algae (seaweeds) in fish diets may improves growth performance and feed efficiency without adverse effects on liver enzymes activity and blood indices. And *Ulva rigida* low-level dietary incorporation has improved growth, feed efficiency, carcass quality, disease resistance, physiological activity and reduced stress response. Also, using of macro algae in fish diets had positive effect on growth performance and reduces of the feed cost.

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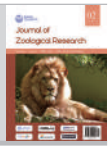

ARTICLE

A Case Report on Canine Transmissible Venereal Tumor

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ABSTRACT

A male Japanese spitz (3 years) was brought at Himalayan Animal Rescue Trust (HART), Pokhara with a complaint of swollen gums and loss of appetite. A lobulated tumorous mass was seen at the gingival region on physical examination. Diagnosis and treatment of condition detected in the dog was the major objective. Impression smear of tumor cell was prepared and was observed under oil immersion microscope (100x). Microscopic examination shows the presence of vacuolations within the cytoplasm and the condition was diagnosed to be CTVT. Chemotherapy was performed using the most effective cytostatic agents I.e. Vincristine sulphate (once a week, I/v). The chemotherapy was repeated for 3 doses till the tumor gets completely regressed. The condition was resolved after third session of chemotherapy. Myelosuppression and gastrointestinal effects like vomiting are the major complications of using vincristine.

1. Introduction

TVT is a tumorous condition transmitted horizontally among dogs after coitus mediated by the viable tumor cells possessing transposons [1]. TVT can also be called as Sticker tumor or sarcoma, transmissible lymphosarcoma, venereal granuloma, infectious granuloma, canine condyloma and contagious lymphosarcoma [2]. Novinsky in 1876 initially described canine TVT and demonstrated that the tumor could be transferred from one host to another via tumoral cells [3]. This viable tumor cells mainly effects the genital region whereas extra-genital cases of TVT has also been discovered and treated. Extra-genital region includes nasal cavity, conjunctiva and eye, skin, buccal and anal mucosa [4–6]. This might also lead to the condition known as phimosis. TVT cells are round with large round nuclei that possess coarse chromatin and single, prominent nucleoli. It also consists of prominent cytoplasm with distinct vacuolation [7]. The tumor growth occurs 15 to 60 days after implantation of tumor cells. Normal canine cells possess 78 chromosomes whereas CTVT cells consists of 57-59 chromosomes [8]. Some cases reports spontaneous regression of tumor mass and recovered dogs were found to acquire humoral and cellular immunity against CTVT [9]. Metastasis was found to be seen in less than 5-17% of cases and was more common in male (15.6%) than in female dogs (1.8 %) [10]. The rapidity of metastases depends on health and immune sta-

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tus of the affected dog.

2. Case Report and History

A 3 years old male Japanese spitz weighing 20 kg was brought to HART, Pokhara. The owner noticed the signs like loss of appetite, difficult feeding and loss of body condition. The owner explained that the dog was habituated to wander around the village with other street dogs. There was no cases of loss of consciousness. The owner visited us to determine and treat the underlying cause. The dog was properly vaccinated.

On physical examination, the nodular, lobulated mass was seen at the gingival region. Grossly, the mass was pink to red, multinodular, raised to pedunculated, soft and hemorrhagic. Temperature, pulse and respiration were found to be 103.8°F, 85 times per min and 22times per min respectively.

Figure 1. tumorous mass in gingiva

3. Materials and Methods of Diagnosis

The condition was tentatively diagnosed to be CTVT based on the expertise of the doctor. The condition could be differentially diagnosed with gingivitis. CTVT are mainly diagnosed by histopathological examination of biopsy, impression smear of the tumor or by using fine needle aspiration cytology (FNAC). So, the impression smear was sent to the regional laboratory located within Pokhara. The smear was then fixed with alcohol and was stained using Giemsa stain. The stained smear was observed using oil immersion microscope (100x). After staining, the confirmatory structure was visible i.e. highly vacuolated cytoplasm.

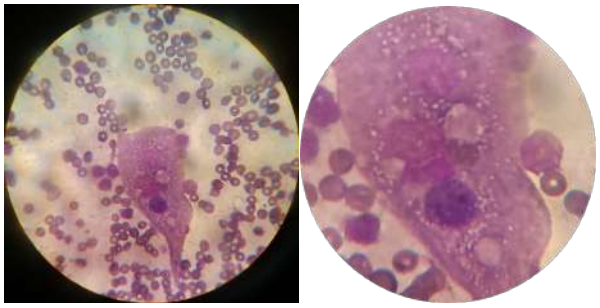

Figure 2. The impression smear was prepared and shows the presence of cytoplasmic vacuolations after microscopic observation

4. Discussion

TVT is mostly benign in nature which is found to be transmitted directly from dog to dog, across major histocompatibility complex (MHC) barriers and also through damaged mucosal surfaces mediated by the viable tumor cells during mating [11]. TVT possess unique characteristic of occurring naturally and transmitted as an allograft which acts more or less like a parasite that grows autonomously from the original host. CTVT cells can avoid its detection from immunological cells by inhibiting MHC-II activity or down-regulating MHC-I activity due to the secretion of inhibitory cytokines i.e. IL-6 and TGF-1[12].

Treatment of TVT mainly includes surgical techniques, radiotherapy and chemotherapy. Surgical method of treatment was being used since last century with lower efficacy. Small, localized TVTs were treated extensively using surgical method inspite of its higher chances of reoccurrence[7]. The use of electro-cautery in surgery proves to have higher success rate. Vincristine Sulfate is obtained as the salt of an alkaloid from a common flowering herb, the periwinkle plant (Vinca rosea Linn) [13]. The exact mode of action of vincristine sulfate is still under investigation. But some researches have showed that it inhibits microtubule formation in the mitotic spindle resulting arrest of mitotic division of cells at metaphase stage. Administration of vincristine alters spermatogenesis either temporarily or permanently [14]. Extravasation of anti-neoplastic agents like vincristine shows symptoms ranging from local pain, inflammation and ulceration vinblastine, vinorelbine [15,16].

5. Treatment

Chemotherapy was found to be the most effective method of treatment among all other. Complete regression of tumor takes 2 to 8 injections in most of the cases and have good prognosis with chemotherapy.

Among various cytostatic agents, vincristine sulphate

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was found to have higher success rate. So, vincristine sulphate (C-VINLON™ @1mg/ml) was administered at the rate of 0.025mg/kg body weight intravenously (Cephalic vein) once a week. Vincristine was given with fluid (i.e. normal saline at the rate of 2drops/second) to reduce the burning sensation in veins. Chemotherapy was performed for three sessions and the condition was resolved.

The prognosis of CTVT of extra-genital region was excellent after the treatment with vincristine.

Figure 3. The size of tumorous mass was reduced after third session of chemotherapy

6. Conclusion

CTVT is the unique type of tumor of benign nature mostly seen in genital regions. Vincristine was found to be effective even in the case of venereal tumor of extra-genital region. Animal must be prevented from having intercourse with random dog to prevent transmission of CTVT. The rate of fluid administration along with chemotherapeutic agent must be maintained at an optimum rate to prevent burning sensation caused by the drug. Regular administration of chemotherapeutic agent must be done weekly till the resolution of the condition.

Conflict of Interest

We have no conflicts of interest to disclose.

Ethical Statement

Handling of the dog during the treatment was performed under the ethical guidelines of HART, Pokhara.

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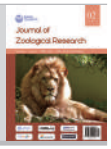

ARTICLE

Effect of Feeding on Fresh (wet) Housefly Maggots (*Musca domestica*) with or without Artificial Diet on Water Quality and Growth Rates of African Catfish (*Clarias gariepinus* Burchell, 1822) Fry under Laboratory Conditions

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ABSTRACT

No or little information on the use fresh (wet) housefly maggots (*Musca domestica*) in African catfish (*Clarias gariepinus*) fry feeding. Therefore, this study was conducted to investigate the effect of feeding on fresh (wet) housefly maggots with or without artificial diet on water quality, growth performance, survival percentage and feed utilization of African catfish fry under laboratory conditions. Housefly maggots produced from a mixture of poultry droppings and foods wastes, it was used to replace artificial feed at 0, 50 and 100% levels. Catfish were fed artificial diet alone (Feed 1), fresh (wet) housefly maggots alone (Feed 2), and 50% fresh housefly maggots with 50% artificial diet (Feed 3) were prepared and tested on triplicate groups of African catfish fry (initial weight of 0.25±0.02 g) for 60 days. Results showed that final weight (g/fish) was significantly ($P \leq 0.05$) higher in fish fed on feed 3 (6.03±0.08), followed by fish fed feed 2 (4.62±0.27), followed by fish fed feed 1 (3.15±0.68). Specific growth rate (%/day) was also significantly higher in fish fed on feed 3 (5.31±0.10), followed by fish fed feed 2 (4.86±0.03), followed by fish fed feed 1 (4.18±0.24). The same trend was observed with total weight gain, percentage weight gain, daily growth rate and relative growth rate. Feed intake and protein intake were significantly ($P \leq 0.05$) higher in fish fed on feed 3 and fish fed on feed 2, followed by fish fed feed 1. While, feed conversion ratio (FCR) and protein efficiency ratio were not significantly ($P > 0.05$), but the improvement in FCR recorded in catfish fry fed feed 3 and feed 2 under the experimental conditions. Survival percentage was within the range 55-75%, with insignificant differences ($P > 0.05$) among treatments. The water quality parameters such as temperature, pH, dissolved oxygen, total ammonia, nitrite and nitrate were not significantly ($P > 0.05$) between the treatments and were tolerable for Catfish culture. Accordingly, use of the 50% fresh (wet) housefly maggots with 50% artificial diet in African catfish fry feeding had positive effect on growth performance and reduce of the feed cost.

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1. Introduction

The African catfish is a chordate animal and belongs to Class Osteichthytes (bony fishes), Family Clariidae. It is a dominant freshwater fish. It can grow up to 1.4 and 2 m long and can weigh anything from 8 kg to 59 kg. The South African angling record is 35 kg; however a 58.9 kg specimen was caught in the Vaal River [1]. It is popular specie grown in many manmade ponds because of high survival ability [2]. The African catfish farming has witnessed an increased production and gained a considerable importance recently in Egypt, turned it from just an undesirable species in tilapia ponds or a 'police-fish' to control overbreeding in mixed-sex tilapia culture in earthen ponds to an important and potential species for aquaculture [3].

Recent high demand and consequent high prices for conventional feed ingredients such as fish meal, groundnut meal and soybean meal etc., has led to the development of insect protein for aquaculture as a new area of research [4]. The increasing cost of fish feed has been at an alarming rate and this has affected the development and expansion of aquaculture in African countries [5]. The need for more research for vital protein augments to make affordable fishmeal and thus increase the production of catfish becomes eminent [2]. The research for suitable and cost-effective alternative protein sources for use in industrial aqua feeds will be the most critical factor in the development of intensive aquaculture [5]. Insect meals are nutritious and healthy alternatives to fishmeal because of its rich nutritional values especially protein, fat and minerals [6].

Housefly maggot (*Musca domestica*) is the larva phase of a housefly which grows extensively on animal dung including cow, sheep, goat and poultry droppings under favorable conditions. Maggot is a potential alternative protein source for fish as reflected in its chemical composition [7]. Also, the ease of maggot production and processing, and acceptability by fish qualifies it as a suitable supplementary feed for fish. Housefly (*Musca domestica*) maggot meal was reported to contain 39-65% protein [8, 9], depending on the age of maggots at harvesting. Such variations in protein content could be attributed to the processing, drying, storage and protein estimation methods employed, or the substrate used for the production of housefly maggots [9, 10]. Maggot has come to be known not only as safe food for fishes, but also as rich protein source for them [11].

Data of Ipinmoroti *et al.* [12] showed that 75% of wet maggots can be recommended as an inclusion level in commercial feed for adequate utilisation by *Clarias gariepinus* juveniles. Moreover, Okore *et al.* [2] implies that the maggot meal can successfully replace fishmeal in fish diets. As well as Fashina-Bombata and Balogun [13] and Ajani *et al.* [14] reported that maggot meal can replace up to 100% of fish meal in the diets of Nile tilapia (*Oreochromis niloticus*). Because of the consumers concern and perceived public health implications, as maggots are associated with decomposing filthy organic matters, the safety and acceptability of fish produced with larvae (maggot) meal need to be ascertained. Therefore, the substitution of expensive fishmeal with cheap maggot meal in fish diet had no negative effects on the quality and acceptability of the final products [15].

Several studies have been reported on the use of housefly maggots (*Musca domestica*) as alternative protein sources in fish feed to partially or completely replace conventional feedstuff such as fishmeal and soybean meal. However there is no or little information on the use fresh (wet) housefly maggots (*Musca domestica*) in African catfish (*Clarias gariepinus*) fry feeding. Therefore, the present study aims to investigate the effects of feeding on fresh (wet) housefly maggots (*Musca domestica*) with or without artificial diet on water quality, growth performance, survival rate and feed utilization of African catfish (*Clarias gariepinus*) fry under laboratory conditions.

2. Material and Methods

The present study was conducted at Shakshouk Fish Research Station, El-Fayoum Governorate, National Institute of Oceanography and Fisheries (NIOF), Egypt, to investigate the effect of feeding on fresh (wet) housefly maggots (*Musca domestica*) with or without artificial diet on water quality, growth performance, survival rate and feed utilization of African catfish (*Clarias gariepinus*) fry under laboratory conditions. The experimental done through July -August and lasting 60 days after start. African catfish (*Clarias gariepinus* Burchell, 1822) fry (0.25±0.02 g initial body weight) were obtained after broodstock hatching in Shakshouk Fish Research Station, NIOF.

2.1 Feeding and Rearing Conditions

This experiment consists of three treatments. The first treatment: catfish fed on artificial feed only. The second treatment: catfish fed on fresh (wet) housefly maggots (*Musca domestica*) only. The third treatment: catfish were fed of half feeding rate on artificial feed and other fresh housefly maggots. Did not take into consideration the percentage of protein feed, but was

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taking the variety feed. Ten fish were randomly stocked in nine glass aquaria (30 L capacity/ aquarium) filled with dechlorinated tap water. Each treatment consisted of three aquaria. The aquaria were provided with air stonws for continuous aeration by electrical air pumps. Fish were fed three times daily (9:30, 13:30 and 16:30 h) for six days a week at a rate of 5% of their wet biomass per day and readjusted bi-weekly after the biomass of fish in each aquarium was determined. Feed was offered by hand on dry weight basis. After weighing, each aquaria was cleaned to prevent accumulation of faeces and to reduce algal growth. The feces and other wastes were siphoned daily from the aquaria immediately before feeding. In addition, about 30% of the water was siphoned and replaced by new, fresh, dechlorinated water that was stocked in fiberglass tank and aerated by electrical air pumps. Feed consumption was recorded daily and rate of mortality was recorded. Water temperature, dissolved oxygen, pH, total ammonia, un-ionized ammonia, nitrite and nitrate were measured during experimental period. Fish were kept in a natural photoperiod condition throughout the experimental period (60 days). At the end of the study, fish in each aquarium were netted, counted and weighed.

2.2 Feed Formulation and Preparation

Artificial diet was formulated based on fish meal as the only animal protein source and a mixture of soybean meal and yellow corn as plant protein sources. Soybean oil was added as the major dietary lipid source to the artificial diet. The artificial feed formulated to be almost containing 40% crude protein (Tables 1 and 2), diet was hand made.

Housefly maggots (the larva stage of the housefly, *Musca domestica*) produced from poultry droppings and foods wastes. Fifteen kilogram of poultry droppings and foods wastes were mixed together and spread on three wood box (40 cm length, 40 cm width and 10 cm height) to a thickness of 7 cm to constitute the substrate. The odor of fresh poultry droppings and foods wastes, fermenting substrate attracted flies, which later laid eggs on it. The eggs hatched into larvae within two days and were allowed 48 hours to develop further. The mature maggots were harvested. Housefly maggots were caught from wood box by using tweezers then stored in plastic bags in the freezer (-18°C ±1°C) until used. Moisture in wood box was maintained high all time during housefly maggot production period [16,17]. Chemical composition of housefly maggots (*Musca domestica*) are shown in Table 2.

Table 1. Percentage composition of the artificial feed

Ingredients, %	Artificial diet
Fish meal, (CP 63%)	47
Soybean meal	18
Yellow corn	28
Soybean oil	4
Commercial yeast	1
Starch	1
Vitamins mixture[1]	0.5
Minerals mixture [2]	0.5

Notes: [1.] Vitamins each 3 Kg contains: 1200 000 IU Vit. A, 300 000 IU Vit. D3, 700 mg Vit. E, 500 mg Vit. K_3, 500 mg Vit. B_1, 200 mg Vit. B_2, 600 mg Vit. B_6, 3 mg Vit. B_{12}, 450 mg Vit. C, 3000 mg Niacin, 3000 mg Methionine, 10 000 mg Cholin chloride, 300 mg Folic acid, 6 mg Biotin, 670 mg Panthonic acid. [2.] Minerals each 1 Kg contains: 1472 mg Manganese sulphat, 1030 mg Zinc sulphat, 2359 mg Iron sulphat, 747 mg Copper sulphat, 5 mg Cobalt sulphat, 33 mg Potassium iodide, 1.28 mg Sodium selenite, 4300 mg Sodium sulphat 32.37%, 4000 mg Potassium chloride 52%.

Table 2. Proximate chemical analysis (% on dry matter basis) of the experimental feeds

Chemical analysis (%)	Artificial diet	Fresh housefly maggots	Diet + maggots
Moisture	9.98	74.43	42.21
Dry matter, DM	90.02	25.57	57.79
Crude protein, CP	40.75	58.60	49.67
Ether extract, EE	9.40	15.82	12.61
Crude fiber, CF	1.93	--	0.97
Ash	8.84	24.18	16.51
Nitrogen free extract, NFE[1]	39.08	1.40	20.24
Gross energy, GE kcal/g[2]	4.922	4.806	4.864
Digestible energy, DE kcal/g[3]	4.117	3.768	3.943

Notes: [1] Calculated by differences. [2] Calculated according to NRC [18]. [3] Calculated according to Garling and Wilson [19].

2.3 Growth Performance Indices

The growth and feed utilization parameters were calculated according the following equations:

Weight gain (g) = final weight, g - initial weight, g.

Percentage weight gain (%) = (weight gain)/ (final weight) × 100.

Daily growth rate (mg/day) = weight gain, mg / experimental period, day.

Relative growth rate (%) = (weight gain)/ (initial weight) × 100.

Specific growth rate (%/day) = [(ln final weight - ln initial weight)/period in days] × 100, where ln is the natural log.

Condition factor (g/cm^3) = (fish weight)/ (fish $length^3$) ×100.

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Survival percentage (%) = (number of fish at end/ number of fish at start) × 100.

Feed conversion ratio (FCR) = dry feed intake, g/ weight gain, g.

Protein intake (g/fish) = total feed intake × protein content of feed.

Protein efficiency ratio (PER)= weight gain, g/ protein intake, g.

Energy intake (Kcal/ fish) = total feed intake × energy content of feed.

Energy efficiency ratio (EER) = weight gain, g/ energy intake, Kcal.

2.4 Water Quality Analysis

Water temperature was measured daily by using centigrade thermometer. Dissolved oxygen (DO) and pH were measured every week by using Tintometer® group (pH/ORP, DO, CD/TDS, Nr: 00724200. Germany 01/16). Water ammonia, nitrite and nitrate were determined every two weeks by using Spectrophotometer model (LKB Bichrom UV visible spectrophotometer) according to the method described by APHA [20]. To determine un-ionized ammonia concentration, multiply total ammonia concentration by the percentage which is closest to the observed temperature and pH of the water sample [21].

2.5 Chemical Analysis of Feeds

Feeds used were analyzed for their proximate composition in triplicates following the methods described by AOAC [22]. Gross energy (GE) content was calculated according to NRC [18] by using factors of 5.65, 9.45 and 4.22 kcal/g of protein, lipid and carbohydrate, respectively. Digestible energy (DE) content was calculated from standard physiological fuel values as 4, 4 and 9 kcal/g of protein, carbohydrate and lipid, respectively [19].

2.6 Statistical Analysis

Data of water quality, growth performance and feed utilization at different feeds were statistically analyzed using a one-way analysis of variance (ANOVA test) using SPSS Statistical Package Program, version 23 [23]. Mean of treatments were compared by Duncan multiple range test when the differences were significant [24]. Level of significance in all tests was $P \leq 0.05$. The results are expressed as means ± standard error (SE).

3. Results

3.1 Water Quality Parameters

Criteria on water of aquarium such as: temperature, pH, dissolved oxygen, total ammonia, un-ionized ammonia, nitrite and nitrate were presented in Table (3). Water quality parameters were not significantly ($P>0.05$). Similar water quality characteristics were observed in all aquaria.

Table 3. Average water quality criteria (mean±SE) recorded during the experimental period

parameters	Artificial diet	Fresh housefly maggots	Diet + maggots	CV, %*
Temperature, °C	30.25±0.75[a]	30.10±0.90[a]	30.20±0.80[a]	2.98
pH	8.20±0.09[a]	8.25±0.02[a]	8.17±0.07[a]	0.97
Dissolved oxygen, mg/l	6.55±0.15[a]	6.90±0.30[a]	6.70±0.20[a]	4.36
Total ammonia, mg/l	0.363±0.10[a]	0.349±0.07[a]	0.351±0.09[a]	27.54
Un-ionized ammonia, mg/l	0.040±0.011[a]	0.039±0.009[a]	0.039±0.009[a]	27.38
Nitrite, mg/l	0.76±0.19[a]	0.66±0.06[a]	0.68±0.11[a]	21.82
Nitrate, mg/l	1.45±0.22[a]	1.44±0.01[a]	1.43±0.19[a]	12.92

Notes: Values are mean of three replicates. Value in the same row having similar superscript are not significantly different from one another ($P>0.05$). * Coefficient of variation (CV, %) = (standard deviation)/ (mean) × 100.

3.2 Growth Performance and Survival Percentage

Results of growth performance and survival percentage of catfish fed on the three different feeds are shown in Table (4). There was no significant difference in the initial length and body weight of the fish between treatments. Survival percentage was within the range 55-75%, with insignificant differences ($P>0.05$) among treatments. Results of the growth performance parameters of catfish fry fed the three feeds showed that final weight (g/ fish) was significantly ($P \leq 0.05$) higher in catfish fed 50% artificial diet + 50% fresh housefly maggots (6.03±0.08), followed by fish fed fresh housefly maggots alone (4.62±0.27), followed by fish fed artificial diet alone (3.15±0.68). Specific growth rate (%/day) was also significantly ($P \leq 0.05$) higher in catfish fed 50% artificial diet + 50% fresh housefly maggots (5.31±0.10), followed by fish fed fresh housefly maggots alone (4.86±0.03), followed by fish fed artificial diet alone (4.18±0.24). The same trend was observed with total weight gain, percentage weight gain, daily growth rate and relative growth rate. But, final length and condition factor were not significantly ($P>0.05$) between treatments. The results indicated that the catfish fed 50% artificial diet + 50% fresh housefly maggots grow better in weights compared to those fed on fresh housefly maggots alone and artificial diet alone, but, catfish fed fresh housefly maggots alone grow better than catfish fed artificial diet alone under the experimental conditions.

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Table 4. Average of the growth performance and survival percentage of catfish fed on the three different feeds for 60 days (mean± SE)

parameters	Artificial diet	Fresh house-fly maggots	Diet + mag-gots
Initial length, cm/ fish	3.3±0.1[a]	3.3±0.1[a]	3.3±0.1[a]
Final length, cm/ fish	8.10±0.70[a]	8.75±0.15[a]	9.75±0.25[a]
Initial weight, g/ fish	0.25±0.02[a]	0.25±0.02[a]	0.25±0.02[a]
Final weight, g/ fish	3.15±0.68[b]	4.62±0.27[ab]	6.03±0.08[a]
Total weight gain, g/ fish	2.90±0.66[b]	4.37±0.25[ab]	5.78±0.06[a]
Percentage weight gain, %	91.79±1.18[b]	94.58±0.11[ab]	95.84±0.26[a]
Daily growth rate, mg/ day	48.32±11.02[b]	72.74±4.10[ab]	96.24±0.94[a]
Specific growth rate, %/day	4.18±0.24[b]	4.86±0.03[ab]	5.31±0.10[a]
Relative growth rate, %	1142.87 ±177.76[b]	1742.95 ±32.86[ab]	2314.19 ±151.58[a]
Condition factor, g/cm^3	0.59±0.03[a]	0.69±0.01[a]	0.66±0.05[a]
Survival percentage, %	55.00±5.00[a]	75.00±5.00[a]	75.00±5.00[a]

Notes: Values are mean of three replicates. (a and b) Average in the same row having different superscripts are differ significantly (P≤0.05). Mean values with the same superscript are not significantly different (P>0.05).

3.3 Feed Efficiency Parameters

As shown in Table (5). Results of the feed efficiency parameters of catfish fed the three feeds showed that feed intake, protein intake and energy intake were significantly highest (P≤0.05) in fish fed 50% artificial diet + 50% fresh housefly maggots and fish fed fresh housefly maggots alone, followed by fish fed artificial diet alone. While, feed conversion ratio (FCR), protein efficiency ratio and energy efficiency ratio were not significantly (P>0.05) different between the three treatments. But the improvement in FCR recorded in African catfish fry fed 50% artificial diet + 50% fresh housefly maggots and fish fed fresh housefly maggots alone.

Table 5. Average of the feed utilization efficiency parameters of catfish fed on the three different feeds for 60 days (mean± SE)

parameters	Artificial diet	Fresh housefly maggots	Diet + maggots
Feed intake, g/ fish/ period	3.84±0.51[b]	5.48±0.25[a]	6.61±0.07[a]
FCR, g feed/ g gain	1.36±0.14[a]	1.26±0.02[a]	1.15±0.01[a]
Protein intake, g/fish	1.56±0.20[b]	3.21±0.15[a]	3.29±0.04[a]
Protein efficiency ratio	1.83±0.19[a]	1.36±0.01[a]	1.76±0.01[a]
Energy intake, Kcal/ fish	18.88±2.43[b]	26.31±1.20[a]	32.15±0.34[a]
Energy efficiency ratio	0.15±0.016[a]	0.17±0.002[a]	0.18±0.001[a]

Notes: Values are mean of three replicates. (a and b) Average in the same row having different superscripts are differ significantly (P≤0.05). Mean values with the same superscript are not significantly different (P>0.05).

Notice: Feed intake was on dry matter basis (3.84g equal to 4.27g on wet weight), (5.48g equal to 21.43g on wet weight) 6.61g equal to 3.68g artificial diet +12.94g maggots on wet weight).

4. Discussion

The high dependence of aqua feeds on prohibitively expensive fishmeal protein has taken its toll on the aquaculture industry. Fishmeal is the most expensive component of fish feeds and several studies have advocated its replacement with plant sources. Plant protein alternatives have their nutritional deficiencies [8] and also their use by humans and other animals make it imperative for a search for other alternatives [15]. Recently, there are several reports on the evaluation of unconventional protein sources in fish feeds, but the use of maggot (housefly larvae) meal that has a comparable nutritive value, especially amino acid profile, with fishmeal [25], holds a promise in fish nutrition. Maggot meal has been found to be rich in protein and essential amino acids [26] and has successfully replaced fishmeal in catfish diets [27, 28].

In the present study, African catfish (*Clarias gariepinus*) fry fed on three feeds, artificial feed only, fresh (wet) housefly maggots (*Musca domestica*) only and 50% artificial feed with 50% fresh housefly maggots. Results obtained for growth performance such as final weight, total weight gain, daily growth rate and specific growth rate showed that there were significantly (P≤0.05) higher in catfish fed 50% artificial diet + 50% fresh housefly maggots, followed by fish fed fresh housefly maggots alone, followed by fish fed artificial diet alone.

Response of catfish to feed was more aggressive with fed on fresh housefly maggots than artificial feed during the feeding trial, probably this is due to the predation behavior of African catfish as the fresh housefly maggots satisfy this natural instinct. The higher growth performance of catfish fed on 50% artificial diet + 50% fresh housefly maggots, this is due to two reasons: the first is that fresh housefly maggots provide satisfying instinct in predators, and the second is that artificial diet contains various ingredients (fish meal, soybean meal, yellow corn, soybean oil, yeast, starch and vitamins and minerals mixture) that contain all the necessary growth promoting factors from protein, energy, vitamins and minerals.

The difference obtained in the growth performance parameters of catfish fed on housefly maggot when compared to artificial diet, this may be attributed to maggot meal is animal protein ingredients are high protein content [5], good sources of amino acids [29], fatty acids [30], minerals [27]. Moreover, Spinelli et al [31] indicated that the amino acid profile of maggot meal contains on the same number and outstanding level of amino acids found in fish meal. Also, Ipinmoroti *et al.* [12] mention that the wet maggot's composition showed the presence of amino acids similar to fishmeal. Also, maggot meal is rich in phosphorus, trace elements and B complex vitamins [32]. In addition, the improvement in growth and feed efficiency recorded in African catfish (*Clarias gariepinus*) fry fed maggot-supplemented diet suggest that maggot contain all the

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necessary growth promoting factors, this opinion was supported by Mustapha and Kolawole [11] for *Oreochromis niloticus*. As well as, Mustapha and Kolawole [11] mention that the reason for the superiority of 100% fresh maggot diet over other diets was attributed to the relatively large amount of soft tissue contain in the whole diet. Also, Adesulu and Mustapha [33] indicated that the maggot meal may be superior to other protein sources in fish diets, it may contribute to this that the maggots easily digestible. This result agree with the report of other authors who have observed a better performance of fish fed diets containing maggot meal over those solely fed on fish meal diets meal. Thus, this is a reflection of the nutritive quality and acceptance of this biomaterial [34]. The result also corroborates previous observation that maggot meal, like other animal protein sources is well accepted and utilised by fish [35].

From results of this study, the use of housefly maggot with artificial feed to fed African catfish appear to be advantageous especially as it produced higher growth performance when compared to the use of housefly maggot only and artificial diet only in feeding. This is similar to that observed by Ipinmoroti *et al.* [12] utilised housefly maggots (*Musca domestica*) at different levels (0, 25, 50, 75 and 100%) to replace fishmeal in the diets of catfish (*Clarias gariepinus*) juveniles. And they observed that 75% of wet maggot gave better growth and feed utilisation and conversion of feed to flesh. Also, Okore *et al.* [2] reported that, supplementing conventional fish feed with *Musca domestic* maggots for *Clarias gariepinus* juveniles. The percentage of the conventional feed to maggot inclusions were 70% to 30%, 55% to 45%, 35% to 65%, and 100% conventional feed. The result shows that a combination of 55% compounded ration and 45% maggot gives the best growth performance. And concluded that using housefly maggot directly as supplementary feed for *Clarias girepinus* at appropriate ration will enhance its growth and haematological performance. In another research conducted by Mustapha and Kolawole [11] indicated that fresh maggot meal can be successfully used to replace fishmeal partially or completely from 50% up to 100% in the diet of *Oreochromis niloticus* fingerlings for optimal growth and nutrient utilization. The results were in partial agree with Mustapha [36], the best growth performance was recorded for fingerling fed with diet containing 75% oven dried maggot meal, followed by 50% maggot inclusion and the least growth performance was exhibited by fingerlings fed diet containing 100% oven dried maggot meal as the protein source.

On the other hand, Arong and Eyo [6] studied the effects of the combination of housefly maggot meal with commercial feed on the growth rates, survival rate and feed utilization of the African catfish (*Clarias gariepinus*). Fish were fed maggot meal (100%), maggot meal with commercial feed (50:50), and commercial feed (100%). Although commercial feed was the best growth performance and feed utilization, the combination of maggot meal with commercial feed as supplementary feed will reduce the cost of fish production.

In this study, housefly maggot (*Musca domestica*) had crude protein of 58.60% which is higher than 22.97% [37], 33.29% [6], 42.00% [38] and 47.45% [12], these variations observed in the chemical composition of maggot meal in the present study with other studies may be attributed to the methods used in processing of housefly maggots.

In fish nutrition studies, amount of feed consumed and feed conversion ratio (FCR) are very useful indices that are used to evaluate feed acceptability, production economics and fish performance in terms of growth [6]. The result of this study showed that feed intake and protein intake were significantly highest ($P \leq 0.05$) in fish fed 50% artificial diet + 50% fresh housefly maggots and fish fed fresh housefly maggots alone, followed by fish fed artificial diet alone. While, FCR and protein efficiency ratio (PER) were not significantly ($P > 0.05$), but the improvement in FCR recorded in African catfish fry fed 50% artificial diet + 50% fresh housefly maggots and fish fed fresh housefly maggots alone. This clearly indicates that the maggots used in the present study as supplementary feed could be consumed and utilized efficiently by catfish in the absence of artificial feed. This confirms the suitability of maggots as supplementary diets for *Clarias gariepinus*. This result corresponds with Okore *et al.* [2] mention that the best FCR was observed in *Clarias gariepinus* fed 55% conventional feed with 45% maggot. The results were in partial agree with Mustapha and Kolawole [11] indicated that the FCR decrease with increasing maggot level from 25% to 100% and PER decreased as the dietary maggot inclusion level increased. FCR was not significantly different between the maggot levels from 50% to 100% in the diet of *Oreochromis niloticus* fingerlings. On the other hand, Arong and Eyo [6] mention that the best FCR was obtained in catfish fed commercial feed.

In the present study, survival percentage was within the range 55-75%, with insignificant differences ($P > 0.05$) among treatments, but the higher survival percentage (75%) recorded in African catfish fry fed 50% artificial diet + 50% fresh housefly maggots and fish fed fresh housefly maggots alone. These results agree with the observation of Okore et al [2] confirmed that the highest survival rate was observed in *Clarias gariepinus* fed 55% conventional feed with 45% maggot. Also, Faturoti and Ifili [39] who indicated that the feeding of *Clarias*

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gariepinus fingerlings on maggot diets made for high survival. The results were in partial agree with Arong and Eyo [6] confirmed that the survival rate was not influenced by experimental feed as catfish fed on 100% commercial feed, 100% maggot meal, and maggot meal with commercial feed (50:50), but the highest survival recorded in African catfish fed commercial feed.

In the present study, water quality parameters such as temperature, pH, dissolved oxygen, total ammonia, un-ionized ammonia, nitrite and nitrate were not significantly ($P>0.05$). Similar water quality characteristics were observed in all aquaria and were within the range recommended for optimal growth of freshwater fishes [40]. Also, the absence of negative effect of water quality parameters on catfish growth confirm that the combination of housefly maggots with artificial diet as a suitable feed combination to be used in culturing *Clarias gariepinus* [6].

5. Conclusion

Findings of this study has shown that growth performance and feed utilization indices were significantly better ($P\leq0.05$) in African catfish fry fed 50% artificial diet + 50% fresh housefly maggots, followed by fish fed fresh housefly maggots alone, followed by fish fed artificial diet alone under the experimental conditions. Use of the 50% fresh (wet) housefly maggots with 50% artificial diet in African catfish fry feeding had positive effect on growth performance and reduce of the feed cost.

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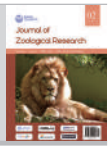

ARTICLE

Elephant Culture Matter for China's Asian Elephants Conservation

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ABSTRACT

Traditional anthropogenic impacts such as hunting, using as war-elephant, trading of ivory, paying tribute to the imperial court and so on, were once thought to be directly responsible for the rapid decline of Asian elephants in China. But in Yunnan Province, China, a unique human factor such as the traditional elephant culture of local ethnic minorities, is an important factor in the conservation of Asian elephants. In these areas, we investigated by means of village interviews, field surveys and data collection, the results show that the elephant culture of ethnic minorities has a great impact on people's thoughts and behaviors, these traditional culture and belief (that mean taking elephant as the God, holding elephant as a belief, worshipping elephant and praise it) urges people to actively protect elephants and avoid more human-elephant conflicts. To enhance the public awareness of Asian elephant conservation, the Chinese Government or international environmental organizations should give higher attention and support to these elephant cultures.

1. Introduction

Asian elephant (*Elephas maximus* L.) is one of endangered (EN) species listed by the International Union for Conservation of Nature (IUCN) and one of the first class state protected species in China [1]. Historically, Asian elephants once distributed widely in China [2]. Due to some reasons, such as hunting, using as war-elephant, trading of ivory, paying tribute to the imperial court [2,3], natural vegetation being massively occupied by agriculture and so on [4]. As a result, both population of Asian elephant and their habitats have been reduced drastically, even disappeared regionally in most parts of China [2].

And now, about 300 individuals of China's Asian elephants merely survive in Xishuangbanna, Pu'er and Lincang in Yunnan Province [4,5], where are also main living places of two ethnic minorities named Dai and Wa people in China [6,7]. Co-existing with Asian elephants, Dai and Wa people have been developing characteristic elephant culture (that is, cultural products and customs are made of elephant as a core), they all take elephant as the God, hold elephant as a belief, worship elephant and praise it [7].

Since 1989, during the 30 years of research on the ecology of Asian elephants, we have been conducting field surveys in these distribution areas of Asian elephants in China - Xishuangbanna, Pu'er and Lincang in Yunnan

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Province, China. During our work, we have come into contact with many friends of the local ethnic minorities and naturally come into contact with their rich and unique production, life and culture. We are curious about these splendid minority cultures, especially the myths, legends and stories about the Asian elephant, such as these various Asian elephant sculptures in streets, decorations with elephant elements in the architecture, the solemn riding elephant parade during the festival, the lively elephant foot drum dance. A great deal of information and data on the culture of the Asian elephant has also been collected with consultation. We found that elephant culture has ultimately slowed down the extinction of China's Asian elephants, and it played a vital role in protection of Asian elephants. To promote the development of elephant cultures for conservation of China's Asian elephants in the future, some suggestions have been given in this paper.

2. Methods

2.1 Study Area

The study area is mainly located in Nangunhe National Nature Reserve (with total area 50887 hm^2) in Cangyuan County, Lincang, Yunnan Province, and it is a national nature reserve with Asian elephants and their habitats as its main tasks. The surrounding communities of the reserve involve 9 towns, 40 villages committees, 271 villages, inhabited by 23 ethnic groups, such as Wa, Dai, Lahu, Han, Lisu, Yi, Jingpo, Bai, Blang, Tu and De'ang and so on, with a total population of 265,479. Among them, 179,925 are ethnic minorities, accounting for 67.8% of the total population. Dai is the largest and Wa is second, accounting for 30.4% and 19.2% respectively. In addition to the primitive worship of the ethnic, the community residents also believe in other four religions: Theravāda Buddhism, Christianity, Islam and Taoism, most of them believe in Theravāda Buddhism.

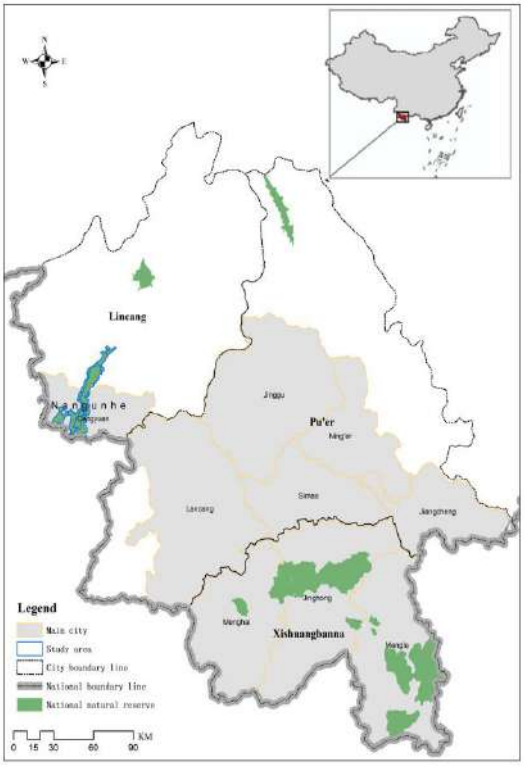

Figure 1. Map of the study area is mainly located in Nangunhe National Nature Reserve (noted as Nangunhe)

2.2 Methods

How does elephant culture motivate people to be more active in protecting elephants, we conducted interviews with community residents in the designated study area. These people we interviewed were mainly staffs of the local reserve administration and the elderly from ethnic minorities who are familiar with their cultural traditions, and also many young people. These interviews were focused on what elephants meant to them and whether they were willing to actively protect elephants, we finally collected a total of 30 valid questionnaires.

3. Result

Among these 30 people, only one is not an ethnic minority, the others include 27 Wa and 2 Dai. And the results of the survey showed that only one Wa teenagers was not concerned about elephant conservation, and all of the others were willing to protect elephants voluntarily, we also found that attitudes toward elephant conservation had nothing to do with the education of the people we interviewed.

Figure 2. The Wa people pray for the blessing of elephants during the "Elephant Worshipping Festival"

Like the surrounding communities of Nangunhe Reserve, Xishuangbanna and Pu'er, where are also main living places of Dai and Wa people. Almost all these people believe in the Theravāda Buddhism [8,9]. In one myth of

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this religion, the Buddha's mother become pregnant by dreaming of a white elephant, and give birth to him [7], that means the Buddha is the embodiment of the elephant. So elephant has the same supreme status, right and power as the Buddha, and so the elephant be worshipped, respected and protected by all believers [7]. A fairy tale of Dai people tells, the earliest female ancestor of them become pregnant by drinking elephant's urine that she had mistaken for water, so all Dai people consider themselves as elephant's descendants [7]. Another myth in the "Sigangli", the Creation Epic of Wa people, says the earliest ancestors of human live originally in a cave, then they can come out and find an ideal place to live, mainly attributed to the help of an elephant [6,10]. In all ages, Wa people inherit their own elephant culture by myths and legends of the "Sigangli", cliff paintings of elephant pictures [10], holding the "Elephant Worshipping Festival" every year [6], "Elephant Day" (that mean three days a month are belongs to elephants) [8], etc. Elephant is is honored as "Da" (means "grandpa", the term for elders by Wa people) in their own language fully expresses the respect of Wa people for elephant [8]. Both in the "Water-splashing Festival" (the New Year of Dai people) held annually by Dai people and the "Elephant Worshipping Festival" by Wa, all people should keep solemn silence in respecting elephant ceremony, while enthusiastic when dancing with elephant-foot drum [6,11]. With the theme of respecting elephant, many works of literature, poems, paintings, dances have been created by artists, and totems of white elephant appear everywhere, especially in temples and other public buildings [7].

4. Discussion

Human behaviors are strongly guided and regulated by these rich and colorful elephant cultures, thus promote people to protect elephants and their habitats consciously [6]. For instance, the forest where elephants live in is regarded as "the God's Forest" forbidding to be cut. Hunting elephant is strictly prohibited in any time and anywhere. In order to avoid disturbing elephants, on the "Elephant Day", all villagers are forbidden to enter any mountain, even cultivate in their own farmland. Whether wildlife could chronically survive in a region mainly depends on attitudes and behaviors of the locals who co-existing with them [12], besides protective efforts of the national and local government. From the ancient times to now, with various positive and significant impacts, elephant culture has played a vital role in protection of Asian elephant. It can protect elephants well, especially in the case of weak protection by local governments, and ultimately slow down the extinction of this endangered species in China.

Figure 3. During the "Elephant Worshipping Festival", people push the white elephant through the streets

Earlier studies have shown that human-elephant conflict results from human's actively harmed elephants [13], and that these injuries involve a large proportion of non-native minority populations, in addition, in recent years, non-native minorities have been hurt more by elephants. Therefore, it is reasonable to believe that the awareness of elephant protection by minority elephant culture may be one feasible way to solve human-elephant conflicts in the future.

To enhance the public awareness of Asian elephant conservation, the Chinese Government or international environmental organizations should give higher attention and support to the elephant culture. For example, the Chinese Government could make more totems and other cultural products of elephant in Xishuangbanna, Pu'er and Lincang. Especially, organizers of "Water-splashing Festival", "Elephant Worshipping Festival" or "Elephant Day" may add more elements of elephant culture in these annual festivities, as well as increase the number of participant villages and villagers. It is urgent to promote people-to-people exchanges, develop elephant culture and strengthen cooperation among government, NGOs and local people in elephant conservation.

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All articles should include a cover letter as a separate document.

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This section offers closure for the paper. An effective conclusion will need to sum up the principal findings of the papers, and its implications for further research.

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