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EFFICACY OF MARIGOLD OLEORESIN PETALS AS NATURAL CAROTENOIDS SOURCES ON SKIN PIGMENTATION AND GROWTH PERFORMANCE IN TICTO BARB PUNTIUS TICTO

Anurag Protim Das and Shyama Prasad Biswas

ABSTRACT

The present study investigated the effect of Marigold *(Tagetes erecta)* Oleoresin petals on skin pigmentation, growth performance and survival of ticto barb *Puntius ticto* under confined environment. A feeding trial of 7 weeks was done using five isonitrogenous experimental diets formulated by using the locally available ingredients and supplementing carotenoids meal at four concentrations viz. 1%, 2%, 3%, 4% and a control devoid of carotenoids. At the end of the feeding trial the total carotenoid concentration in fish muscle found significantly higher ($p < 0.05$) in both male (3.74±0.07 µgg-1) and female (3.09±0.05 µgg-1) fishes fed diet incorporated with 3% marigold oleoresin. However, there was no significant effect observed on body indices and survival rate of the fishes. Positive correlation, (0.88) in male and (0.92) female is observed between elevated levels of dietary carotenoids and body pigmentation which revealed that incorporation of dietary carotenoids resulted in a significant increase in total carotenoid concentration. The results revealed that marigold oleoresin meal as natural colour enhancer source can be safely supplemented at 3% levels in the diets of ticto barb to increase their skin pigmentation without any xenobiotic effect on ticto barb *Puntius ticto.*

Key Words: Marigold Oleoresin petals, carotenoids, *Puntius ticto*, skin pigmentation, xenobiotic affect.

INTRODUCTION

In the 21st century aquarium fish keeping has evolved as an indispensible part of interior decoration and ornamental fish culture has emerged as a million dollar industry (Das & Biswas, 2016 & Katia, 2001). Vibrant body pigmentation is one of the major quality attributes for market acceptability of the ornamental fish (Das & Biswas, 2016 & Saxena, 1994). Research established that fish do not possess the ability to synthesize carotenoids (Goodwin, 1951). The carotenoid pigmentation of fish is attributed to the

pigment present in the diet (Hata and Hata, 1973). Hence, a direct relationship between dietary carotenoids and pigmentation exists in them (Halten *et. al.,* 1995). Carotenoids are a group of naturally occurring lipid soluble organic pigments that are responsible for the red, orange and yellow colour in the skin, flesh, shell and exoskeleton of aquatic animal (Pailan *et. al.,* 2012). The ornamental fishes, when kept under captivity for long duration has experienced the problem of faded coloration. The diet for aquarium fish should be nutritionally balanced, palatable, and resistant to crumbling, water stable, and buoyant but it should also enhance body pigmentation in the fish in captivity (Das & Biswas, 2016).

Detail studies on colouration enrichment in indigenous ornamental fish are lacking (Das & Biswas, 2016). Plant sources have been harnessed for inducing pigmentation in fish. For example, *Arthrospira platensis* (Spirulina) have been used as a source of carotenoid pigments for rainbow trout and fancy carp (Choubert, 1979; Boonyaratpalin and Phromkunthony, 1986; Alagappan *et. al.,* 2004). Gouveia *et. al.,* (2003) worked with microalgal biomass supplementation have shown that *Chlorella vulgaris* is as efficient as synthetic pigments in the pigmentation of *Cyprinus carpio* and *Carassius auratus*. Alagappan *et. al.,* (2004) studied the utilization of algae *Spirulina* sp. as a source of carotenoid pigment for *Trichogaster trichopterus*. Marigold *(Tagetes erecta)* is a hardly annual branching herb grown in tropical regions of India as loose flower and landscape plant but also as a source of natural carotenoid pigment (Usha Bharathi *et. al.,* 2014). Marigold *(Tagetes erecta)*, a bright orange tropical flower are a significant source of xanthophylls (Verghese, 1998) and contains lutein as a principal component (Bolanos *et. al.,* 2005). Marigold petal meal was used for the tiger barb *(Puntius tetrazona)*, red swordtail *(Xiphophorus helleri)* and gold fish *(Carassius auratus L.)* (Boonyarapatin and Lovell, 1977; Ezhil *et. al.,* 2008; Alma *et. al.,* 2013) to enhance body pigmentation. The present study was carried out to evaluate the efficacy of supplementation of graded levels of marigold oleoresin *(Tagetes erecta)* on the coloration and body indices of the Ticto barb *Puntius ticto,* one of the beautiful barb species for tropical aquarium culture.

MATERIALS AND METHODS

Experimental Design

Ticto barb *Puntius ticto,* of uniform size group were collected from different water bodies such as wetlands and small ditches of Dibrugarh district for the experiment. Following collection, were transferred to aquaria of 50 litre capacity with continuous aeration and were acclimatized for 10 days in laboratory conditions before the onset of the experiment. The experiment was conducted for a period of 7 weeks and was carried out in 15 aquaria of 50 litre capacity. These 15 aquaria were grouped into 5 sets

and each set consists of 3 replicates. The fishes were stocked at the rate of 15 per aquarium in which 10 were males and 5 were females in each trough. The length and weight of fishes were recorded at weekly intervals. The fishes were fed twice daily viz. morning and evening at the rate of 3% of body weight per day. The feed was adjusted after every 10 days according to the body weight of the fish. Aquaria were cleaned properly by siphoning off feed particles and metabolic wastes daily from the bottom. Water exchange was carried out weekly for the sufficient supply of oxygen to the fish.

Diet Formulation

For the feeding trial dry pelleted feed was formulated following Pearson Square method and trail & error method of Hardy (1980). The control feed was prepared using the basic ingredients like fish meal 25%, soya bean meal 22%, groundnut oil cake 15%, rice bran 20% and wheat flour 12%. The selected ingredients were ground, thoroughly mixed and dough was prepared by adding required amount of water. This dough was steamed in water bath for 40 min. at 70°C. After this the dough was cool for a while and added other ingredients like Soya oil 2%, starch powder 3%, vitamins and minerals premixes 1% were added and mixed thoroughly. Marigold *(Tagetes erecta)* Oleoresin petals were dried in Lypholizer and finely powdered and subsequently added in respective concentration of 1, 2, 3 and 4g/100g of basal diet by replacing same quantity of rice bran for the preparation of treatment diets (C1 to C4). This was done to protect these micro nutrients from heat. The dough was taken into hand pelletizer to make 2.0 mm pellets. The pelleted feed was air dried and kept in airtight containers until further use.

Assessment of carotenoid concentration, proximate composition of feed, feed conversion ratio (FCR), protein energy ratio (PER), survival and water quality parameters

Proximate compositions of the experimental diets were analyzed for moisture, crude protein, ether extract and total ash following AOAC, 2005. Quantitative estimation of crude fat was done following Das and Biswas (2019). Estimation of carotenoids in fish feed was carried out following Britton (1995) with improvisations. Total carotenoid concentration (TCC) in the muscle tissue of both male and female fishes was analyzed at an interval of 15 days and after the completion of experiment following Olson (1979). All procedures were performed in low light and temperature as carotenoids are very sensitive to light, temperature and oxygen. Feed conversion ratio (FCR) was calculated by relating the feed consumption to gain in wet weight of fish following Vasudhevan *et. al.,* (2013), protein energy ratio (PER) was calculated by relating wet weight gain to protein fed and the survival rate was estimated following Francis (1995). Water quality parameters viz. water temperature; pH, dissolved oxygen, total hardness,

and alkalinity from all the aquaria's were analyzed at fortnightly intervals as per standard methods of APHA (1998) and light intensity was measured using software Lux Meter of Microsoft Lumia.

RESULTS

Water Quality Parameters

Physico-chemical parameters of water in the experimental tanks was estimated weekly during the experimental period are presented in Table 1. The water quality parameters were maintained within the normal range required for tropical fishes viz., temperature 24 to 30°C, dissolved oxygen > 5 mg and pH 7 to 8.5 (Santhosh and Singh, 2007) throughout the experimental period. High adaptability of ornamental fishes to culture conditions enhances their capability of living in environments having wide ranges (Chapman, 2000).

Proximate Composition Analysis, Growth and Survival

Analyzing the proximate composition all the experimental diets was almost similar (Table 2). The similarities in the nutrients composition can be traced to the feed ingredients inclusions, which were only differentiated by the level of carotenoids. In the present experiment the protein content of the diets varied from 30.39 to 31.47. In general for rearing and maintenance of ornamental fishes high protein feed is not required. For the growth of guppy *(Poecilia reticulate)* 30% protein in diet is optimum (Fah and Leng, 1986). Shirley *et. al.,* (2006) reported 30% protein and 12% lipids level are optimum for growth and of sword tail *(Xiphophorus helleri)*. Loachmann and Phillips (1994) found 29% protein sufficient for optimum weight gain, feed conversion, protein efficiency ratio of goldfish *(Carrasius auratus)*. and the protein content in the diets was approximately 30 %. The survival and growth rates of the fishes at end of the experimental period are shown in Table 3. There were no significant effects of marigold oleoresin supplementation on growth parameters of Pool barb. However, fishes fed with 3% marigold oleoresin supplemented diet showed marginally increased values in net weight gain (0.71±0.01 g) in comparison to the other treatments and control group. Feed conversion ratio (FCR) in control group was 1.81±0.05 and the corresponding values in different experimental groups ranged from 1.74±0.06 to 1.79±0.04, highest being in CF3 and lowest being in CF4. The FCR values in CF1, CF2 and CF4 groups was significantly lower ($P<0.05$) than the other groups (Table 3). Feed conversion ratio and feed efficiency ratio are considered as indices for feed utilization. In the present study results clearly showed that increased level of supplementation of carotenoids in diets reduced FCR in the fish to a considerable extent. Optimum FCR was obtained by Christiansen and Torrissen, (1996) in Atlantic salmon juveniles fed with astaxanthin

supplemented diet. Similarly, in *Penaeus monodon,* better FCR was observed with prawn fed on the diet containing spirulina *(Arthrospira platensis)*. Dietary supplementation of marigold oleoresin did not show significant affect on the survival rate of the Pool barb with treatment diets and control diet ranged from 90 % to 100 %. The results showed that different level of supplementation of marigold oleoresin in the treatment diets has no effect on survival % of fish. Lower survival of Atlantic salmon *(Salmo salar)* fry (Christiansen *et al.,* 1995) and juveniles (Christiansen and Torrissen, 1996) was found when fed with diets without asthaxanthin supplementation in comparison to the groups that were fed diets containing asthaxanthin. The results obtained in the present investigation may be attributed to lower densities and /or the hardiness of species, since ticto barb are very hardy species and survive under extreme conditions.

Effects of the Experimental Diets on Total Carotenoid Concentration (TCC) of Ticto Barb Skin

At the beginning of the experiment the total carotenoids concentration in the muscle and skin of Pool barb was 1.28g/g wet weight. Total carotenoids concentration in the muscle and skin of Pool barb after 45 days of experimental feeding trial clearly showed that the total carotenoids concentration increased with the supplementation of marigold petals meal in the diet, highest being in 3% levels (3.74±0.07 g/g wet weights) in male and (3.09±0.06 g/g wet weights) in case of female, and beyond that no further significant increase in carotenoids content was found in both the sexes (Table 3). Positive correlation is observed between increasing dietary carotenoids level to increasing skin and muscle carotenoids concentration in both male (0.88) and female (0.92) fishes. Kalinowski *et. al.,* (2005) described the effect on growth and skin colour in *Pagrus pagrus* when fed with different carotenoid sources with their dietary levels. Ezhil *et al.,* (2008) observed enhancement of pigmentation in *Xiphophorus helleri* when fed with formulated feed containing *Calendula officinalis* concluding that this lutein can be used as pigmenting source of natural origin.

Table 1 Ecological parameters recorded in experimental tanks

Parameter	Minimum	Maximum
Temperature (°C)	19±1	24±2
pH	7.4±0.3	7.8±0.2
Dissolved oxygen (ppm)	5.3±0.9	7.6±0.5
Light intensity (Lux)	320.6±13.5	344.0±2.4

Table 2 Proximate composition (%) of basal and experimental diets

Diet	Moisture	Crude fat	Crude protein	Crude Fibre	NFE	Total Ash
Control	7.56±0.02	8.3±0.01	30.67±0.02	6.28±0.02	35.77±0.03	11.42±0.02
CF1	7.54±0.03	8.1±0.03	30.63±0.01	6.26±0.01	36.02±0.02	11.45±0.01
CF2	7.63±0.01	8.3±0.01	30.60±0.03	6.24±0.02	35.80±0.02	11.43±0.02
CF3	7.59±0.02	8.1±0.02	30.59±0.02	6.26±0.01	35.80±0.01	11.41±0.01
CF4	7.56±0.02	8.3±0.01	30.67±0.02	6.28±0.02	35.77±0.03	11.42±0.02

CF- Carotenoids feed with numbers referring to percentage of carotenoids incorporated per 100gm of basal ingredients, FCR- Feed conversion ratio, PER- Protein energy ratio
Nitrogen free extract = 100 - (% moisture + % crude protein + % crude lipid + % crude fibre + % ash)

Table 3 Mean survival, weight, length, and FCR of Pool barb after 45 days of trial

Diet	Increase in weight(gm)	Increase in length(cm)	FCR	PER	Survival %	Carotenoids% (g/gm wet weight)	
						Male	Female
Control	0.45 ±0.01	0.33±0.02	1.81±0.05	0.34	100	2.67±0.08	2.24±0.09
CF 1	0.62±0.01	0.40±0.01	1.74±0.06	0.37	100	3.39±0.04	2.66±0.06
CF 2	0.67±0.01	0.44±0.02	1.77±0.03	0.39	100	3.56±0.10	2.74±0.03
CF 3	0.71±0.01	0.50±0.02	1.79±0.04	0.37	100	3.74±0.07	3.09±0.06
CF 4	0.69±0.01	0.47±0.01	1.76±0.04	0.38	100	3.71±0.04	2.99±0.07

The means with different superscript in each row indicate a significant difference ($p < 0.05$).

DISCUSSION

Dietary carotenoids play an important role in the regulation of skin and muscle colour in fish (Ahilan et. al., 2008). Feeding is a complex behavior that is closely associated with food intake. The present study enunciates formulated diets incorporated carotenogenic biomass revealed stabilization and enhancement of body pigmentation

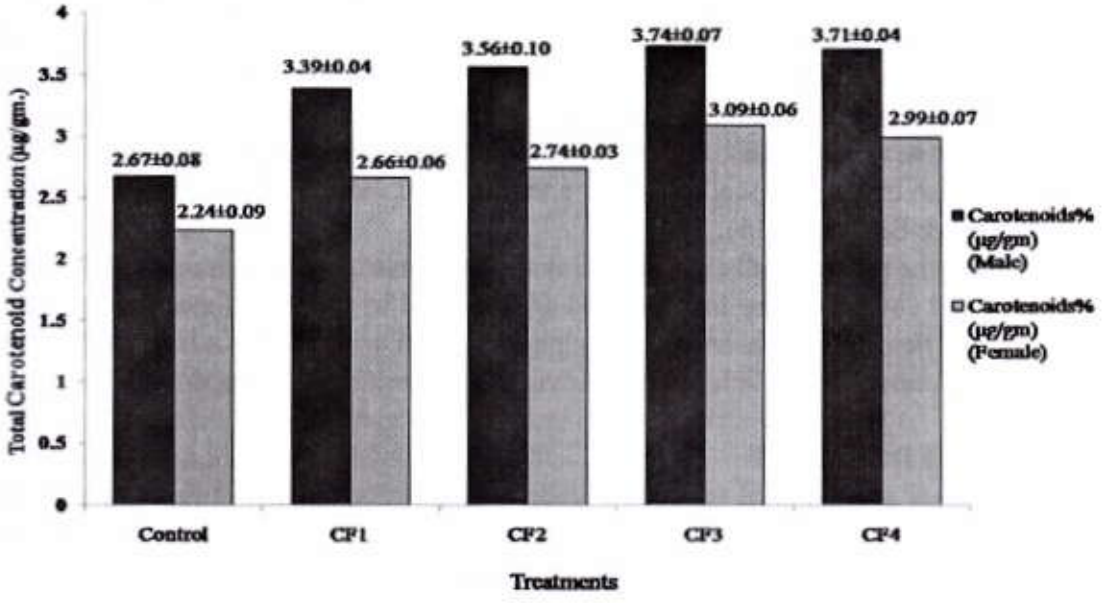

Figure 1 Total Carotenoid Concentration (g/gm) of male and female fishes fed with diets containing graded levels of marigold oleoresin.

during the maximum study period. The carotenoid source and its effectiveness on deposition of pigments in fish body is species specific (Ha *et. al.*, 1993). Also sexual dichromatism is often common in several fish species. In general attire males are more brightly coloured than their female counterparts (Gogoi *et. al.*, 2013). In the present feeding trial even female fishes fed with carotenoid diets also exhibit the remarkable pigmentation due to increased total carotenoid concentration in their body. Fish species may exhibit varying pathways for carotenoid metabolism (Matsuno, 2001). Alma *et. al.*, (2013) reported that 200 mg of carotenoids from marigold meal is optimum to increase the pigmentation in skin of goldfish and over that level they have not found any additional accumulation of carotenoids. Similarly, in the present study, fish fed with 3% marigold oleoresin diet showed increased level of carotenoids in their muscle and skin tissue than other treatment fishes. The elevated levels of marigold oleoresin did not show any increase in the colour of the fishes. The dietary supplementation of marigold oleoresin did not have any remarkable effects on the body indices of ticto barb. The results obtained in the present study are comparable with the recent studies conducted by Ramamoorthy *et al.* (2010) and Alma *et. al.* (2013) where they reported that incorporation of marigold meal in fish diet do not promote the growth of ornamental fish. Similar studies on *Etroplus*

maculatus fed with diet having marigold oleoresin contained the highest total carotenoid concentration (Jagadeesh *et. al.*, 2014). Due to the adverse effects of synthetic carotenoids on aquatic environment, many natural plant sources can be harnessed and incorporated in formulated feeds for colour retention or enhancement of ornamental fishes in captive environment. It will aid in creation of avenues for promotion of the ornamental fish industry as well as colour enhancer feed industry and employment generation (Das & Biswas, 2016).

From the present study it is recommended that marigold oleoresin meal as natural carotenoid source can be incorporated @ 3% level in the formulated diet of ticto barb *Puntius ticto* to obtain desired body pigmentation without any adverse effect on growth, feed conversion efficiency and survival in aquarium conditions.

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REFERENCES

Ahilan, B., Jegan, K., Felix, N. and Ravaneswaran, K. (2008). Influence of botanical additives on the growth and colouration of adult goldfish, Carassius auratus (Linnaeus). Tamil Nadu Journal of Veterinary and Animal Sciences. 4(4): 129-134

Alagappan, M.,. Vijula, K and Sinha, A. (2004). Utilization of spirulina algae as a source of carotenoid pigment for blue gouramis (Trichogaster trichopterus Pallas). Journal of Fisheries and Aquatic Science. 10: 1-11

Alma, A. del Villar-Martinez., Juan., Orbe-Rogel., E. C, Vanegas- Espinoza., P, Quintero- Gutierrez, A. G. and Lara-Flores, M. (2013). The effect of marigold (Tagetes erecta) as natural carotenoid source for the pigmentation of goldfish (Carassius auratus L.). Research Journal of Fisheries and Hydrobiology. 8(2): 31-37

AOAC, (2005). Official Methods of Analysis of AOAC, 18th edn. (Horwitz, W. Ed), AOAC, Washington. D.C. 1094

PHA, (1998). Standard Methods for the Examination of Water and Waste Water. Association of Official Waste Water. 20th ed. American Public Health Association, Washington, DC, USA

Bolanos, N., Rangel-Cruz, J. L., Jimenez-Islas, C. L., Botello-Alvarez, H. E. and Rico-Martinez, R. (2005). Pre-treatment effects on the extraction efficiency of xanthopylls from marigold flower (Tagetes erecta) using hexane. Food Research International. 38: 159-165

Boonyarapatin, M. and. Lovell, R. T. (1977). Diet preparation for aquarium fish. Aquaculture. 12: 53-62

Boonyaratpalin, M. and Phromkunthong, W. (1986). Effects of carotenoid pigments from different sources on colour changes of fancy carp, Cyprinus carpio Linn. Journal of Food Science and Technology. 8: 11-20

Britton, G. (1995). UV/Visible Spectroscopy, Carotenoids: Spectroscopy, Vol 1B. Birkhäuser Verlag, Basel

Chapman, F.A. (2000). Ornamental fish culture, freshwater. Page 602-610 in Encyclopedia of Aquaculture Stickney, R.R. Ed., John Wiley & Sons, New York

Choubert, G. 1979. Tentative utilization of spirulina algae as a source of carotenoid pigments for rainbow trout. Aquaculture. 18: 135-143

Christiansen, R., Lie, O. and Torrissen, O.J. (1995). Growth and survival of Atlantic salmon, Salmo salar L., fed different dietary levels of astaxanthin. First-feeding fry. Aquaculture Nutrition, 1: 189-198

Christiansen, R. and Torrissen, O.J. (1996). Growth and survival of Atlantic salmon, Salmo salar L. fed different dietary level of astaxanthin. Juveniles. Aquaculture Nutrition. 2: 55-62

Das, A. P., and Biswas, S.P. (2016). Carotenoids and Pigmentation in Ornamental Fish. Journal of Aquaculture & Marine Biology 4(4): 00093. DOI:10.15406/jamb.2016.04.00093

Das, A. P., and Biswas, S.P. (2019). Improvised protocol for quantitative determination of crude fat in fish feeds. Journal of Aquaculture & Marine Biology. 8(4):144146. DOI: 10.15406/jamb.2019.08.00253

Ezhil, J., Jeyanthi, C. and Narayanan. M. (2008). Marigold as a carotenoid source on pigmentation and growth of red swordtail, Xiphophorus helleri. Turkish Journal of Fisheries and Aquatic Sciences. 8: 99-102

Fah, S.K. and Leng, C.Y. (1986). Some studies on protein requirement of the guppy, Peocilia reticulata (Peters). Journal of Fisheries and Aquatic Science 4:325-335

Francis, O.N. (1995). Hatchery Propagation of Five Hybrid Groups by Artificial Hybridization of Clarias gariepinus (B.) and Heterobranchus longifillis (Val.) Clariidae, using Dry Powdered Carp Pituitary Hormone. Journal of Aquaculture in the Tropics, 10: 1-11

Gogoi, R., Behera, S., Chetia Borah, B. and Bhuyan, S. (2013). Sexual dimorphism and gonadal development of a rare murrel species Channa bleheri (Bleher) in Assam. The Bioscan. 8(4): 1265-1269

Goodwin, T.W. (1951). Carotenoids in fish. In: The biochemistry of fish. Biochemical Society Symposia

Gouveia, L. P., Rema, O., Pereira. and Empis J. (2003). Colouring ornamental fish (Cyprinus carpio and Carassius auratus) with micro algal. Aquaculture Nutrition. 19: 123-129

Ha, B. S., Kang, D. S., Kim, J. H., Choi, O. S. and Ryu, H. Y. (1993). Metabolism of dietary carotenoids and effects to improve the body colour of cultured flounder and red sea bream. Bulletin of the Korean Fisheries Society. 26: 91-101

Halten, B., Aas, G.H., Jorgensen, E.H., Storebakken, T. and Goswami, U.C. (1995). Pigmentation of 1, 2 and 3 years old Arctic charr (Salvelinus alpinus) fed two different dietary astaxanthin concentrations. Aquaculture. 138: 303-312

Hardy, R. (1980). Fish feed formulation, In: Fish feed Technology, Agriculture development coordination programme. FAO/ADCP/Rep. 80(1-1): 233-239

Hata, M. and Hata, M. (1973). Studies on astaxanthin formation is some freshwater fishes. Tohoku. Journal of Agricultural Research. 24(4): 192-196

Jagadeesh, T. D., Shivananda Murthy, H., Swain, H., Chetan, N., Manjunatha, A.R. and Baglodi, V. (2014). Effect of marigold oleoresin on growth, survival and pigmentation in orange chromide, Etroplus maculatus (Bloch, 1795). Fishery Technology. 51: 25-30

Kalinowski, C.T., Robaina, L.E., Fernadez-Palacios, H., Schuchardt, D. and Izquierdo, M.S. (2005). Effect of different carotenoid sources and their dietary levels on red porgy (Pagrus pagrus) growth and skin colour. Aquaculture, 244: 223-231

Katia, O. (2001). Ornamental fish trade. INFOFISH International 3:14-17

Lochmann, R.T. and Philips, H. (1994). Dietary protein requirement of gold shines (Notemegonus crysoleucas) and gold fish (Carassius auratus) in Aquaria & Aquaculture. 128:277-285

Matsuno, T. (2001): Aquatic animal carotenoids. Fisheries Sciences. 67: 771-783.
Olson, J.A. (1979). A simple dual assay for Vitamin A and carotenoids in human liver. Nutrition Reports International. 19: 808-813

Pailan, G. H., Sinha, A. and Kumar, M. (2012). Rose Petals Meal as Natural Carotenoid Source in Pigmentation and Growth of Rosy Barb (Puntius Conchonius). Indian Journal of Animal Nutrition. 29(3): 291-296

Ramamoorthy, K., Bhuvaneswari, S., Sankar, G. and Sakkaravarthi, K. (2010). Proximate composition and carotenoid content of natural carotenoid sources and its colour enhancement on marine ornamental fish Amphiprion ocellaris (Cuveir 1880). World Journal of Fish and Marine Sciences. 2(6): 545-550

Santhosh, B. and Singh, N. P. (2007). Guidelines for water quality management for fish culture in Tripura, ICAR Research Complex for NEH Region, Tripura Centre, Publication no. 29

Saxena, A. (1994). Health; colouration of fish. Page No. 94 in Proc. of Int. Symposium on Aquatic Animal Health. University of California, School of Veterinary Medicine, Davis, CA, U.S.A. Saxena

Shirley, L., Roshada, H., Sagiv, K. and Chong Shu-Chien, A. (2006). Effect of varying dietary lipid and protein levels on growth and reproductive performance of female swordtail, Xiphophorus helleri (Poeciliidae). Aquaculture Research, 37: 1267-1275

Usha Bharathi, T., Jawaharlal, M., Kannan, M., Manivannan, N. and Raveendran, M. (2014). Correlation and path analysis in African marigold (Tagetes erecta L.). The Bioscan. (Supplement on Genetics and Plant Breeding) 9(4): 1673-1676

Vasudhevan, I., Asokan, K., Rinna Hamlin, S. and Rama Devi, P. (2013): Feed utilization, Growth parameters and Coloration of Different Feeds in Gold Fish (Carassius auratus). International Journal of Research in Biotechnology and Biochemistry ISSN: 2277-3827

Verghese, J. (1998): Focus on xanthophylls from Tagetes erecta L. the giant natural complex I. Indian Spices. 33(4): 8-13

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A GENETIC STUDY OF THE WAR JAINTIAS OF NONGTALANG VILLAGE, WEST JAINTIA HILLS DISTRICT, MEGHALAYA

Merry C. Passah and D. K. Limbu

ABSTRACT

In the present study an attempt has been made to study the genetic variation among the War Jaintia of Nongtalang village with the help of Genetic markers such as ABO and Rh(D) blood groups, Phenylthiocarbamide (PTC) taste sensitivity and colour blindness. A total of 207 individuals were tested, which comprised 101 males and 106 females. Blood samples were collected following standard slide methods suggested by Lawler and Lawler (1951) and Bhatia (1977) for ABO and Rh(D) blood grouping. Serial dilution method suggested by Harris and Kalmus (1949) was followed to collect data on PTC taste sensitivity and Ishihara Chart (1959) was used to collect data on Colour blindness. In the present population, it was found that with respect to ABO blood group, the percentage frequencies of O, A, B and AB blood groups were 42.03%, 26.57%, 26.57% and 4.83% respectively. It shows that O blood group has the highest frequency, followed by A, B and least is the AB. Out of 207 subjects tested, only 3 (1.45%) were reported to have Rh-negative blood group. The frequency of colour blindness in the present population is 2.42% which is quite high compared to the other Khasi sub tribes. In respect of PTC taste sensitivity, the allele frequency of taster (T) was found to be 0.3480 and non-taster (t) was 0.6520. The War Jaintias of Nongtalang deviate significantly from other neighbouring populations of Meghalaya.

Key Words: ABO and Rh(D) Blood Group, PTC Taste Sensitivity, Colour Blindness, War Jaintia, Nongtalang

INTRODUCTION

Biological Anthropology deals with the comparative biogenetics of man. Within the various fields of the Biological Anthropological research, the study of human evolution as well as the study of genetic variation holds an eminent place. Population Genetics, an important branch of Biological Anthropology deals on the one hand with exact genetic descriptions of human population and on the other hand it tries to find out the

reasons for genetic differences among them. Such exact and comprehensive descriptions are the basic requirements for the understanding of genetic variation in man and thus for the analysis of the various evolutionary factors, which caused this variation in the course of time. To study these ongoing genetic differentiation processes in man, reliable population data are necessary.

Anthropological studies have generated huge amounts of biological data among Indian populations, which in turn can be used to understand the peopling of India. A number of genetic markers have been usefully employed in investigation of population genetic studies. ABO blood group system is the most studied trait in human genetics followed by the ability to taste phenylthiocarbamide (PTC) and colour-blindness traits. These three traits have been extensively used in describing genetic variations among human populations around the world. In the present study an attempt has been made to describe the genetic composition of the War Jaintias of Nongtalang village with the help of Genetic markers such as ABO and Rh(D) blood groups, Phenylthiocarbamide (PTC) taste sensitivity and colour blindness and also to compare the findings of the present study with those reported on other populations of Meghalaya and Assam.

OBJECTIVES

1. To describe the genetic composition of the War Jaintias of Nongtalang village with the help of some genetic markers such as ABO and Rh (D) blood groups, PTC taste blindness and colour blindness traits.
2. To compare the findings of the present study with those reported on other populations of Meghalaya and Assam.

LAND AND PEOPLE

West Jaintia Hills District is one of the eleven districts of the state of Meghalaya. With the bifurcation of the erstwhile Jaintia Hills District into East and West Jaintia Hills Districts, West Jaintia Hills District came into existence on 3 1st July 2012 with its Head Quarter at Jowai. Jowai is the host of all the heads of important governmental offices and establishments, educational institutions, hospitals, banking institutions, etc. The total area of the district is 1693 Sq.kms. The district has a total population of 2, 70,352.

The present study was conducted among the War Jaintias of the Nongtalang village which falls under the West Jaintia Hills District of the state of Meghalaya. The village is about 45 km from Jowai which is the Headquarters of West Jaintia Hills District. It is approximately 95 km away from the State Capital City, Shillong and just 13 km away from Bangladesh. The War Jaintias apart from being a sub tribe of Khasi Jaintia, they

also have their own distinct dialect (also called War) that neither the Pnar of Jaintia nor the Khasi can understand except those who come in daily contact with them. The War Jaintias of the Nongtalang Village are matrilineal form of society. Descent is always through the female line; succession is also through the female line. Majority of the people in Nongtalang village believe in the indigenous religion known as "NiamTre". There are also Christian followers but they form the lesser number of the population. Rice is the staple food of the people. Nongtalang is predominantly an agrarian society. The main agricultural products include areca nut (Areca catechu), commonly called as betel nut and betel leaves (Piper betel). The economic structure of the village depends mainly on the trading of these crops.

MATERIALS AND METHODS

The present study has been carried out among the War Jaintias of Nongtalang village which is located in the West Jaintia Hills District of Meghalaya. Blood samples from 207 subjects (101 males and 106 females) were collected following the standard slide methods suggested by Lawler and Lawler (1951) and Bhatia (1977) for ABO and Rh(D) blood grouping. Data on PTC taste sensitivity were collected, following the serial dilution method suggested by Harris and Kalmus (1949). For collecting data on colour- blindness, the Ishihara Chart (1959) was used.

For calculating the gene frequencies of the ABO blood groups, PTC taste sensitivity and colour-blindness, the method suggested by Bernstein (1930) was followed. The test for goodness of fit was also applied for testing the genetic equilibrium of the present population with respect to the allele frequencies of the ABO blood groups, following the method described by Bernstein (1930). Chi square test was used for determining the differences between sexes or populations with respect to the distribution of the ABO and Rh (D) blood groups and PTC taste sensitivity and Colour-blindness. The overall genetic difference between the present population and the neighbouring populations was calculated, using the above mentioned genetic markers and the secondary data available from the neighbouring populations with a view to understand the genetic affinity of the present population.

RESULTS

ABO Blood Groups

Table1 shows that the percentage frequencies of A, B, AB and O are 24.75%, 24.77%, 7.92%, 44.55% and 28.30%, 30.19%, 1.89% and 39.62% in males and females respectively. These differences between sexes in respect of the phenotype distribution of the

Table 1 Phenotypic and genotypic allele frequencies of ABO blood groups

Sl. No.	Phenotype	Males (N101)		Females (N106)		Total (N207)		Phenotype Frequency
		No.	%	No.	%	No.	%	
1	A	25	24.75	30	28.30	55	26.57	0.2657
2	B	23	24.77	32	30.19	55	26.57	0.2657
3	AB	8	7.92	2	1.89	10	4.83	0.0483
4	O	45	44.55	42	39.62	87	42.03	0.4203
Allelic Genotype Frequency p=0.1724 q=0.1724 r=0.6552 Difference between sexes: χ^2=5.5146, d.f.=3, p>0.05 Goodness of fit for Hardy-Weinberg equilibrium: χ^2=131.031, d.f.=1, p<0.05								

ABO blood groups are statistically insignificant (χ^2=5.5146, d.f. = 3, p > 0.05). Since the ABO locus is an autosomal in character, data on both the males and females were pooled together to find out the allele frequencies of the ABO blood groups. Thus, combining the data of both the sexes, the percentage frequencies of A, B, AB and O are found to be 26.57%, 26.57%, 4.83% and 42.03% respectively. It is also observed that, in respect to the ABO blood groups, the frequency of 'O' blood group is highest (42.03%) followed by blood group 'A' and 'B' being 26.57% each.

Following the methods given by Bernstein (1930) and Balakrishnan (1988), the calculated gene frequencies of p, q and r are 0.1724, 0.1724 and 0.6552, respectively. Applying the test of goodness of fit for Hardy-Weinberg equilibrium, it is found that the difference between these allele frequencies in the present population are statistically significant (χ^2 =131.031, d.f. =1, P<0.05). Thus, it indicates that the War Jaintia is not in genetic equilibrium in respect of ABO locus.

Rh (D) Blood Group

Table 2 Phenotype and allele frequencies of Rh(D) blood group

Sl. No.	Phenotype	Males (N101)		Females (N106)		Total (N207)		Phenotype Frequency
		No.	%	No.	%	No.	%	
1	Rh-Positive	99	98.02	105	99.06	204	98.55	0.9855
2	Rh-Negative	2	1.98	1	0.94	3	1.45	0.0145
Allele Frequency D=0.8796 d=0.1204 χ^2=6.1101, d.f.=1, p<0.05								

Table 2 shows that there are only three Rh-negative subjects in the present population, out of which two are male and only one is female. The percentage distribution of Rh negative phenotypes in the present population is 1.45%. The allele frequency of D and d are 0.8796 and 0.1204 respectively. The chi-square value for sex difference is statistically significant (χ^2=6.1101, d.f. =1, p<0.05) which reveals that there are some differences exist between sexes.

PTC Taste Sensitivity

Table 3 shows the data on taste sensitivity to PTC for both males and females. It is found that the mean threshold value is 5.71 ± 0.62 and 6.29 ± 0.70 for males and females respectively. Thus, it indicates that the mean threshold value is higher in females than in males however, the differences between the two sexes are not statistically significant (t=0.6237, d.f. =205, p>0.05). Therefore, the present data for both the sexes were pooled together for classifying the population into tasters and non-tasters. Moreover the gene responsible for not being able to taste PTC compound is believed to be an autosomal one.

In order to classify the individuals into tasters and non-tasters, we followed the method suggested by Harris and Kalmus (1949) in which the number of individuals being able to taste PTC compound solution was plotted against the serial dilution numbers of PTC solution which was presented as shown in the Figure 1.

Table 3 Distribution of PTC taste sensitivity

Threshold Solution Number	Males (N=101)	Females (N=106)	Total (N=207)
1	11	3	14
2	14	12	26
3	5	4	9
4	18	17	35
5	15	25	40
6	7	9	16
7	7	9	16
8	2	6	8
9	1	2	3
10	0	0	0
11	0	1	1
12	0	0	0
13	0	0	0
14	0	0	0
Mean Threshold ± S.E	5.71 ± 0.62	6.29 ± 0.70	12 ± 0.90
Student's t test = 0.6237, d.f.=205, p>0.05			

Figure 1 The distribution of PTC tasters and non-tasters

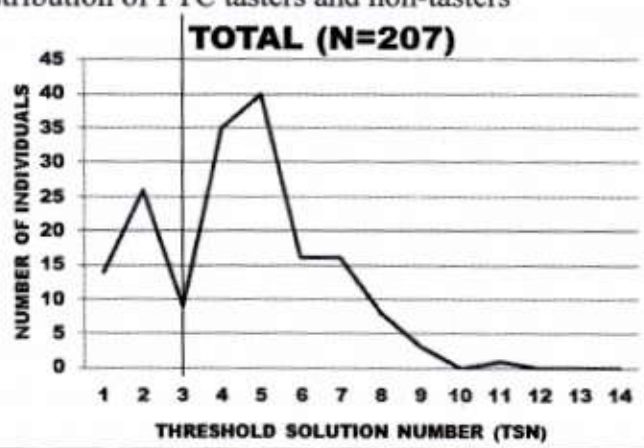

Figure 1 shows that the distribution of PTC taste sensitivity in the present population follows a bimodal distribution in which the antimode falls on 3. Thus the cut-off point of 3 was considered for classifying the individuals into tasters and non-tasters. Thus, individuals who had the threshold value above 3 or who perceived taste in a more diluted solutions were considered as tasters while those with the threshold value of 3 and below or who perceived taste in a more concentrated solutions were regarded as non-tasters. The frequency of tasters and non-tasters is given in Table 3.

Table 4 Frequency of Tasters and Non-tasters for PTC

Sl. No.	Phenotype	Males (N=101)		Females (N=106)		Total (N=207)		Phenotype Frequency
		No.	%	No.	%	No.	%	
1	Tasters	50	70.30	69	82.08	119	76.33	0.7633
2	Non-tasters	51	29.70	37	17.92	88	23.67	0.2367
Allele Frequency T=0.3480 t=0.6520 χ^2=5.1395, d.f.=1, p<0.05								

Table 5 Frequency distribution of Colour Blindness

Sl. No.	Phenotype	Males (N=101)		Females (N=106)		Total (N=207)		Phenotype Frequency
		No.	%	No.	%	No.	%	
1	Normal	97	96.04	17705	99.06	202	97.58	0.9758
2	Red-Green Deficiency	4	3.96	1	0.94	5	2.42	0.0242
Allele Frequency C=0.8444 c=0.1556 χ^2=1.9962, d.f.=1, p>0.05								

Table 4 shows that the frequency of non-tasters is higher among the males than in females. However, the chi square test indicates that the difference between males and females are statistically significant (X^2=5.1395, d.f. =1, P<0.05). The gene allele frequencies for tasters (T) and non-tasters (t) were found to be 0.3480 and 0.6520 respectively.

Colour Blindness

It is observed from Table 5, that only 5 subjects that is, 2.42% were colour-blind. The gene frequencies for colour blindness for red-green deficiency (c) and normal (C) found to be 0.1556 and 0.8444 respectively.

Genetic Affinity

Here we shall compare and describe our findings with those published on other neighbouring populations of Meghalaya and Assam with a view to understanding the genetic affinity of the present population.

ABO Blood Group

Table 6 Phenotypic frequencies of ABO blood group of war Jaintia of Nongtalang village and other populations of Meghalaya and Assam

Sl.No	Population	Sample Size (N)	Phenotype Frequency				Reference
			A	B	AB	O	
1	Garo	107	30	36	14	27	Nchang (2006)
2	Bodo	402	113	141	45	103	Das *et. al.* (1980)
3	Mikir	245	81	65	25	74	Das *et. al.* (1980)
4	Rabha	834	275	249	105	205	Das (1987)
5	Bhoi	192	59	44	15	74	Das (1978)
6	Marngar	160	58	34	21	47	Chumikam(2002)
7	Khynriam	222	65	41	7	109	Das (1978)
8	Pnar	197	66	23	4	104	Das (1978)
9	War	230	66	28	8	128	Das (1978)
10	Lyngam	120	47	34	15	24	Ahmed *et. al.* (1997)
11	War Jaintia of Nongtalang	207	55	55	10	87	Present study

Table 6 shows that the War Jaintias are characterized by a high frequency of blood group 'O' and low frequency of blood group 'AB' as compared to Garo, Bodo, Mikir, Rabha and Marngar and is by and large similar to the other Khasi populations as reported by Das (1978).

In order to have a better understanding of the genetic relationship of the War Jaintia with the compared populations, the Chi-square test was done to test the differences between them if any with respect to distribution of the ABO blood groups.

Table 7 Chi-square value of ABO blood group between War Jaintia of Nongtalang village and the other populations of Meghalaya and Assam

Sl. no.	Population Compared	Chi-square (2)Value	d.f.	Significance Level
1	War Jaintia vs Garo	13.04*	3	Significant
2	War Jaintia vs Bodo	21.10*	3	Significant
3	War Jaintia vs Mikir	10.16*	3	Significant
4	War Jaintia vs Rabha	29.80*	3	Significant
5	War Jaintia vs Bhoi	2.85	3	Insignificant
6	War Jaintia vs Marngar	15.12*	3	Significant
7	War Jaintia vs Khynriam	5.36	3	Insignificant
8	War Jaintia vs Pnar	17.97*	3	Significant
9	War Jaintia vs War	16.65*	3	Significant
10	War Jaintia vs Lyngam	20.66*	3	Significant

*Significant at 5% level of probability

It is observed from Table 7, that the difference between the War Jaintia of Nongtalang village and majority of the compared populations are statistically significant in respect to the frequencies of the ABO blood groups that is, the present population deviates significantly from all the populations except with Khynriam and Bhoi in respect of the above mentioned blood group.

Rh(D) Blood Group

Table 8 Frequency distribution of Rh(D) blood group of War Jaintia of Nongtalang village and other populations of Meghalaya and Assam

SL. No.	Populations	Sample Size (n)	Rh-negative	Rh-positive	Reference
1	Garo	107	-	107	Nchang (2006)
2	Bodo	402	1	401	Das *et. al.* (1980)
3	Mikir	134	2	132	Das *et. al.* (1980)
4	Lalung	114	1	113	Das *et. al.* (1980)
5	Rabha	126	1	125	Das *et. al.* (1980)
6	Kachari	131	-	131	Das *et. al.* (1980)
7	Koch	104	-	104	Sengupta (1991)
8	Chutiya	64	1	63	Das *et. al.* (1985)
9	Marngar	160	4	156	Chumikam (2002)
10	Lyngam	120	2	118	Ahmed *et. al.* (1997)
11	Pnar (Jatinga)	120	-	120	Khongsdier (2001)
12	War Jaintia (Nongtalang)	207	3	204	Present study

Table 8 shows that there are only three subjects who have Rh-negative blood group. The findings of the present study shows a slight variation in the occurrence of Rh-negative blood group with other Khasi populations and the neighbouring populations compared, where the incident of Rh-negative is very low. Among all the populations compared, the present study shows high frequency of Rh-negative except the Marngar while Khynriam, Pnar, Garo, Koch and Kachari shows complete absence of Rh-negative.

Table 9 Chi-square value of Rh(D) blood group between War Jaintia with other populations of Meghalaya and Assam.

Sl No.	Population compared	Chi-Square (X^2) value	d.f.	Significance level
1	War Jaintia vs Bodo	3.02	1	Insignificant
2	War Jaintia vs Lalung	0.20	1	Insignificant
3	War Jaintia vs Rabha	0.28	1	Insignificant
4	War Jaintia vs Chutiya	0.01	1	Insignificant
5	War Jaintia vs Marngar	0.53	1	Insignificant
6	War Jaintia vs Lyngam	0.03	1	Insignificant

Table 9 shows that the differences between the present population and all the neighbouring populations compared are statistically not significant. The occurrence of Rh– negative blood is found in very low frequency or absent in all the above populations. Thus, the present findings seem to confirm the earlier observations that the frequency of Rh-negative blood group is negligible or very rare in many populations of Northeast India (Das, 1974).

Phenylthiocarbamide (PTC) taste sensitivity

Table 10 Frequency of PTC taste sensitivity among the War Jaintias of Nongtalang village and other populations of Meghalaya and Assam.

SL. No.	Populations	Sample Size(n)	Taster	NonTaster	Reference
1	Garo	107	88	19	Nchang (2006)
2	Mikir	245	205	40	Das *et. al.* (1980)
3	Lalung	114	81	33	Das *et. al.* (1980
4	Bhoi	210	164	46	Das (1978)
5	Marngar	160	113	47	Chumikam (2002)
6	Khynriam	222	197	25	Das (1978)
7	Pnar	178	148	30	Das (1978)
8	War	236	207	29	Das (1978)
9	Lyngam	120	84	36	Ahmed *et. al.* (1997)
10	War Jaintia of Nongtalang	207	119	88	Present study

The frequency distribution of tasters and non-tasters of PTC taste sensitivity among the present population and some neighbouring populations is given in Table 10. The above Table indicates that the war Jaintia of Nongtalang is characterized by low frequency of tasters compared to the other Khasi subtribes. An important observation noted here is that though the chewing habit of areca nut, betel leaf and lime along with tobacco ingredients is prevalent in the food habits of the War Jaintia of Nongtalang village, yet this influences did not seem to interfere on the perception of taste to PTC solution. The chi-square values between the War Jaintia of Nongtalang in comparison to other neighbouring populations in respect of PTC taste sensitivity are given in the Table below.

Table 11 Chi-square values of PTC taste sensitivity between War Jaintia of Nongtalang village and other neighbouring populations of Meghalaya and Assam

Sl No.	Population compared	Chi-Square (X^2) value	d.f.	Significance level
1	War Jaintia vs Garo	19.24*	1	Significant
2	War Jaintia vs Mikir	37.90*	1	Significant
3	War Jaintia vs Lalung	5.76*	1	Significant
4	War Jaintia vs Bhoi	20.30*	1	Significant
5	War Jaintia vs Marngar	6.70*	1	Significant
6	War Jaintia vs Khynriam	53.93*	1	Significant
7	War Jaintia vs Pnar	29.65*	1	Significant
8	War Jaintia vs War	51.83*	1	Significant
9	War Jaintia vs Lyngam	5.05*	1	Significant

* Significant at 5% level of probability

Table 11 shows that the War Jaintia deviates significantly from all other populations taken into consideration for comparison in the present study.

Red-Green colour vision

The frequency distribution of colour-blindness among the War Jaintia and other neighbouring populations of Assam and Meghalaya are shown in Table 12.

Table 12 Defective Red-Green colour vision distribution among the War Jaintia of Nongtalang village and other neighbouring populations of Meghalaya and Assam

Sl. No	Populations	Sample Size (N)	Normal	Red-Green Deficiency	Reference
1	Garo	52	50	2	Nchang (2006)
2	Mikir	125	125	-	Mukherjee (1963)
3	Bhoi	100	100	-	Mukherjee (1963)
4	Khynriam	100	99	1	Lama (1998)
5	Pnar	142	142	-	Mukherjee (1963)
6	Bodo-kachari	201	186	15	Mukherjee & Guha (1990)
7	Hajong	183	176	7	Barua (1985)
8	War Jaintia (Nongtalang)	207	202	5	Present study

Table13 Chi-square value of red-green colour deficiency between War Jaintia (Nongtalang) and the neighbouring populations

Sl No.	Population compared	Chi-Square (X^2) value	d.f.	Significance level
1	War Jaintia vs Garo	0.32	1	Insignificant
2	War Jaintia vs Khynriam	0.70	1	Insignificant
3	War Jaintia vs Bodo-Kachari	5.58*	1	Significant
4	War Jaintia vs Hajong	0.65	1	Insignificant

* Significant at 5% level of probability

Comparative study indicates that the War Jaintia is characterised by high frequency of Red-Green deficiency as compared to the other Khasi sub-groups. This is present in a very low frequency among the Hajong, Khynriam, Garo and Bodo-Kachari, and is absence among the Pnar, Bhoi and Mikir.

The prevalence of colour deficiency was found to vary in various degrees among the War Jaintias. Table 12 shows the distribution of colour-blindness among the Garo, Khynriam, Hajong and Bodo-Kachari.

Table 13 shows the chi-square values between War Jaintias of Nongtalang and other neighbouring populations of Assam and Meghalaya in respect of colour vision deficiency. The War Jaintia does not differ significantly with all the populations compared except the Bodo-Cachari.

CONCLUSION

Present study reveals that the War Jaintias of Nongtalang village is not in genetic equilibrium in respect of ABO blood group. It shows variations with the neighbouring populations except to the Khynriam and the Bhoi Khasi .Therefore, with respect to the present study it is difficult to give a proper explanation whether evolutionary forces such as mutation and natural selection are playing significant roles in regulating the gene frequencies in the present population or not. When the studied population was compared with the neighbouring populations with respect to the ABO blood group, it deviates significantly from them which indicate that there is likely a low admixture rate or intermarriage with other populations which might have brought about genetic difference between them. However, when we compared with the other sub-groups of Khasi, the War Jaintia seems to deviate significantly from the Pnar but shows similarities with the Khynriam and Bhoi supporting the work of Prof. B. M. Das (1970). Thus, it may be said that no selection or evolutionary forces are working on at different degrees. It shows that there is a genetic equilibrium of the present population with the other sub-group of Khasi populations resulting in similarity between the ancestral and descendant populations. This indicates that the present population still acquires the common Khasi characteristics of the original stock. The findings on Rh(D) blood groups seems to confirm the general prevailing trend among the mongoloid populations of Northeast India where the Rh-negative gene is either absent or present in a very low frequency (Das,1997 and Battacharjee,1968).

On the other hand, genetic markers like PTC taste sensitivity shows that the War Jaintias of Nongtalang deviate significantly from the compared populations. However, in respect of the deficiency of colour vision, the present population is significantly

different only from the Bodo-Kacharis of Assam. It may, however, be noted that genetic markers like PTC taste sensitivity and colour blindness are considered to be as weak genetic markers in comparison to the ABO blood groups system. Nevertheless, on the basis of the present findings, it is difficult to interpret the genetic affinity of the War Jaintias of Nongtalang. However, it shows similarity with the Khasi sub-groups with respect to the ABO blood groups and Colour blindness, but significant different in respect of the PTC taste sensitivity. Thus, the present finding on genetic markers among the War Jaintia population of Nongtalang needs to be further studied with the larger sample size along with the inclusion of the additional genetic markers which could provide more insight into the genetic composition of the Khasi population.

REFERENCES

Ahmed, T.J., Sengupta, S. and A.K. (1997). A genetic study on the Lyngam of Meghalaya. J. Hum. Ecol.108:473-475

Alimba. C.G., Adekoya, K.O. and Obeh, B.O. (2010). Prevalence and gene frequencies of phenylthiocarbamide (PTC) taste sensitivity, ABO and Rhesus factor (Rh) blood group, and haemoglobin variants among a Nigerian population, The Egyptian Journal of Medical Human Genetics, 11:153-158

Balakrishnan, V. 1988. Hardy-Weinberg equilibrium and allele frequency estimation.In: Statistical Methods in Human Populations Genetics, (Ed.) Malhotra, K. C. Indian Statistical Institute, Calcutta: EKA press

Barua, S.(1985). Red-Green colour blindness and Earlobe Attachment among the Hajongs of Meghalaya. Indian Journal of Physical Anthropology and Human Genetics, 11:151-154

Bernstein, F. (1930). Forgesetzie Unterchungen aus der theorie der blutgruppen (cited in Balakrishnan, 1988)

Bhasin. M. K and Walter, H. (2013). Genetic Markers in Human Blood, Kamla-Raj, Journal of Life science, 5(2):71-121

Bhatia, H. M. (1977). Procedures in Blood Banking and Immunohaematology. Blood Group Reference Centre, Bombay

Bhattacharjee, P.N. (1968). Sero-genetical variations in the Lepcha of Darjeeling district with special reference to the Indian mongoloid. Bull. Anthropological Survey of India. 17:382-392

Chumikam, B.T. (2002). A Genetic study on the Marngar population of Ri-Bhoi District, Meghalaya. Unpublished M.Sc. Dissertation, Department of Anthropology, NEHU, Shillong

Das, B. M. (1974). Physical anthropology in Northeast India: A short review. In: Contemporary anthropological research in Northeast India, edited by Das, B. M. Dibrugarh: Department of Anthropology, Dibrugarh University

Das, B. M. (1997). Outline of physical anthropology. Kitab Mahal Distributors, New Delhi

Das, B.M. (1978). Variation in Physical Characteristics in the Khasi population of North-East India. Guwahati: Dutta Barua and Co.

Das, B.M., Das, P.B., Das, R., Walter, H. and Danker-Hopfe, H. (1985a). Anthropological studies in Assam, India. 1. Observations on five mongoloid populations. Anthrop. Anz. 43:193-204

Das, B.M., Das, P.B., Das, R., Walter, H. and Danker-Hopfe, H. (1985b). Anthropological studies in Assam, India. 2. Observations on Muslims. Anthrop. Anz. 43:299-310

Das, B.M., Das, P.B., Das, R. (1980b). A Comparative study of physical anthropology of five mongoloid populations. of Assam. (Mimeographed). Anthropology Department, Gauhati University, Guwahati

Das, B.M., Deka, R. and Das R. (1980). ABO blood groups and Rh factor in six populations of Assam. Bull. Anthropological Survey of India. 29:1-3

Harris, H and Kalmus, H., (1949). The Measurement of Taste Sensitivity to PTC. Ann. Eugene, 15:24-31

Ishihara, S. 1959. Tests for colour Blindness. Japan:Kannchara Shuppan Co. Ltd. Tokyo

Khongsdier, R., Nsarangbe, A. and Phimu, A. (2001). A Bio-Anthropological study of the Pnar of Jatinga in North Cachar Hills of Assam. Journal of Indian Anthropological Society.36:213-222

Lama, R. (1998). A genetic study among the Khynriam of West Khasi Hills District, Meghalaya. Unpublished M.Sc. Dissertation, Department of Anthropology, NEHU, Shilong

Lawler, S.D. and Lawler, L.J. (1951). Human blood groups and inheritance. London: William Heinemann

Meitei, S.Y., Asghar, M., Achoubi, N.D., Murry, B., Saraswaty, K.N. and Sachdeva, M.P. (2010). Distribution of ABO and Rh(D) blood group among four populations in Manipur, North-East India. Anthrop. Not. 16(2):19-28

Mukherjee, D.P and Guha, A. (1990). Influence of cultural traditions and social movements on the genetic structure of the Boro Kachari population. Journal of Indian Anthropological Society. 25:73-81

Mukherjee, D.P. (1963). Variation in defective colour vision among some tribes of Assam. Bull. Anth. Surv. India. 12:39-42

Mukherjee, D.P. and Guha, A. (1990). Colour blindness, manual mid-phalangeal hair and attached earlobe among Boro Kacharis. J. Indian Anthrop. Soc. 25:153-163

Nchang, H. (2006). A Genetic study among the Garo population of Rangsakona village of Ri-bhoi District, Meghalaya. Unpublished M.Sc. dissertation, Department of Anthropology, NEHU, Shillong

Sengupta, S. (1991). A physical anthropological profile of the Koch of Darrang district, Assam. Indian Journal of Physical Anthropology and Human Genetics. 17:65-72

Web resources:
http://megartsculture.gov.in/herit-war-jaintias.htm. DAO 13/11/2017
http://westjaintiahills.gov.in/DAO 13/11/2017
https://www.revolvy.com/main/index.php?s=Meghalaya DAO 13/11/2017

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BACTERIAL DEGRADATION OF DELTAMETHRIN PESTICIDE

Jahangir Alom and Sangeeta Borchetia

ABSTRACT

Deltamethrin, a synthetic pyrethroid pesticide is a widely used insecticide and is applied to a wide range of commercial crops like tea, cotton, etc. It rapidly paralyzes the insect nervous system giving a quick knockdown effect; however, this insecticide is highly toxic to humans and animals as it acts as carcinogen as well as a neurotoxin. In order to reduce the environmental as well as public health risks associated with the use of deltamethrin, there is a need to develop rapid and effective methods to remove or minimize the concentrations of insecticides used. Biological-based approaches such as utilising pesticide-degrading bacteria's seems to be the most promising and effective strategy. For the bacterial degradation, it is extremely important to determine the potential of these bacteria for the degradation of deltamethrin under optimal conditions in liquid media. In present study, it was observed that bacterial strains are capable of degrading deltamethrin in liquid cultures with varying pesticide concentration.

Key Words: Pyrethrins, Deltamethrin, Bacterial Degradation, Deltamethrin

Introduction

Pyrethroid insecticides are derived from natural compounds known as pyrethrins, has been isolated from *Chrysanthemum*. One of the broad-spectrum insecticide belonging to pyrethroids is Deltamethrin [(S)-α-cyano-3-phenoxybenzyl (1R, 3R)-3-(2, 2-dibromovinyl)-2, 2-dimethylcyclopropane carboxylate] (Fig. 1), and has been used for the management of a variety of insect pests (Akre and MacNeil 2006, Cycon 2014). Pyrethroids act as neurotoxins and target the central nervous system of insects (Burr and Ray 2004). The insecticidal potency of deltamethrin is due to induction of a toxic effect

in the cells of the nervous system, by permitting a flux of sodium ions, which alters the activity of the sodium channels responsible for the signal transmissions of nerve impulses (Burr and Ray 2004, Akre and MacNeil 2006). Deltamethrin can also bind to other target channel proteins and can disrupt the proper function of the nervous cells leading to paralysis and eventually leads to the death of insects (Davies *et al.* 2007, Hintzen *et. al.* 2009).

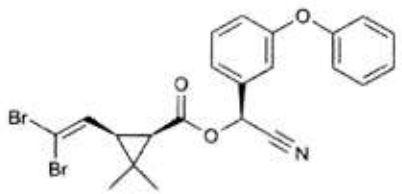

Fig.1: Chemical structure of Deltamethrin

Although, deltamethrin is considered to be safe when compared to other insecticides, however, excessive use of these compounds can cause severe contamination and ecological concern. Deltamethrin have a strong ability to adsorption to particles and therefore, may negatively affect non-target organisms such as fish, aquatic insects, beetles, bees, parasitic wasps, and microorganisms (Wendt-Rasch *et. al.* 2003, Cycon *et al.* 2014, Das *et. al.* 2016). Deltamethrin is highly toxic to humans as well as animals and long term exposure may lead to some chronic diseases (Pavel *et. al.* 1999, Munoz-Leoz et al. 2009, Cycon 2014).

In order to reduce the environmental and public health risks associated with pyrethroid use, there is a need to develop rapid and effective methods for the removal or inactivation of deltamethrin in the environment. Biological approaches based on the catabolic activity of pesticide-degrading bacteria, seems to be the most promising and effective strategy. In liquid media, the biodegradation process of deltamethrin is strongly dependent on factors such as temperature, pH, nutrients, inoculum size, moisture, organic matter content, the initial pesticide concentration, and additional carbon sources as well as the properties of the bacterial strains (Mestres and Mestres 1992, Munoz-Leoz *et al.* 2009, Therefore, the present study is aimed to assess the capability to degrade deltamethrin by indigenous isolated-bacteria.

MATERIALS AND METHODS

Study Area

For the present study Tocklai Tea Research Institute, Jorhat, was chosen as the study site.

Insecticide

Deltamethrin was obtained from Pesticide Store of Tocklai Tea Research Institute, Jorhat. Deltamethrin-degrading microorganisms were studied with varying concentrations of pesticide 100ppm, 200ppm, 300ppm, 400ppm.

Bacterial Culture

Bacterial strains (S2, S3 and S6) were isolated from Deltamethrin contaminated tea garden of Tocklai Tea Research Institute, Jorhat. For the present study, the bacterial strains were grown in nutrient broth. Pure cultures were grown and maintained using nutrient agar media plates. The plates were then incubated at 37°C for 24 hours.

Biodegradation of Deltamethrin

For studying biodegradation of Deltamethrin, Mineral Salt Medium (MSM) with varying concentration of Deltamethrin (100 ppm, 200 ppm, 300 ppm, 400 ppm) was used. In one set bacterial strain S3 was used singly and the other set contains a consortium of S2, S3 and S6 were inoculated to the MSM with varying deltamethrin. The cultures were grown in two batches one containing just MSM, and the other supplemented with other souces of carbon, and they were then incubated at an ambient temperature of 30°C for 3 days in a shaker incubator. The optical density of the cultures was measured after every 24 hours at 600 OD, to check the adaptability of the microorganisms, as well as degradation of Deltamethrin.

Extraction and degradation of Deltamethrin

After 72 hours of incubation of the S3 and consortia bacterial strains, the cultures were- centrifuged at 1000 rpm for 5 min. The supernatant was taken and an equal volume of dichloromethane was added and incubated overnight at room temperature. The organic phase was extracted -and dried over anhydrous Na_2SO_4. The dried extract was dissolved in HPLC grade methanol and then analysed by spectrophotometer. The degradation activity was expressed as percentage of degradation which was calculated by using the formula as:

$$\textbf{Percentage of degradation} = A_b - A_a / A_b \times 100$$

Where, A_b is absorbance of compound at 264 nm before degradation and A_a is absorbance at same wavelength after degradation.

Kinetic analysis

Kinetic analysis using the extract of various bacterial strains were studied by incubating the extracts in nutrient broth along with the bacterial stain S-3 and consortium of bacterial stains (S2, S3, and S6) for about 30 minutes. Non-inoculated sample kept as control. The analyses was carried out using a by spectrophotometer for 15 min, and the reaction rate was observed at every 5 min intervals.

RESULTS

Deltamethrin acts as a sole carbon source in MSM. The S3 bacterial strain and consortium of S2, S3 and S6 strains were able to grow in MSM with varying concentration of deltamethrin. Degradation of deltamethrin using S-3 strain with 0 ppm deltamethrin showed a limited growth. On the other hand, the same bacterial the strain was able to grow in 100 ppm, 200 ppm, 300 ppm, and 400 ppm concentration deltamethrin. The bacterial growth was increasingly rapid during 72 hrs of incubation as observed in Table 1.

Table 1 Absorbance of bacterial growth with deltamethrin at OD 600nm

Bacterial Strain	Concentration of Deltamethrin	24 Hours	48 Hours	72 Hours
S3	0 ppm	0.048	0.064	0.065
	100 ppm	0.080	0.077	0.086
	200 ppm	0.066	0.086	0.084
	300 ppm	0.100	0.110	0.109
	400 ppm	0.133	0.132	0.132
S2, S3 and S6	0 ppm	0.183	0.162	0.174
	100 ppm	0.184	0.165	0.178
	200 ppm	0.160	0.156	0.153
	300 ppm	0.202	0.172	0.190
	400 ppm	0.213	0.201	0.208

The biodegradation of deltamethrin performed using consortium of S2,S3 and S6 strains for a period of 72 hours in MSM showed a limited growth at 0 ppm concentration of deltamethrin, however, at 100 ppm, 200 ppm, 300 ppm, and 400 ppm concentration, they were able to grow well. The bacterial growth increased rapidly during 24 hrs of incubation (Table 1).

The degradation of deltamethrin perform by S-3 strain for a period of 72 hours in Mineral Salt Medium with additional carbon source showed a better growth as compared to samples using deltamethrin as the sole carbon source for culture (Table 2).

Table 2 Absorbance of bacterial growth with deltamethrin and additional nutrient at OD 600nm

Bacterial Strain	Concentration of Deltamethrin	24 Hours	48 Hours	72 Hours
S3	100 ppm	0.386	0.374	0.551
	400 ppm	0.312	0.422	0.479
S2, S3 and S6	100 ppm	0.219	0.599	0.652
	400 ppm	0.213	0.479	0.519

Similarly, biodegradation of deltamethrin by consortium of S2, S3 and S6 strains for a period of 72 hours in MSM with additional carbon source increased rapidly during 48 hrs of incubation.

The S-3 strain able to degrade the deltamethrin pesticide with 100 ppm and 400 ppm concentration. At 100 ppm, the percentage degradation was found to be 74% and 400 ppm the percentage of degradation was 76% (Table 3). However, when supplemented with an additional carbon source, the percentage of degradation at 100 ppm was 0% and it increases to 68 % at 400 ppm deltamethrin concentration.

Likewise, the consortium of S2, S3 and S6 strains were able to degrade the deltamethrin pesticide with 100 ppm and 400 ppm concentration, and the percentage of degradation of 42% and 77% respectively. The bacterial degradation was more with 400ppm of initial concentration than 100 ppm of initial concentration of pesticide as compared to when a single bacterial strain was used.

The consortium of S2, S3 and S6 strains were able to degrade the deltamethrin pesticide in 400 ppm concentration of deltamethrin and there is no reduction of deltamethrin in 100 ppm concentration. The percentage degradation at 100 ppm

deltamethrin was found to be 0 %; however it increases to 51 %. The most probable reason might be that bacteria utilized only the additional carbon source for growth, however, as the additional carbon source gets depleted, it starts metabolising deltamethrin.

Table 3 Percentage degradation of deltamethrin after 72 hours

Bacterial Starins	Percentage Degradation of deltamethrin (%)			
	MSM		MSM + Additional Carbon Source	
	100ppm	**400ppm**	**100ppm**	**400ppm**
S3	74	76	0	68
S2, S3, S6	42	76	0	51

The biodegradation of deltamethrin was also confirmed by kinetic studies in real time. The S3 strain and consortium of S2, S3 and S6 were found to efficiently utilize deltamethrin in 15 mins of degradation reaction (Figs. 2 and 3). Moreover, the degradation rate and the concentration were in direct proportion to the reaction kinetics observed. The rate of degradation was higher in both S3 and consortium of S2, S3 and S6 strains when supplemented with an additional carbon source at at 400 ppm deltamethrin

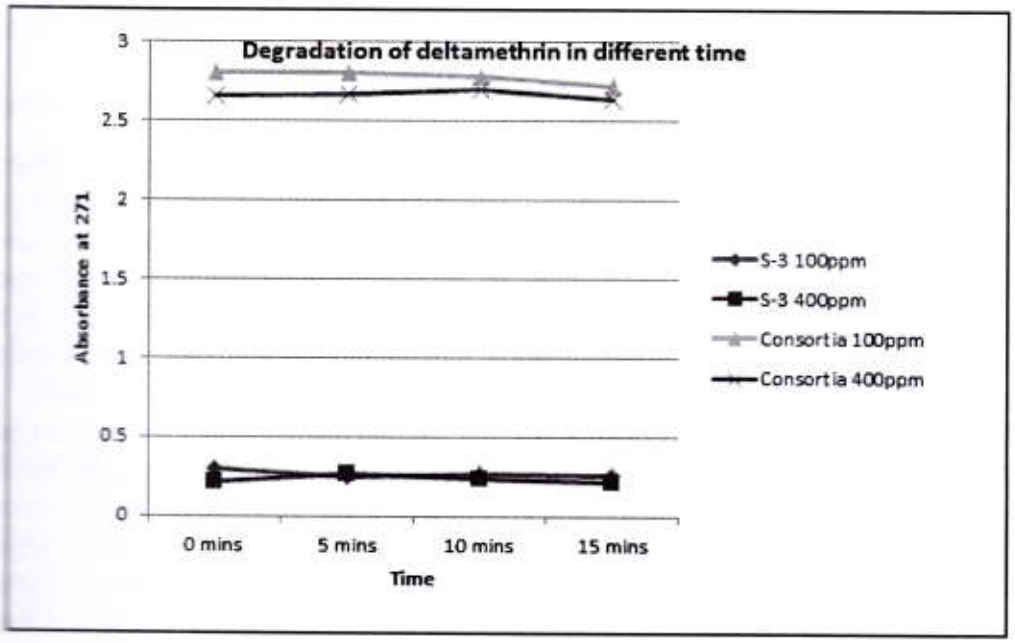

Fig. 2 : Degradation of deltamethrin by S3 and S2, S3 and S-6 without additional carbon source

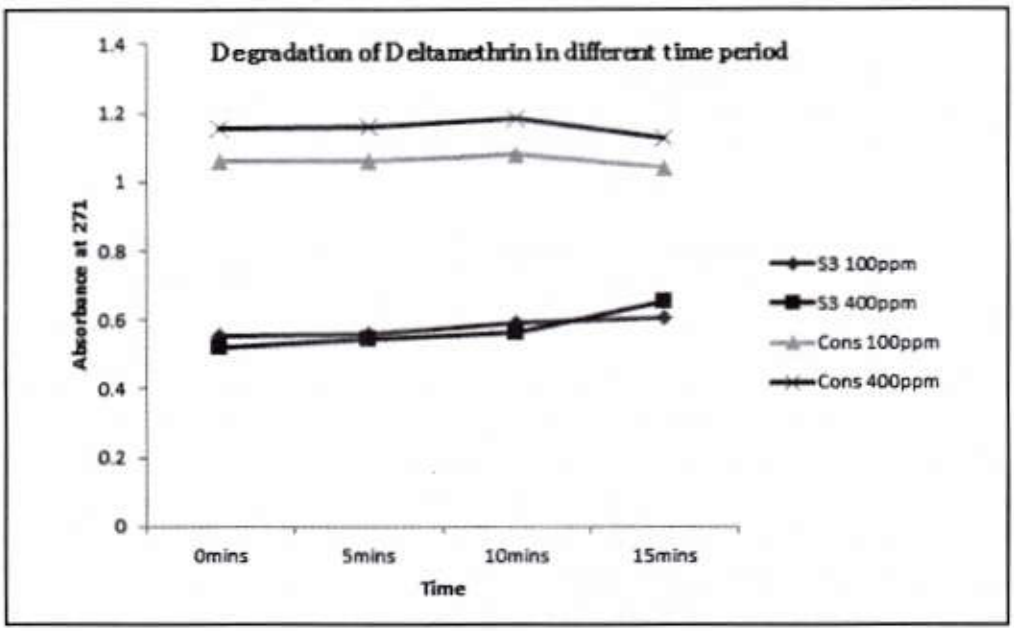

Fig. 3: Degradation of deltamethrin by S3 and S2, S3 and S-6 with additional carbon source

DISCUSSION

Deltamethrin is one of the most commonly and extensively used commercial insecticide in tea garden, it is, therefore, important to remove any Deltamethrin contamination, as it poses many hazardous effects not only to the environment but also to humans and animals as well (Cycon *et. al.* 2014). Biological methods using soil microorganism might be the best method to remove and/or degrade such contaminants as they can convert such hazardous compound non-toxic metabolites, without causing any detrimental effects to the human health and environment (Munoz-Leoz *et. al.* 2009).

Deltamethrin is used to control *Buzura suppresseria* (Looper caterpillar) in tea garden, and kills insect on contact and through digestion. Deltamethrin rapidly paralyze the insect nervous system giving a quick knockdown effect by induces the inhibition of the sodium channel activation gate (Cycon *et. al.* 2014). Deltamethrin is highly toxic as it acts as a neurotoxin as well as a carcinogens, it has deleterious effects to humans, aquatic organisms and other non-targetted organisms. Due to its extensive and exces-

sive usage, it is therefore necessary to degrade deltamethrin, as it can contaminate and seep to water sources. For the efficient bioremediation of deltamethrin, it would be advantageous if microorganisms could be used to degrade deltamethrin. Nevertheless, it is extremely important to determine the potential the different microorganisms to degradation using liquid media. Thus, in the present study, an attempt has been made to assess the potential of soil bacteria to degrading deltamethrin in liquid cultures. Two approaches were adopted where a single S3 and a consortium of S2, S3 and S6 strains were isolated from soil and studied. Also varying concentrations of deltamethrin in MSM and MSM supplemented carbon source was used to study the percentage of degradation and its effect on the growth of bacteria. From our study, it was observed that the growth of bacterial strain and rate of the biodegradation process were enhanced when the liquid media supplemented with additional carbon source or nutrients.

When the bacterial strains were grown in MSM media only, they were capable of metabolising deltamethrin as the sources of carbon and energy. Similar reports were observed in *Serratia marcescens* and some bacteria, and these were found to be capable of degrading a wide spectrum of synthetic pyrethroids (Muir *et. al.* 1985, Cycon *et. al.* 2014). The information presented in this study, indicated that the bacterial strains and their consortia were proficient in biodegradation of deltamethrin, can be an efficient tool for bioremediation of the synthetic pyrethroids (deltamethrin). However, further research studies, such as molecular characterization, interactions of environment with the strains, and the toxicological and genetic aspects from the strains and their ability to degrade other pyrethriods and its derivatives, should be carried out before the application of these strains in the field-scale bioremediation.

REFERENCES

Akre, C.J. and MacNeil, J.D. (2006). Determination of eight synthetic pyrethroids in bovine fat by gas chromatography with electron capture detection. J AOAC Int 89: 1425

Burr, S.A. and Ray, D.E. (2004). Structure-activity and interaction effects of 14 different pyrethroids on voltage-gated chloride ion channels. Toxicol Sci 77: 341–346

Cycon, M., Mijowska, A.Z. and Piotrowska-Seget, Z. (2014). Enhancement of deltamethrin degradation by soil bioaugmentation with two different strains of Serratia marcescens. Int J Environ Sci Technol 11:1305–1316

Das, R., Das, S.J. and Das, A.C. (2016). Effect of synthetic pyrethroid insecticides on N2 fixation and its mineralization in tea soil. Eur. J. Soil Biol 74: 9–15

Davies, T.G.E., Field, L.M., Usherwood, P.N.R. and Williamson, M.S. (2007). DDT, pyrethrins, pyrethroids and insect sodium channel. IUBMB Life 59: 151–162

Hintzen, E.P., Lydy. M.J. and Belden, J.B. (2009). Occurrence and potential toxicity of pyrethroids and other insecticides in bed sediments of urban streams in central Texas. Environ Pollut 157: 110–116

Mestres, R. and Mestres, G. (1992). Deltamethrin: Uses and environmental safety. Reviews of Environmental Contamination and Toxicology 124

Muir, D.C.G., Rawn , G.P. and Grift, N.P. (1985). Fate of the pyrethroid insecticide deltamethrin in small ponds: A mass balance study. J Agric Food Chem 33: 603-609

Munoz-Leoz, B., Garbisu, C. Antiguedad, I., Alonso, M.L., Alonso, R.M. and Ruiz-Romera, E. (2009). Deltamethrin degradation and soil microbial activity in a riparian wetland soil. Soil Science. doi: 10.1097/SS.0b013e3181a09ea8

Narahashi, T., Ginsburg, K.S., Nagata, K., Song, J.H. and Tatebayash, H (1998). Ion channels as targets for insecticides. Neurotoxicology 19: 581

Pavel, E.W., Lopez, A.R., Berry, D.F., Smith, E.P., Reneau, R.B. and S. Mostaghimi. (1999). Anaerobic degradation of dicamba and metribuzin in riparian wetland soils. Water Res. 33: 87-94

Wendt-Rasch, L., Friberg-Jensen, U., Woin, P. and Christoffersen, K. (2003). Effects of the pyrethroid insecticide cypermethrin on a freshwater community studied under field conditions. II. Direct and indirect effects on the species composition. Aquat Toxicol 63: 373-389

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A REVIEW ON NON CONVENTIONAL LEAFY VEGETABLES OF MEGHALAYA

Devajyoti Bokolial

ABSTRACT

This paper lists 134 species of underutilized, non conventional leafy vegetables in the state of Meghalaya. These species belongs to 100 genera and 60 families. Rutaceae contains 8 species of wild edibles followed by Asteraceae, Polygonaceae and Rubiaceae, each with 7 species. These plants are mainly collected from wild habitats; some of them are semi domesticated.The wild edibles are rich in essential nutrients necessary to maintain a healthy nutritional profile of an individual. Due to a change in the food habits of the people and habitat destruction of the wild edible species, the latter are disappearing from the diet. People should be made aware of the health benefits from the wild edibles and sustainable use of these species should be encouraged.

Key Words: Leafy Vegetable, Non Conventional, Wild Edible SpeciesTribes, Meghalaya.

Introduction

The northeasern state of Meghalaya is one of the biodiversity rich states of India. Its geographical location is 25°47′-26°10′ N latitude and 89°45′-92°47′ E longitude and covers an area of 22429 sq km. The state is surrounded by Bangladesh in south and southwest and by Assam in north and northeastern side. The state is a part of Indo-Burma biodiversity hot spot (Meyer *et. al.* 2000). The state has a forest cover of Meghalaya 17217 sq km which is 76.76% of the total geographical area of the state. Meghalaya is a predominantly tribal state, and has a tribal population of 86.1% with three main tribes-Khasi, Jaintia and Garo. The Khasi and Jaintia are believed to have migrated from Cambodia. The Garo are believed to be of Tibeto-Burman origin. These people are very

close to nature and largely dependent on forest for their livelihood (Sawian *et. al.*, 2007), which is reflected in the food habit and other socio cultural practices of these communities.

Wild edible plants grow naturally without much care and attention. These are termed as non conventional food plants as all of them lack large scale or organized cultivation. Some of these non conventional food plants are semi domesticated due to their usefulness. They play an important role in lives of people of indigenous communities in different parts of the world. Apart from providing important ecological services, non timber forest products such as fruits, tubers, rhizomes, leaves, shoots, flowers and other parts of wild plants are extracted from the forests and utilized for various purposes including their use as food. These non conventional sources of food are rich in essential nutrients and vitamins and are very important dietery supplements to a tribal individual. Wild plants also give several other useful products like medicine, fiber, fodder, dyes etc. (Kayang 2007). These also provide economic security to the rural people at the time of need. Rural people earn some amount by selling them in the markets and thus are also important in rural economy (Sawian *et. al.*, 2007). Many of these plants are also used in traditional and religious rituals and thus intimately associated with socio-economic-cultural-religious lives of the tribal people.

During the last decade, considerable interest has been given to research and documentation of wild edible plants. Scientific researches across the world have revealed that due to intensive agriculture practices, our commercial crops are slowly losing their original taste, nutritional qualities, etc. Thus interest has been shifted towards alternative food plants, which are available naturally and have not been commercially exploited yet. Around the world, a number of such plants are still being collected and consumed as dietery supplements, especially by indigenous communities. Leafy vegetables are one such important wild edible extracted from forests. Leafy vegetables are comparatively easy to cook, in the sense that these require less processing unlike roots, tubers, stems or other edible parts of a plant. Leafy vegetables are also packed with essential minerals, antioxidants and other nutrients and thus an ideal wild edible product from the forest.

A number of workers have studied the ethnobotany of different tribes of the state (Rao and Neogi, 1980, Rao, 1981, Maikhuri and Gangwar, 1993, Ahmed and Borthakur 2005, Jaiswal, 2010). Works documenting wild edible plants of the state have also been carried out (Pandey *et. al.* 1993, Kayang 2007, Sawian *et. al.* 2007, Jeeva 2009, Chhetri 2010, Kar *et. al.* 2012, Singh *et. al.* 2012, Lyngdoh and Kharshandi 2014, Kharshandi *et. al.* 2015). Present account deals with the description of recorded wild leafy vegetables from Meghalaya.

Non-conventional leafy vegetables of Meghalaya

A list of recorded non conventional vegetable species of Meghalaya with their family names, local names, habit and usage is given in Table 1. A total of 134 species of wild edibles belonging to 100 genera and 60 families are included in the list. Out of these, gymnosperms are represented by one species and pteridophytes with two species belonging to two families and two genera. Rutaceae contains the highest number of wild edible species (8 spp.) followed by Asteraceae, Polygonaceae and Rubiaceae, each with 7 species. Araceae, Moraceae and Primulaceae have 6 species of wild edible leafy species each, followed by Acanthaceae, Begoniaceae, Vitaceae and Verbenaceae with 4 species each (Figure 1). *Zanthoxylum* contains the highest number of species (6 spp.) followed by Ficus (5 spp.), *Clerodendrum* and and *Persicaria* with four species each (Figure-2). Habit distribution shows that 43.28% (58 spp.) of the wild edibles are herbs, 22.34% (30 spp.) each are shrubs and trees, and 11.94% (16 spp.) are climbers (Figure 3).

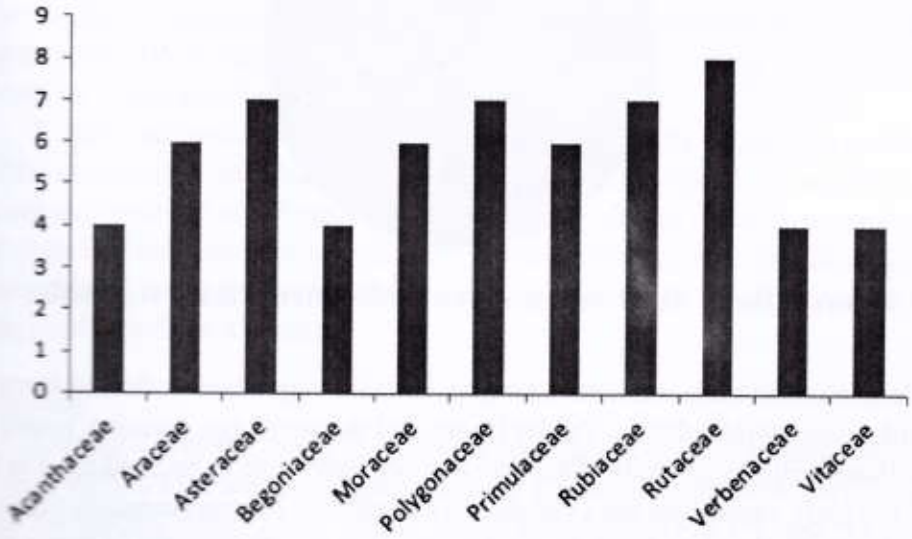

Figure 1 Dominant families of non conventional leafy vegetables

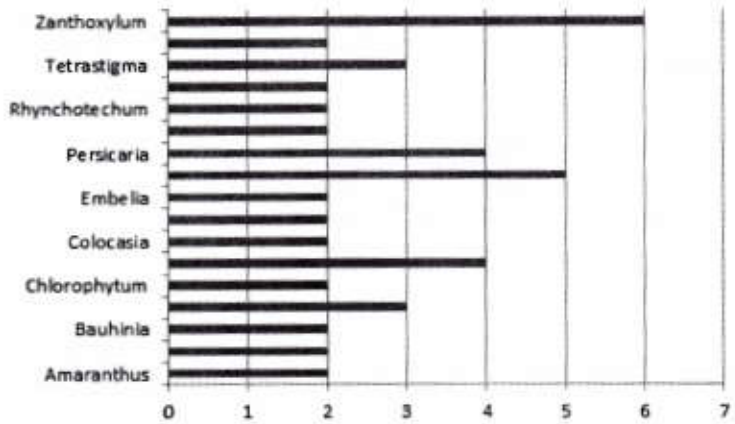

Figure 2 Dominant genera of non conventional leafy vegetables

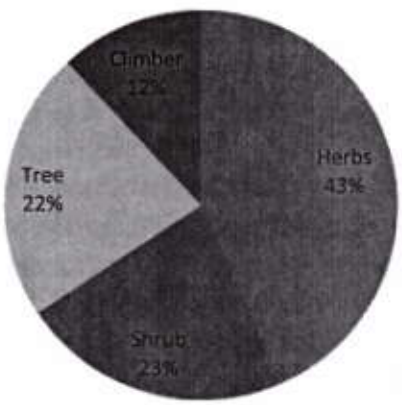

Figure 3 Habit distribution of non-conventional leafy vegetables

People of the state consume a number of leafy vegetables collected from a variety of wild or semi wild habitats. Tender leaves and shoots, twigs, petioles, young fronds and sometimes entire plant body of herbaceous vegetables are consumed fried or boiled. Many of the leafy vegetables are available in a particular season (annuals) while others are available throughout the year. Several species are weeds growing in home gardens, roadsides, fallow agricultural lands, etc. (e.g. *Centella asiatica, Chenopodium album, Diplazium esculentum, Drymaria cordata, Emilia sonchifolia, Paederia foetida, Plantago asiatica, Rorippa indica, Sonchus arvensis,* etc.). Some species though grow naturally, due to their usefulness and economic utilities, are grown in homestead gardens.

Houttuynia cordata and *Centella asiatica* are probably the most commonly used herbs, eaten raw, as chutney or vegetable by the ethnic people of the state. Both have medicinal properties and also used traditionally for curing certain ailments. *Amaranthus tricolor, A. viridis, Alocasia indica, Colocasia affinis, C. esculanta, Chenopodium album, Diplazium esculentum, Fagopyrum acutatum, Rumex nepalensis,* etc. are some of the common wild leafy vegetables consumed in the state. Leaves of *Allium ramosum, Eryngium foetidum, Murraya koenigii, Zanthoxylum armatum, Z. limonella Z. oxyphyllum and Z. rhetsa* are used as spice to flavor fish, meat or other curries. A number of these wild leafy vegetables are available in local vegetable markets. *Amaranthus viridis, Asparagus racemosus, Centella asiatica, Chenopodium album, Colocasia esculenta, Corchorus olitorius, Diplazium esculentum, Eryngium foetidum, Houttuynia cordata, Oenanthe linearis, Zanthoxylum rhetsa* are the leafy vegetables mainly found in local markets. Not only the rural people, but the city dwellers too prefer these non conventional wild vegetables and thus wild leafy vegetables have good demands in both rural and urban markets. However some of the wild leafy vegetables are consumed only in rural areas. Leaves of species like *Ardisia neriifolia, Commelina benghalensis, Desmodium trifolium, Garcinia cowa, Lasia spinosa, Oroxylum indicum, Piper malamiris, Sarchochlamys pulcherrima, Xanthium strumarium*, etc. are collected and consumed mainly by rural people; these wild edibles are either not available or not preferred by urban population.

Nutritional quality analysis of some of the wild leafy vegetables available in the state has been carried out. Reports reveal that these wild vegetables are rich in essential nutrients and antioxidants. Some of the wild edibles can be easily compared with cultivated ones for their nutritional qualities (Agrahar-Murugkar, 2006; Seal, 2011). Thus consumption of these leafy vegetables from the wild plays an important role in maintaining good health of a person.

Conclusion

Around the world, there are only 30 species of cultivated plants which provide more than 90% of the food production and only 12 domesticated species provide around 80% of the calorie intake of the entire human population (FAO, 1996). Thus our crops will be under immense pressure in near future. In such a situation these non conventional and wild edible plants can be a good alternative to ease the pressure on our agricultural system. The non conventional wild vegetables have other significances too. The potential wild edible species can be picked up for domestication (Kayang, 2007). Many of the wild species contain agronomically desirable characters, which can be in-

corporated in plant breeding programme to improve commercial varieties (Bokolial and Syiemlieh, 2014). There is a lack of awareness among the general masses about the role of the wild edibles in maintaining good health. Sustainable use of these wild leafy and other edible species should be encouraged. At the same time, conservation of these plants is important, as population of some of the wild edible species is declining due to overexploitation (mainly for timber and medicinal purposes) and habitat destruction (Lyngdoh and Kharshandi, 2014; Kharshandi, *et. al.*, 2015). In such a situation, domestication can be considered as a low cost conservation measure for the wild edible species (Chhetri, 2006). The need of the hour is to make people aware about the health benefits of the wild edibles and importance of sustainable utilization and conservation of these species.

Table 1 List of non conventional leafy vegetables of Meghalaya

SI No	Botanical name	Family	Local names *	Habit	Usage
1.	*Abelmoschus manihot* var. *pungens* (Roxb.) Hochr. (=*Hibiscus pungens* Roxb.)	Malvaceae	Galda (G)	Herb	Leaves, shoots used as vegetable.
2.	*Allium ramosum* L. (=*A. odorum* L.)	Liliaceae	Raseng (G)	Herb	Leaves are used as condiments.
3.	*Alocasia indica* (Roxb.) Schott.	Araceae	Kimchit nokam (G)	Herb	Leaves cooked as vegetable.
4.	*Alternanthera philoxeroides* Griseb.	Amaranthaceae	Ong-put (K)	Herb	Tender leaves and shoots used as vegetable.
5.	*Amaranthus tricolor* L. (=*A. gangeticus* L.)	Amaranthaceae	Jada saw (K), Chantili (G)	Herb	Leaves and shoots cooked as vegetable.
6.	*Amaranthus viridis* L.	Amaranthaceae	Jada saw (K), Chendoli (G)	Herb	Twigs or stems are used as vegetable.
7.	*Amblyanthus glandulosus* (Roxb.) A. DC.	Primulaceae	Jia herew (J)	Shrub	Twigs cooked as vegetable.
8.	*Antidesma acidum* Retz. (= *Antidesma diandrum* (Roxb.) B.Heyne ex Roth)	Phyllanthaceae	Dieng japew, Khouding (K), Aburok (G)	Tree	Boiled shoots are used as vegetable.
9.	*Ardisia neriifolia* Wall ex A.DC. (=*A. floribunda* Wall.)	Primulaceae	Theilangrong (K), Bhaujawa (J)	Tree	Leaves used as vegetable.
10.	*Ardisia polycephalla* Wall.	Primulaceae	Dieng-soh-sying (K)	Tree	Leaves and shoots used as vegetable.
11.	*Argyreia nervosa* (Burm. f.)	Convolvul	Jatap-masi, Soh- ryng-	Climb	Young leaves eaten raw.

	Boj. (=*A. speciosa* Sweet)	aceae	kang (K)	er	
12.	*Arisaema consanguineum* Schott	Araceae	Saru-bsein (K)	Herb	Leaves used as vegetable.
13.	*Artemisia indica* Willd.	Asteraceae	Praprukur bijak (G)	Herb	Tender shoot used as vegetable.
14.	*Asparagus racemosus* Willd.	Liliaceae	Bat soh-phlang (K), Phlang chokria (J), Sam-riching (G)	Herb	Tender shoots are eaten as vegetable.
15.	*Azadiracta indica* A. Juss.	Meliaceae	Dieng-neem (K, J), Aja-neemu (G)	Tree	Tender shoots with leaves cooked as vegetable.
16.	*Baliospermum micranthum* Muell.-Arg.	Euphorbia ceae	Thylli-skei (K)	Shrub	Twigs cooked as vegetable.
17.	*Bauhinia purpurea* L.	Fabaceae	Dieng long (K), Jiat-iong (J), Megong (G)	Tree	Boiled shoots are consumed as vegetable.
18.	*Bauhinia variegata* L.	Fabaceae	Dieng-tharlong (K), Bol megong (G)	Tree	Leaves used as vegetable.
19.	*Begonia josephii* A. DC.	Begoniace ae	Jajew (K)	Herb	Leaves cooked as vegetable.
20.	*Begonia palmata* D. Don.	Begoniace ae	Sla-lajaw (K)	Herb	Leaves and shoots consumed as vegetable.
21.	*Begonia roxburghii* A. DC.	Begoniace ae	Jajew jylwang (K), Kamchal (G)	Herb	Leaves used as vegetable. Petioles eaten raw.
22.	*Cardamine macrophylla* Willd.	Brassicace ae	--	Herb	Leaves consumed as vegetable.
23.	*Casearia graveolens* Dalz.	Salicaceae	Soh-kyian (J), Bolong-miandok (G)	Tree	Leaves and twigs cooked as vegetable.
24.	*Centella asiatica* (L.) Urban	Apiaceae	Khliang-syiar, Kynbat moina (K), Mesena chil (G)	Herb	Leaves, petiole and the whole plant used as chutney or as boiled vegetable.
25.	*Chenopodium album* L.	Chenopodi aceae	Ja- ut- pudar-saw (K), Butia sak (G)	Herb	Tender shoot, leaves cooked as vegetable.
26.	*Chlorophytum arundinaceum* Baker	Asparagac eae	Soh kyian (J), Bol kian (G)	Herb	Twigs cooked as vegetable.
27.	*Chlorophytum nepalense* (Lindl.) Baker (=*C. [illegible]* Hk. f.)	Asparagac eae	Bol chamsko (G)	Herb	Twigs cooked as vegetable.

28.	*Cissus repens* Lam. (=*Vitis repens* (Lam.) Wight. & Arn.)	Vitaceae	Mei hur jarap (K)	Climber	Tender shoots used as vegetable.
29.	*Clausena excavata* Burm. f.	Rutaceae	Dieng tyrur (K), Sam sweng (G)	Shrub	Leaves cooked as vegetable.
30.	*Clerodendrum colebrokianum* Walp.	Verbenaceae	Sla-jarem (K), Dieng risai (J)	Shrub	Leaves eaten as vegetable.
31.	*Clerodendrum infortunatum* L. (=*Clerodendrum viscosum* Vent.)	Verbenaceae	Dieng-jarem synrang (K), Sam-maki (G)	Shrub	Leaves consumed as vegetable.
32.	*Clerodendrum serratum* (L.) Moon	Verbenaceae	Rilong-phlang (K), Dieng-la-myrsiang (J), Sam-seng (G)	Shrub	Leaves, young shoots eaten as vegetable.
33.	*Clerodendrum wallichii* Merr.	Verbenaceae	Medongdi (G)	Shrub	Leaves eaten as vegetable.
34.	*Colocasia affinis* Schott	Araceae	Shriew (K), Geneusu (G)	Herb	Leaves, petiole cooked as vegetable.
35.	*Colocasia esculanta* (L.) Schott	Araceae	Wang, Shriew (K), Matchita-Ngong (G)	Herb	Leaves with petioles are cooked with dry fish or lentil.
36.	*Combretum album* Pers. (=*Combretum roxburghii* Spreng.)	Combretaceae	Mei long-kha- saw (K), Dugrak (G)	Climber	Leaves used as vegetable.
37.	*Commelina benghalensis* L.	Commelinaceae	Bat- pied (K)	Herb	Leaves cooked as vegetable.
38.	*Conocephalus suaveolens* Bl.	Moraceae	Dudiblok (G)	Climber	Leaves used as vegetable.
39.	*Corchorus olitorius* L.	Tiliaceae	Meka (G)	Herb	Leaves cooked as vegetable.
40.	*Crateva nurvala* Buch.-Ham.	Capparaceae	Jong sia (G)	Tree	Shoots used as vegetable.
41.	*Crateva religiosa* G. Frost (= *C. magna* (Lour.) DC.)	Capparaceae	Jong sia (G)	Tree	Shoots cooked as vegetable.
42.	*Cycas pectinata* Buch.-Ham.	Cycadaceae	Dieng sia-goda (K)	Tree	Tender leaves, young shoots used as vegetable.
43.	*Cyclocodon parviflorus* (Wall. ex A. DC.) Hk. f. & Thomson (=*Codonopsis*	Campanulaceae	Ja-tyndong (J)	Herb	Leaves are cooked as vegetable.

	parviflora DC.)				
44.	*Desmodium trifolium* DC.	Fabaceae	Memang mong arabok (G)	Herb	Leaves cooked as vegetable.
45.	*Diplazium esculentum* (Retz.) Sw.	Athyriacea e	Jhur-tyrkhang (K), Agachi (G)	Herb	Frond is fried and consumed.
46.	*Drymaria cordata* (L.) Willd. ex R.& S	Caryophyll aceae	Bat-nongrim (K), Sliaslia (J	Herb	Leaves or whole plant eaten raw.
47.	*Dysoxylum excelsum* Bl. (= *Dysoxylum gobara* (Buch.-Ham.) Marr.)	Meliaceae	Sla lushai (K)	Tree	Leaves cooked as vegetable.
48.	*Elatostema dissectum* Wedd.	Urticaceae	Jhur khlow (J)	Herb	Leaves eaten raw or cooked as vegetable.
49.	*Eleutherococcus trifoliatus* (L.) Hu (=*Acanthopanax trifoliatus* Seem.)	Araliaceae	Shi -soh-satkhlaw (K), Kenbut (G)	Shrub	Young shoots used as vegetable.
50.	*Embelia nutans* Wall.	Primulacea e	Mei ja jew khlaw (K)	Climb er	Leaves eaten as vegetable.
51.	*Embelia vestita* Roxb.(= *Embelia nagushia* D.Don)	Primulacea e	Mei jajew-khlaw (K)	Climb er	Leaves eaten as vegetable.
52.	*Emilia sonchifolia* (L.) DC	Asteraceae	Jangew, Jalyngshor (K)	Herb	Leaves used as vegetable.
53.	*Erioglossum rubiginosum* Bl.	Sapindace ae	Abigran (G)	Tree	Shoot eaten as vegetable.
54.	*Eryngium foetidum* L.	Apiaceae	Dhonia-khlaw (K), Sam skal (G)	Herb	Leaves as spice or as vegetable. Also used to prepare chutney.
55.	*Eurya acuminata* DC.	Pentaphyla caceae	Dieng shit (K), Murmura (G)	Tree	Leaves, tender shoots cooked as vegetable.
56.	*Fagopyrum acutatum* (Lehm.) Mansf. ex K. Hammer (= *F. cymosum* Meisn. = *F. dibotrys* (D.Don) Hara= *Polygonum dibotrys* D. Don)	Polygonac eae	Jarain (K), Yarain (J), Sambodom Bong (G)	Herb	Tender shoots cooked as vegetable.
57.	*Ficus clavata* Wall. ex Miq.	Moraceae	Slileshiat (J)	Shrub	Leaves eaten as vegetable.
58.	*Ficus geniculata* Kurz	Moraceae	Prap-agar (G)	Tree	Young leaves eaten as

					vegetable.
59.	*Ficus hispida* L. f.	Moraceae	Dieng lapong (K), Thamusa (G)	Tree	Leaves are used as vegetable.
60.	*Ficus subincisa* Buch.-Ham. ex J.E. Sm.	Moraceae	Samch-blang (K)	Shrub	Leaves cooked as vegetable. Fruits eaten raw.
61.	*Ficus virens* Ait.	Moraceae	Dieng-soh poklao (K), Dieng-chiri (J)	Tree	Leaf buds along with stipules are eaten as vegetables or pickle.
62.	*Garcinia cowa* Roxb. ex Choisy	Clusiaceae	Soh-khylleng,Soh-kwang (K, J), Tekra (G)	Tree	Tender twigs used as vegetable.
63.	*Gomphogyne cissiformis* Griff.	Cucurbitaceae	Jhur thliem (K)	Climber	Leaves cooked as vegetable.
64.	*Houttuynia cordata* Thunb.	Saururaceae	Jamyrdoh (K), Myrdoh (J), Machelongbeng (G)	Herb	Leaves used as vegetable or used to prepare chutney. Whole plant is eaten raw.
65.	*Ilex guianensis* (Aubl.) Kuntz. (= *I. acuminata* Willd.)	Aquifoliaceae	Dieng tilut (K), Jiakeng (J)	Shrub	Leaves cooked as vegetable.
66.	*Ipomoea batatas* (L.) Lam.	Convolvulaceae	Phan-karo (K), Sa lah (J), Thamlang bejak (G)	Herb	Shoots used as boiled vegetable.
67.	*Ixeris gracilis* (DC.) Stebbins	Asteraceae	Khmut sim (K)	Herb	Leaves used as vegetable.
68.	*Ixora subsessilis* Wall.	Rubiaceae	Dieng-jowat (K), Sangrura (G)	Shrub	Leaves, shoots cooked as vegetable.
69.	*Justicia adhatoda* L. (=*Adhatoda vasica* Nees)	Acanthaceae	Jalymmuh (K), Devglameh (G)	Shrub	Leaves cooked as vegetable.
70.	*Lasia spinosa* (L.) Thw.	Araceae	Timulana, Chongay (G)	Herb	Leaves are eaten as vegetable.
71.	*Maesa indica* Wall	Primulaceae	Dieng-soh-jala-tyrkhai (K), Dieng-pyllein-dakha (J), Samnakatok (G)	Tree	Leaves eaten as vegetable.
72.	*Medinilla rubicunda* (Jack) Bl. (=*M. erythrophylla* Wall. ex Lindl.)	Melastomataceae	Ja-jaotler (J), Megong-appal (G)	Shrub	Leaves cooked as vegetable.

73.	*Meliosma pinnata* (Roxb.) Maxim.	Sabiaceae	Dieng knot (K), Bol mechik (G)	Tree	Leaves used as vegetable.
74.	*Monochoria hastata* (L.) Solms.	Pontederiaceae	Garopoksi gachli (G)	Herb	Young leaves and petioles are used as vegetable.
75.	*Moringa oleifera* Lam. (= *Moringa pterygosperma* Gaertn.)	Moringaceae	Dieng jhur sasina (K), Sojona (G)	Tree	Leaves cooked as vegetable.
76.	*Murraya koenigii* (L.) Sprengel	Rutaceae	Nolsing, Samkatsi (G)	Shrub	Leaves used as spice or cooked as vegetable.
77.	*Mussaenda roxburghii* Hk. f.	Rubiaceae	Dieng jalong tham (K), Dieng-pnah (J), Gardek (G)	Shrub	Tender leaves cooked as vegetable.
78.	*Nasturtium officinale* R. Br.	Brassicaceae	Ayr-soh-um (K)	Herb	Leaves are used as vegetable.
79.	*Natsiatum herpeticum* Buch.-Ham.	Icacinaceae	--	Shrub	Leaves, shoots used as vegetable.
80.	*Oenanthe linearis* Wall.	Apiaceae	Jatira (K, J)	Herb	Young shoots cooked as vegetable.
81.	*Olax acuminata* Wall. ex Benth.	Olacaceae	Dieng-tyrut (K)	Shrub	Leaves consumed as vegetable.
82.	*Oldenlandia diffusa* (Willd.) Roxb. (=*Hedyotis diffusa* Willd.)	Rubiaceae	Chenong, Mangaluk (G)	Herb	Leaves eaten as vegetable.
83.	*Oroxylum indicum* (L.) Vent.	Bignoniaceae	Ja-rang-hon (K), Dieng-kawaitblai (J), Kiring (G)	Tree	Young shoot boiled and eaten as vegetable.
84.	*Oxalis corniculata* L.	Oxalidaceae	Kynbat khnai (K), Kiakna (G)	Herb	Leaves and whole plant eaten raw, also cooked as vegetable.
85.	*Oxyspora paniculata* (D. Don) DC.	Melastomataceae	Lang-tang (K)	Shrub	Leaves used as vegetable.
86.	*Paederia foetida* L.	Rubiaceae	Mei-soh-myrsem (K, J), Gandharadal (G)	Climber	Leaves, shoots used as vegetable.
87.	*Pavetta subcapitata* Hk. f.	Rubiaceae	--	Shrub	Leaves eaten as vegetable.
88.	*Pedicularis carnosa* Wall.	Scrophulariaceae	Sam thapar (K), Sam dipo (G)	Herb	Leaves used as vegetable.

89.	*Pegia nitida* Colebr.	Anacardiaceae	Du-chengbrup (G)	Shrub	Leaves used as vegetable.
90.	*Peperomia pellucida* (L.) Kunth.	Piperaceae	Bithe (G)	Herb	Leaves, shoots used as vegetable.
91.	*Persicaria capitatum* (Buch.-Ham. ex D. Don) H. Gross (=*Polygonum capitatum* Buch.-Ham. ex D. Don)	Polygonaceae	Sambodombong (G)	Herb	Twigs cooked as vegetable.
92.	*Persicaria chinensis* (L.) H. Gross (=*Polygonum chinense* L.)	Polygonaceae	Ja- lynnong (K), Syndem (J), Samitchang (G)	Herb	Leaves and shoots are eaten as vegetable.
93.	*Persicaria orientalis* (L.) Spach (=*Polygonum orientale* L.)	Polygonaceae	Jalynnoh (K), Slydem sniang (J)	Herb	Leaves cooked as vegetable.
94.	*Persicaria nepalensis* (Meisn.) Miyabe (= *Polygonum alatum* Buch.-Ham. ex D.Don = *Polygonum nepalense* Meisner)	Polygonaceae	Jakyrphuh (K), Achiak (G)	Herb	Tender leaves and shoots cooked as vegetable.
95.	*Phlogacanthus thyrsiformis* (Roxb. ex Hardw.) Mab.	Acanthaceae	Dieng-soh-ka-jut (K), Jia-merembut (J), Veru kaincheit (G)	Shrub	Young leaves fried and used as vegetable.
96.	*Phyllostachys mannii* Gamble	Poaceae	U siej naka (K)	Herb	Young shoots eaten as vegetable.
97.	*Phytolacca acinosa* Roxb.	Phytolaccaceae	Jada-phareng (K)	Herb	Leaves used as vegetable.
98.	*Piper diffusum* Blume ex Miq.	Piperaceae	Soh-mrit (K)	Climber	Shoots used as boiled vegetable.
99.	*Piper malamiris* L.	Piperaceae	--	Climber	Leaves cooked as vegetable.
100.	*Plantago asiatica* subsp. *erosa* (Wall.) Z.Yu Li (=*Plantago erosa* Wall. ex Roxb.)	Plantaginaceae	Shkhorblang (K)	Herb	Leaves used as vegetable.
101.	*Plectranthus mollis* (Aiton)	Lamiaceae	Chichithoni (G)	Herb	Leaves cooked as vegetable.

	Spreng. (=*P. incanus* Link.)				
102.	*Polygonum muricatum* Meisn.	Polygonaceae	Jabuit (K)	Herb	Leaves are used as vegetable.
103.	*Portulaca oleracea* L.	Portulacaceae	Jiahusia (K), Stilchi (G)	Herb	Leaves are eaten as vegetable. Shoots used to prepare chutney.
104.	*Psychotria denticulata* Wall.	Rubiaceae	Sonopincyl (G)	Shrub	Leaves cooked as vegetable.
105.	*Pteridium aquilinum* (L.) Kuhn	Pteridaceae	Tyrkhang shatri (K), Shatri (G)	Herb	Tender fronds cooked as vegetable.
106.	*Rhododendron arboreum* Sm.	Ericaceae	Tiew-saw (K)	Tree	Leaves used as vegetable.
107.	*Rhynchotechum ellipticum* (Wall. ex Dietrich) A. de Candolle	Gesneriaceae	Dieng ja mahek (K), Regong (G)	Shrub	Leaves eaten as vegetable.
108.	*Rhynchotechum vestitum* Wall. ex C.B. Clarke	Gesneriaceae	Regong chu (G)	Shrub	Leaves cooked as vegetable.
109.	*Rorippa indica* (L.) Hiern. (=*Nasturtium indicum* (L.) DC.)	Brassicaceae	Jhurktieh (K)	Herb	Leaves used as vegetable.
110.	*Rumex nepalensis* Spreng.	Polygonaceae	Jhur sniang (J)	Herb	Young leaves cooked as vegetable.
111.	*Sambucus javanica* Bl.	Adoxaceae	--	Tree	Leaves cooked as vegetable.
112.	*Sarcochlamys pulcherrima* (Roxb.) Gaud.	Urticaceae	Dieng-langshir (K), Mechaki (G)	Shrub	Tender shoots cooked as vegetable.
113.	*Schima wallichi* (DC.) Korthals	Theaceae	Dieng-ngan (K)	Tree	Tender leaves cooked as vegetable.
114.	*Smilax perfoliata* Lour.	Smilacaceae	Shiah krot (J)	Climber	Shoots are consumed as vegetable.
115.	*Sonchus arvensis* L.	Asteraceae	Ki-lan-jiat (K)	Herb	Leaves used as vegetable.
116.	*Sonchus asper* (L.) Hill	Asteraceae	Jalynniar (J)	Herb	Leaves cooked as vegetable.
117.	*Strobilanthes hamiltoniana* (Steud.) Bosser & Heine (=*S. colorata* T. Anders)	Acanthaceae	Soh umiuw (K), Sumet cheng (G)	Shrub	Leaves used as vegetable.
118.	*Tamilnadia uliginosa* (Retz.) Tirveng. & Sastre (=*Randia uliginosa* DC.)	Rubiaceae	Suskeng (G)	Tree	Leaves used as vegetable.

119.	*Tapiria hirsuta* Hk. f.	Anacardiaceae	Da cheng brap (G)	Shrub	Leaves eaten as vegetable.
120.	*Tetrastigma leucostaphylum* (Dennst.) N.P.Balakr.	Vitaceae	Syrpung (J)	Climber	Leaves eaten as vegetable.
121.	*Tetrastigma serrulatum* (Roxb.) Planch.	Vitaceae	Mei soh myn-jiriang (K), Sla ngnar (J)	Climber	Leaves eaten as vegetable.
122.	*Tetrastigma angustifolium* (Roxb.) Planch. (= *Vitis angustifolia* (Roxb.) Wall.)	Vitaceae	Dudhi-kansau (G)	Climber	Leaves and stem cooked as vegetable.
123.	*Thunbergia grandiflora* Roxb.	Acanthaceae	Jyrmi-khnong (K), Khakkhu (G)	Climber	Tender leaves cooked as vegetable.
124.	*Typhonium trilobatum* (L.) Schott.	Araceae	Makbol java (G)	Herb	Leaves and petiole used as boiled vegetable.
125.	*Vaccinium donianum* Wight.	Ericaceae	Dieng-soh-rong-kham (K)	Shrub	Leaves eaten as vegetable.
126.	*Vaccinium sprengelii* (G. Don) Sleumer	Ericaceae	Dieng jing (K)	Tree	Leaves used as vegetable.
127.	*Vernonia cinerea* (L.) Less.	Asteraceae	Ja long (K)	Herb	Tender shoots eaten as vegetable.
128.	*Xanthium strumarium* L.	Asteraceae	Lokra (G)	Herb	Shoots used as vegetable.
129.	*Zanthoxylum acanthopodium* DC.	Rutaceae	Jaiur khlaw (K)	Tree	Tender leaves are eaten as vegetable.
130.	*Zanthoxylum armatum* DC. (=*Zanthoxylum alatum* Roxb.)	Rutaceae	Jaiur (K)	Tree	Leaves used as spice.
131.	*Zanthoxylum khasianum* Hook. f.	Rutaceae	Jaiur khasi (K, J), Sumet cheng (G)	Climber	Leaves cooked as vegetable.
132.	*Zanthoxylum limonella* (Dennst.) Alston	Rutaceae	Hajor (G)	Tree	Leaves used as vegetable and flavouring agent.
133.	*Zanthoxylum oxyphyllum* Edgw.	Rutaceae	Jaiur blai (J), Miching (G)	Shrub	Leaves used as flavouring agent.
134.	*Zanthoxylum rhetsa* DC.	Rutaceae	Dieng soh-mirik (K), Bol-micheng (G)	Tree	Young leaves and shoots used as condiments or as vegetable.

• (K)- Khasi, (J)- Jaintia, (G)- Garo • (--) not recorded.

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REFERENCES

Agrahar-Murugkar, D. (2006). "Interventions using wild edibles to improve the nutritional status of Khasi tribal women". Journal of Human Ecology 14: 83-88.

Ahmed, A. A. and Borthakur, S. K. (2005). Ethnobotanical wisdom of Khasis (HynniewTreps) of Meghalaya. Bishen Singh and Mahendra Pal Singh, DehraDun.

Bokolial, D. and Syiemlieh, S. M. (2014). Non-conventional vegetables used by Khasis of Meghalaya, North East India. Advances in Plant Sciences 27(1): 249-252.

Chhetri, R. B. (2006). Trends in ethno-domestication of some wild plants inMeghalaya, northeast India. Indian Journal of Traditional Knowledge 5(3):342-347.

Chhetri, R. B. (2010). Ethnobotany of Moraceae in Meghalaya, northeastIndia. Kathmandu University Journal of Science, Engineering and Technlogy 6(1): 5-10.

Food and Agricultural Organization. (1996). The sixth world Food survey. FAO, United Nations, Rome.

Jaiswal, V. (2010). Culture and ethnobotany of Jaintia tribal community of Meghalaya, northeast India- A mini review. Indian Journal of Traditional Knowledge 9 (1): 38-44.

Jeeva, S. (2009). Horticultural potential of wild edible fruits used by the Khasi tribe of Meghalaya. Journal of Horticulture and Forestry 1(9): 182-192.

Kar, A., Goswami, N. K. and Saharia, D. (2012). Wild edible plants sold in the local market of Garo hills, Meghalaya. Journal of Frontline Research in Arts andScience 2:69-78.

Kayang, H. (2007). Tribal knowledge on wild edible plants of Meghalaya, northeast India. Indian Journal of Traditional Knowledge 6(1):177-181.

Kharshandi, D.D., Lyngdoh, D. and Bokolial, D. (2015). Minor fruits used by locals in two hill districts of Meghalaya, northeast India. Asian Journal of Pharmaceutical and Biological Research 5(1):4-15.

Lyngdoh, D. and Kharshandi, D. (2014). Enumeration and threat status of wild edible fruits in Mawsynram Community Development Block, Meghalaya, India. NeBIO 5(2):13-19.

Maikhuri, R. K. and Gangwar, A. K. (1993). Ethnobiological notes on the Khasi and Garo Tribes of Meghalaya, North East India. Economic Botany 47(4): 345-357.

Myers, N, Mittermeier, R. A., Mittermeier, C. G., Fonseca, G. A. B. and Kent, J. (2000) Biodiversity hotspots for conservation priorities. Nature 403: 853-858.

Pandey, G., Sharma, B. D., Hore, D. K. and. Rao, N. V (1993). Indigenous minor fruit genetic resources and their marketing status in north-eastern hill of India. Journal of Hill Research 6(1): 1-4.

Rao, R. R. (1981). Ethnobotany of Meghalaya: Medicinal plants used by Khasi and Garo Tribes. Economic Botany 35(1): 4-9.

Rao, R. R., and Neogi, B., (1980). Observation on the ethnobotany of the Khasi and Garo tribes of Meghalaya (India). Journal of Economic and Taxonomic Botany 1:157-162.

Sawian, J. T., Jeeva, S., Lyndem, F. G., Mishra, B. P. and Laloo, R.C., (2007). Wild edible plants of Meghalaya, Northeast India. Natural Product Radiance 6(5): 410-426.

Seal, T., (2011). "Determination of nutritive value, mineral content and antioxidant activity of some wild edible plants from Meghalaya state, India." Asian Journal of Appllied Sciences 4(3): 238-246.

Singh, B., Sinha, B. K., Phukan, S. J., Borthakur, S. K. and Singh, V. N., (2012). Wild edible plants used by Garo Tribes of Nokrek Biosphere Reserve in Meghalaya, India. Indian Journal of Traditional Knowledge 11(1):166-171.

http://www.theplantlist.org The Plant List version 1.1 (2013)

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REPETITIVE TRANSCRANIAL MAGNETIC STIMULATION IMPROVED GROSS AND FINE MOTOR FUNCTIONS IN SPASTIC CEREBRAL PALSY CHILDREN

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ABSTRACT

Repetitive Transcranial magnetic stimulation (rTMS) being a non-invasive stimulation technology has proved to induce motor functions in patients suffering from neuromuscular disorder. Spastic cerebral palsy (sCP) is one such neuromuscular disorder that affects movement and posture of a developing child. In order to treat these spastic patients, various therapeutic interventions are currently being used with limited result and newer ones that can yield better results are continuously been explored; rTMS is one such intervention. In this study, we employed rTMS as an assistive tool with physical therapy (PT) to improve motor functions of sCP children. Twenty sCP children were selected and divided into two groups of ten each in reference group (RG) and interventional group (IG). RG was provided with only PT for 30 minutes daily for 20 days and IG was administered with rTMS (frequency 5Hz) for 15 minutes followed by PT of same duration as in RG. To evaluate the gross and fine motor performances of these patients, universally accepted scales namely gross motor function (GMFM) and quality of upper extremity skill test (QUEST) were used. Analysis of pre versus post GMFM and QUEST scores revealed an improvement of 0.55% in gross motor function and 0.45% in fine motor functions in RG. However, both gross and fine motor function was higher in IG with 1.95% and 0.99% respectively as compared to RG. The result showed that rTMS followed by PT was more effective in improving motor ability in sCP patients.

Key Words: Cerebral Palsy (CP), Gross Motor Function Measure (GMFM), Quality of Upper Extremity Skill Test (QUEST), Physical Therapy (PT), Transcranial Magnetic Stimulation (TMS)

INTRODUCTION

With the introduction of repetitive Transcranial Magnetic Stimulation (rTMS) as brain stimulation technique in 1989, there had been a number of investigational studies that demonstrated its ability to stimulate corticospinal and intracortical motor cortex (Valero-Cabre and Pascual-Leone, 2005). This excitability phenomenon of rTMS was used as therapeutic tool for a variety of neurological and related movement disorders (Kamble *et. al.,* 2014). rTMS is a non-invasive brain stimulation technique where generated magnetic field from the coil is delivered through the skull deep into the brain tissue. The repetitive pulses of the magnetic field stimulate neuronal activity of the target brain area by changing the pre stimulus dynamics of neuronal firing in the stimulated region (Ridding and Rothwell, 2017). Studies have established that brain stimulation using rTMS can stimulate motor neurons that facilitate motor function in animals (Adkins-Muir and Jones, 2003; Plautz *et. al.,* 2003) and humans (Kumru, 2013). Kirton (2013) demonstrated the use of high frequency rTMS on stroke and CP patients' that facilitated motor function by stimulating their motor cortex. Cerebral palsy (CP) is a neurodevelopmental disorder caused due to injury to the developing brain either during birth or within two years of life (Rosenbaum *et. al.,* 2007). CP is of different types – ataxic, spastic and dyskinetic; among which spastic CP is most common. Spastic cerebral palsy is a neuromuscular impairments that limits the movement and posture of the body due to increase in the tonic stretch reflex or exaggerated tendon reflex in the muscles (Odeen, 1981). These patients are not able to perform activities of daily living (ADL) that involve movement and coordination of arm, leg and other body parts and thus fail to achieve developmental activities such as rolling, crawling, sitting, standing and walking.

In order to reduce muscle tightness for improving their motor performance, various interventions such as intrathecal baclofen, dorsal rhizotomy, hyperbaric oxygen therapy, electric stimulation, etc. (Sénéchal *et. al.,* 2007) constrained induced movement therapy (Taub *et. al.,* 1998), rTMS (Gupta *et. al.,* 2016) are combined with physical therapy. Physical therapy (PT) teaches day-to-day movement skills such as sitting, walking, playing and dressing using cast's orthotics and provides muscle strengthening exercises (Damiano, 2006). PT decreases muscle tightness, strengthen underlying muscles, and learn proper or functional motor patterns. Thus, in the present study we aim to evaluate the effectiveness of rTMS over PT to improve motor function in spastic CP children.

MATERIALS AND METHOD

Material

In this study, Neuro-MS/D Variant-2 therapeutic (Neurosoft, Russia) with angulated

coil in the figure of eight (AFEC-02-100-C) and two channels of Neuro-EMG–MS digital system (for determining the motor threshold) were employed. The eight shaped coil generates a magnetic field of up to 4 Tesla at the center of the coil that easily penetrates the cranium and enters into the soft tissue of the brain.

Participants

Patients diagnosed as spastic CP (hemiplegic, diplegic or quadriplegic) by consultant physician and neurologists that met our inclusion criteria were selected for the study. Inclusion criteria followed were – willingness to participate; age group between 2 to 15 years; muscle tightness mild to moderate and cognitive deficiency nil to moderate. The exclusion criteria were – any metallic implant; uncontrolled seizures or unstable physical condition and any congenital disorders. Twenty sCP patients from the out-patient department of UDAAN-for the different abled, New Delhi, were selected for the study. Informed consent was procured from the parents or guardians of the selected children after briefing them about the effects of rTMS. The patient were divided into two groups – reference group (RG) and interventional group (IG). The participant's characteristics are presented in table 1.

Table 1 Characteristics of study group

	Reference Group (RG)	Interventional group (IG)
Mean Age; years (SD)	8.41 ± 4.31	7.93 ± 4.85
Mean Height; cm (SD)	107.9 ± 26.33	109.4 ± 28.04
Mean Weight; kg (SD)	21.4 ± 12.63	22.4 ± 12.31
Gender; Male : Female	7 : 3	7 : 3

Assessment Scales

Gross Motor Function Measure (GMFM) and Quality of Upper Extremity Skill Test (QUEST) were used as assessment scales in this study. Both these scales are widely used by trained physiotherapists to monitor improvement in sCP patients after administering any intervention. Both the scales are performance based measures for lower and upper limbs. GMFM reflects developmental milestones of a growing child (rolling, crawling, sitting, standing, walking/running) referred to as gross motor abilities of CP patients (Russel et al., 1989) and QUEST reflects hand functions (grasping, lifting, weight bearing) referred to as fine motor abilities (DeMatteo et al., 1993).

GMFM has total 88 assessment items which are grouped into five domains,

namely A - lying and rolling (17 items), B - sitting (20 items), C - crawling and kneeling (14 items), D - standing (13 items), and E - walking, running and jumping (24 items). QUEST was specially developed to overcome the limitation of measures of hand function in children with motor disabilities. It comprises of descriptive and impairment based measures which are designed to evaluate the fine movement patterns in CP patients. QUEST evaluates 36 items of upper extremity in four domains, namely, A - dissociated movements, B - grasping, C - protective extension and D - weight bearing.

Method

In this study, the RG comprising of ten patients were provided only PT for 30 minutes daily for 20 days (5 days per week for 4 weeks) and in the IG, rTMS frequency of 5Hz (1500 pulses) was administered daily for 15 minutes for 20 days followed by PT. In IG, prior to starting the rTMS therapy, motor threshold (MT) of the subject was determined. Motor threshold is defined as the minimum intensity of single pulse of TMS which was required to produce a predefined motor evoked potential in abductor pollicis brevis (ABP) muscle in at least 50% of the trials (Rossini et al., 1994). Determination of MT is of great importance in TMS studies because it is a way to calibrate the coil output energy (magnetic field) for dose and safety limits (Rajapakse and Kirton, 2013).

Statistical Analysis

Paired t-test on pre versus post GMFM and QUEST scores of different groups was performed using MS-Excel. The alpha value of 0.05 was fixed for all statistical analysis. Additionally, the mean and median values of assessment scales were used to evaluate the percentage of improvement in different groups by different therapeutic approaches.

RESULTS

The statistical analysis is summarized in table 2. The p-value obtained from the analysis of both the assessment scales (GMFM and QUEST) of different groups (RG and IG) showed statistically significant result ($p<0.05$). Additionally, the change in median and mean scores in both the groups (RG and IG) signifies improvement in gross and fine motor abilities of spastic CP children.

Table 2 Descriptive statistics of reference and interventional groups

Scales	Group	Median		Mean ± SD		p-value
		Pre	Post	Pre	Post	
GMFM	RG	66.54	67.11	52.52 ± 33.21	53.07 ± 33.52	0.002
	IG	52.29	53.24	44.23 ± 27.57	46.18 ± 26.65	0.006
QUEST	RG	58.70	60.50	60.14 ± 13.16	60.59 ± 13.04	0.039
	IG	64.45	66.08	66.68 ± 14.47	67.67 ± 14.74	0.049

p-value < 0.05 taken as significant.

Gross Motor Function

The improvement in gross motor function in different groups as evaluated from change in mean score (%) and it was found to be 0.55% and 1.95% in RG and IG respectively (figure 1). This improvement is further validated by the change in median scores of GMFM (table 2). Significant improvement in motor function of patients in IG was observed where patients were administered rTMS followed by PT as compared to RG where only PT was administered.

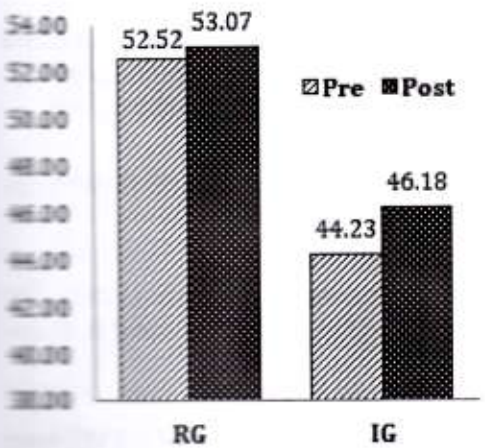

Figure 1: Mean change between pre and post **GMFM** score in different groups

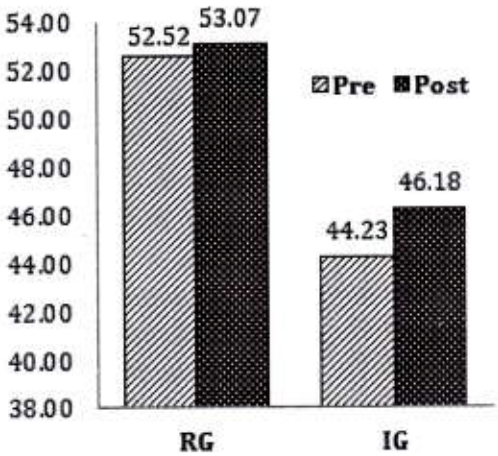

Figure 1: Mean change between pre and post **GMFM** score in different groups

Fine Motor Function

The improvement in fine motor function among both the groups as evaluated from change in mean score (%) was found to be 0.45 and 0.99 in RG and IG respectively (figure 2). This improvement is further validated by the change in median scores of QUEST (table 2). Appreciable improvement in hand function in patients of IG was observed due to the stimulating effect of rTMS.

CONCLUSION

The study demonstrated the effectiveness of rTMS over PT alone. Those patients who were administered rTMS followed by PT showed appreciable progress both in GMFM and QUEST scores in limited 20 sessions as compared to those patients that were provided only PT. The improved gross and fine motor performance in sCP children must be attributed to the stimulating effect of the magnetic pulses delivered from the rTMS on the motor cortex area of the brain that led to the activation of related muscles which

otherwise might have been dormant.

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REFERENCES

Adkins-Muir, D. L., and Jones, T. A. (2003). Cortical electrical stimulation combined with rehabilitative training: enhanced functional recovery and dendritic plasticity following focal cortical ischemia in rats. Neurological Research, 25(8), 780-788

Damiano, D. L. (2006). Activity, activity, activity: rethinking our physical therapy approach to cerebral palsy. Physical therapy, 86(11), 1534-1540

DeMatteo, C., Law, M., Russell, D., Pollock, N., Rosenbaum, P., Walter, S. (1993). The reliability and validity of the Quality of Upper Extremity Skills Test. Physical & Occupational Therapy in Pediatrics, 13(2), 1-18

Gupta, M., Lal Rajak, B., Bhatia, D., Mukherjee, A. (2016). Effect of r-TMS over standard therapy in decreasing muscle tone of spastic cerebral palsy patients. Journal of Medical Engineering and Technology, 40(4), 210-216

Kamble, N., Netravathi, M., and Pal, P. K. (2014). Therapeutic applications of repetitive transcranial magnetic stimulation (rTMS) in movement disorders: a review. Parkinsonism & Related Disorders, 20(7), 695-707

Kirton, A. (2013). Modulation of Developmental Plasticity with Non-Invasive Brain Stimulation in Cerebral Palsy. International Journal of Physical Medicine and Rehabilitation, 1(135), 2

Kumru, H., Benito, J., Murillo, N., Valls-Sole, J., Valles, M., et al. (2013). RETRACTED: Effects of High-Frequency Repetitive Transcranial Magnetic Stimulation on Motor and Gait Improvement in Incomplete Spinal Cord Injury Patients. Neurorehabilitation and Neural Repair, 27(5), 421-429

Odeen, I. (1981). Reduction of muscular hypertonus by long-term muscle stretch. Scan-

dinavian Journal of Rehabilitation Medicine, 13(2-3), 93-99

Plautz, E. J., Barbay, S., Frost, S. B., Friel, K. M., Dancause, N., et al. (2003). Post-infarct cortical plasticity and behavioral recovery using concurrent cortical stimulation and rehabilitative training: a feasibility study in primates. Neurological Research, 25(8), 801-810

Rajapakse, T., & Kirton, A. (2013). Non-invasive brain stimulation in children: applications and future directions. Translational Neuroscience, 4(2), 217-233

Ridding, M. C., and Rothwell, J. C. (2007). Is there a future for therapeutic use of transcranial magnetic stimulation? Nature Reviews Neuroscience, 8(7), 559

Rosenbaum, P., Paneth, N., Leviton, A., Goldstein, M., Bax, M., et al. (2007). A report: the definition and classification of cerebral palsy April 2006. Developmental Medicine and Child Neurology. Supplement, 109, 8-14

Rossini, P. M., Barker, A. T., Berardelli, A., Caramia, M. D., Caruso, G., et al. (1994). Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee. Electroencephalography and Clinical Neurophysiology, 91(2), 79-92

Russell, D. J., Rosenbaum, P. L., Cadman, D. T., Gowland, C., Hardy, S., Jarvis, S. (1989). The gross motor function measure: a means to evaluate the effects of physical therapy. Developmental Medicine & Child Neurology, 31(3), 341-352

Sénéchal, C., Larivée, S., Richard, E., Marois, P. (2007). Hyperbaric oxygenation therapy in the treatment of cerebral palsy: a review and comparison to currently accepted therapies. Journal of American Physicians and Surgeons, 12(4), 109

Taub, E., Crago, J. E., Uswatte, G. (1998). Constraint-induced movement therapy: A new approach to treatment in physical rehabilitation. Rehabilitation Psychology, 43(2), 152

Valero-Cabre, A., and Pascual-Leone, A. (2005). Impact of TMS on the primary motor cortex and associated spinal systems. IEEE Engineering in Medicine and Biology Magazine, 24(1), 29-35

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