ISSN 2221-6421 (Print), ISSN 2223-2567 (Online)
Coden: PJSIC6 61(3) 121-186 (2018)

Pakistan Journal of Scientific and Industrial Research

Series B: Biological Sciences

Vol. 61, No. 3, September-December, 2018

(for on-line access please visit web-site http://www.pjsir.org)

Published by
Scientific Information Centre
Pakistan Council of Scientific and Industrial Research
Karachi, Pakistan

Pakistan Journal of Scientific and Industrial Research

Series B: Biological Sciences

Pakistan Journal of Scientific and Industrial Research is published triannually into two series:

Series A: Physical Sciences [**ISSN 2221-6413 (Print); ISSN 2223-2559 (Online)]** (appearing as issues of January-April, May-August and September-December) and
Series B: Biological Sciences [**ISSN 2221-6421 (Print); ISSN 2223-2567 (Online)]** (appearing as issues of January-April, May-August and September-December).

This Journal is indexed/abstracted in Zoological Record, BIOSIS Preview, NCBI, Pub Med, Scimago, Research Gate, Clarivate Analytics.
Subscription rates (including handling and Air Mail postage): ***Local:*** Rs. 2500 per volume, single issue Rs. 425
Foreign: US$ 450 per volume, single issue US$ 75
Electronic format of this journal is available with: ProQuest Information and Learning, 789 E. Eisenhower Parkway, P.O. Box 1346, Ann Arbor, MI 48106-1346, U.S.A.; Fax.No.+1.734.997.4268; http://www.proquest.com.
Photocopies of back issues can be obtained through submission of complete reference to the Executive Editor against the payment of Rs. 25 per page per copy (by Registered Mail) and Rs. 115 per copy (by Courier Service), within Pakistan; US$ 10 per page per copy (by Registered Mail) and US$25 per page per copy (by Courier Service), for all other countries.

Printed and Published by: PCSIR Scientific Information Centre, PCSIR Laboratories Campus, Shahrah-e-Dr. Salimuzzaman Siddiqui, Karachi-75280, Pakistan.

Editorial Address

Executive Editor
Pakistan Journal of Scientific and Industrial Research, PCSIR Scientific Information Centre,
PCSIR Laboratories Campus, Shahrah-e-Dr. Salimuzzaman Siddiqui, Karachi-75280, Pakistan
Tel: 92-21-99261914-99261916, 99261949-99261917; Fax: 92-21-99261913; Web: http://www.pjsir.org, E-mail: info@pjsir.org

AIMS & SCOPE

Pakistan Journal of Scientific and Industrial Research (PJSIR) was started in 1958 to disseminate research results based on utilization of locally available raw materials leading to production of goods to cater to the national requirements and to promote S&T in the country. Over the past 60 years, the journal conveys high quality original research results in both basic and applied research in Pakistan. A great number of major achievements in Pakistan were first disseminated to the outside world through PJSIR.

It is a peer reviewed journal and published in both print and electronic form. Original research articles, review articles and short communications from varied key scientific disciplines are accepted however, papers of Pure Mathematics, Computer Sciences, Engineering and Medical Sciences are not entertained.

From 54th Volume in 2011, it has been bifurcated into Series A: Physical Sciences & Series B: Biological Sciences. Each series appeared three times in a year from 2011 to 2017.

From 61st Volume in 2018 both Series will be published Triannually with the following sequence:

Physical Sciences in April, August & December each year. It includes research related to Natural Sciences, Organic Chemistry, Inorganic Chemistry, Physical Chemistry, Environmental Sciences, Geology, Geography, Glass Technology, Material Sciences, Physics, Polymer Sciences and Technology.

Biological Sciences in April, August & December each year. Papers included in this series are from Agriculture, Agronomy, Botany, Biochemistry, Biotechnology, Food Sciences, Genetic Engineering, Pharmaceutical Sciences, Microbiology, Marine Sciences, Soil Sciences, Food Sciences, Tissue Culture, Natural Products, Toxicology, Ecology, Veterinary Sciences, Zoology and Technology.

Due to many global issues, we are encouraging contributions from scientists and researchers from all across the globe with the sole purpose of serving scientific community worldwide on the whole and particularly for our region and third world countries.

Pakistan Journal of Scientific and Industrial Research
Series B: Biological Sciences
Vol. 61, No. 3, September-December, 2018

Contents

Special Paper

Reviews

Pak. j. sci. ind. res. Ser. B: biol. sci. 2018 **61B**(3) 121-125

Special Paper

A Bibliometric Portrait of Pakistan Journal of Scientific and Industrial Research (PJSIR) During the Period of 1958-2007

Zameer Hussain Baladi
King Saud Bin Abdulaziz University for Health Sciences, College of Applied Medical Sciences, Ministry of National Guard Health Affairs Riyadh, Kingdom of Saudi Arabia

(received September 17, 2018; revised October 3, 2018; accepted October 5, 2018)

Abstract. Pakistan Journal of Science and Industrial Research (PJSIR) had celebrated its sixtieth anniversary in 2017. Inspired by this occasion, this observational study presents a bibliometric review on the quantity of all published materials under the caption of Physical, Biological and Technological Sciences with Short Communications during the period of 1958-2007 in Pakistan. The data of 340 issues of PJSIR was downloaded and collected to tabulate from the website of electronic journal: (http: www.pjsir.org/arc.php) during January-July, 2018. This study expressed that n=4929; 14.4% articles were published in 340 issues of PJSIR during the period of 1958-2007. Total 4417 (1790; 36.3%, 1651; 33.5%, and 976; 19.8%) articles published under the caption of Physical, Biological, and Technology out of 4929 articles. Remaining 512; 10.3% articles were short communications. Maximum articles n=1375; 28% were published in the fourth decade and n=694; 14% articles in the first decade as a minimum. The short communications n=208; 4.2% related to biological science take a position with the slight margin to other disciplines. PJSIR published regularly from 1958 to this day. It is counted a teamwork of the management of Journal and supported by Pakistan Council of Scientific and Industrial Research (PCSIR) Government-owned body. There are few examples in the world to publish a scientific journal which covers three major disciplines of science.

Keywords: bibliometrics, PJSIR, PCSIR, Ministry of Science and Technology, Pakistan

Introduction

Pakistan Journal of Scientific and Industrial Research (PJSIR) regularly published from 1958 under the Pakistan Council of Scientific and Industrial Research (PCSIR) Government-owned body. It covers the research in basic and applied sciences of physical, biological and technological sciences with their sub-specialties. Bibliometric is the branch which measures the information regarded to the book, an article or a text. This application handles the information mathematically and statistically of a written article published as a text in the book or another format (Wilson, 2014). A journal plays a vital role in disseminating the knowledge to update the researchers, institutes, and countries around the world journals (Mohan and Raigoly, 2017). Publishing articles in journals is a powerful method and provides the help to institutes for more attention in progress of individual talent and funding from the donors (Rawat and Meena, 2014).

E-mail: baladiz@ksau-hs.edu.sa & zameer.baladi@gmail.com

Literature review. A quantitative study was carried out to estimate IEEE scientific journals on the Google Scholar, Web of Science and Scopus databases by traditions about the value of citations, the reliability of search engine statistics and the similarity. This study found 250,000 authors which published their research in 110 IEEE journals. This study also provides bibliometric as a methodological tool for monitoring a large number of scientific journals (Canavero *et al*., 2014). Computers and Industrial Engineering (CIE) is a leading international journal in the field of industrial engineering published research regularly from 1976 to-date. With the help of Web of Science (WoS) database, a study was conducted to know the prominent participators in this journal. The United States of America (USA) was most productive country followed by the People Republic of China (PRC) publishing in CIE (Cansino *et al*., 2017). A research was examined to discover critical themes with collaboration in international construction, the patterns of development and active institutes. Only 87 articles were published in six journals in this field from

2003-2013. The risk management, measuring performance, competencies and foreign market were top trends in industrial research. The National University of Singapore, the Hong Kong Polytechnic University, and Middle East Technical University, Turkey were top in publications (Li *et al*., 2018; Gundes and Aydogan 2016) conducted bibliometric investigations based on the literature covering terms of solid waste reuse and recycling published in Web of Science and its sub-databases; Science Citation Index (SCI), Social Sciences Citation Index (SSCI), Conference Proceedings Citation Index-Science (CPCI-S) and Conference Proceedings Citation Index-Social science & Humanities (CPCI-SSH) during the period of 1992-2016. Study finds 6289 articles published in 1402 journals. Department of Computer Science and Engineering, University of Bohemia, Czech Republic conducted an interested study on the title of computer science with artificial intelligence, interdisciplinary applications, hardware and architecture with software engineering as sub-titles and it reveals that 1,922,652 (1.9 million) articles published all-around the world from 1945-2014 and available on Web of Science (Fiala and Tutoky, 2017). A comparative study was carried out on the journals of Pakistan Heart Journal (PHJ) and Journal of Saudi Heart Association (JSHJ), it was revealed that 393 (207; 906% by PHJ and 186; 10.7% by JSHA) articles were published during the year 2012-2016 with the contribution of 1840 researchers (Baladi and Satti, 2018).

Materials and Methods

This retrospective study started with the aim to evaluate the number of published articles under the title of physical, biological and technological sciences with short communications, volume, decade, and year-wise. The data of all research items published during 1958 to 2007 in the form of abstracts was downloaded and collected from the website of an electronic journal: (http://www.pjsir.org/arc.php) during January to July, 2018 in the library of College of Applied Medical Sciences King Saud bin Abdulaziz University of Health Affairs, Riyadh Kingdome of Saudi Arabia. Microsoft Excel 2010 spreadsheet had been prepared for data analysis.

The objectives were set to examination:

(a) To identify the year, volume and issue wise distribution of publications;
(b) To calculate the share of disciplines:
    (i) Physical, biological, technological sciences;
    (ii) Short communications wise;
(c) To recognize the decade wise publications.

Results and Discussion

Figure 1 and Table 1 reveals the results of this study it shows the results that PJSIR published 4929 articles in 340 issues and 50 volumes with an average of 14.4% articles per issue and 98.5% articles per volume. PJSIR published 156; 45.8% issues as bi-monthly followed by 132; 38.8% issues as monthly and 52; 15.2% issues as on quarterly basis.

Figure 2 and Table 2-3 summarized the position of distribution of publications in PJSIR during the studied period, it reveals that research on physical sciences shows influences with n=1790; 36.3% articles followed by biological science n=1651; 33.5% articles and Table 2 elaborate the picture of publications decade wise from 1958-2007. The fourth decade (1988-1997) published 1375; 28% articles out of 4929 articles followed (1968-1977) by 1005; 20.3% as maximum.

Figure 3 and Tables 4-8 explained the decade and year wise breakdown of PJSIR publishing research during the period of 1958-2007. Maximum n=238; 4.8% articles were published in the year 1987 followed by n=235; 4.7% articles in the year 1989. The minimum n=29; 0.5% articles published in the year 1997 and followed by n=36; 0.7% in the year 1959.

Table 1. Nomenclature of PJSIR 1958 - 2007

PJSIR volumes	Total issues
1 to 13 (Quarterly)	52 (1.05%)
14 to 29 (Bi-Monthly)	96 (1.95%)
30 to 40 (Monthly)	132 (2.68%)
41 - 50 (Bi-Monthly)	60 (1.22%)
Total volumes	50 (98.5 articles published per volume
Total issues	340 (14.4 articles published per issue)
Total articles	4929

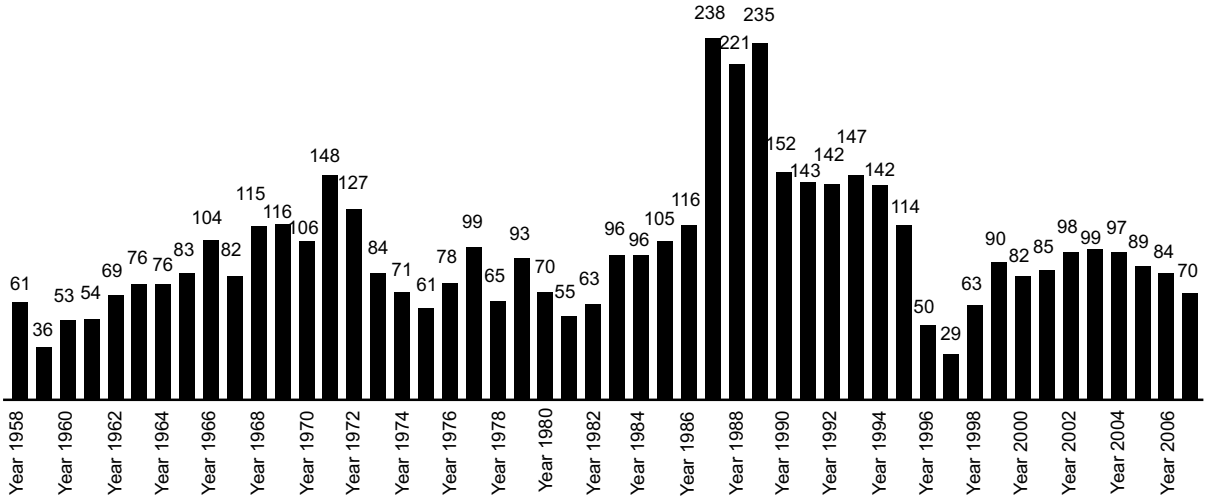


Fig. 1. Resarch Published in PJSIR during the period of 1958 - 2007

Table 2. Decade wise distribution of articles from 1958 - 2007

Decades	1958-1967	1968-1977	1978-1987	1988-1997	1998-2007	Total	PDA*	%
Physical Sciences	284	297	358	509	342	1790	358	36.3%
Short Communications	13	51	34	38	50	186	37.2	3.7%
Biological Sciences	234	295	316	473	333	1651	330.2	33.5%
Short Communications	12	49	26	75	46	208	41.6	4.2%
Technological Sciences	147	261	246	248	74	976	195.2	19.8%
Short Communications	4	52	17	32	13	118	23.6	2.3%
Total (It include 17 Special Articles and 18 Review Papers)	694 14%	1005 20.3%	997 20.2%	1375 28%	858 14.4%	4929		

* PDA: per decade average.

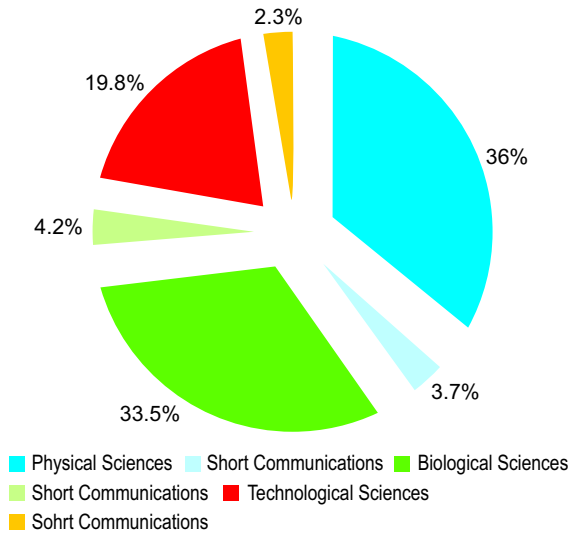


Fig. 2. Resarch articles published in PJSIR from 1958 - 2007.

Table 3. Special Articles/Review Papers published in PJSIR during 1958 - 2007

PJSIR	Phys. Sci.	Biol. Sci.	Tech. Sci.	Total
Special Articles	5	4	8	17
Review Papers	1	1	16	18
Total	6	5	24	35

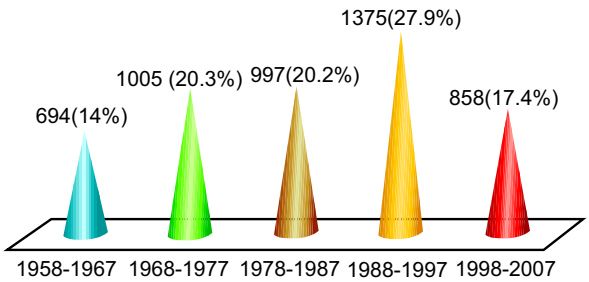


Fig. 3. Resarch published in fifty years of PJSIR (1958 - 2007).

Table 4. Distribution of articles during the period of 1958 - 1967

Years	1958	1959	1960	1961	1962	1963	1964	1965	1966	1967	Total	PDA*
Physical Sciences	26	17	19	21	23	36	32	33	42	35	284	28.4
Short Communications			0	2	0	2	1	2	3	3	13	1.62
Biological Sciences	21	13	21	24	23	19	25	27	34	27	234	23.4
Short Communications			4	1	1	1	3	1	1	0	12	1.5
Technological Sciences	14	6	9	6	20	17	15	19	24	17	147	14.7
Short Communications			0	0	2	1	0	1	0	0	4	0.5
Total	61	36	53	54	69	76	76	83	104	82	694	69.4

* PDA: per decade average.

Table 5. Distribution of articles during the period of 1968 - 1977

Years	1968	1969	1970	1971	1972	1973	1974	1975	1976	1977	Total	PDA*
Physical Sciences	38	44	27	39	28	26	24	19	26	26	297	29.7
Short Communications	6	7	2	5	9	4	3	6	7	2	51	5.1
Biological Sciences	34	31	45	64	33	20	14	17	16	21	295	29.5
Short Communications	7	6	2	7	9	8	1	3	3	3	49	4.9
Technological Sciences	21	22	30	23	37	23	29	14	22	40	261	26.1
Short Communications	9	6	0	10	11	3	0	2	4	7	52	5.2
Total	115	116	106	148	127	84	71	61	78	99	1005	

Table 6. Distribution of articles during the period of 1978 - 1987

Years	1978	1979	1980	1981	1982	1983	1984	1985	1986	1987	Total	PDA*
Physical Sciences	21	24	27	22	29	41	36	37	38	83	358	35.8
Short Communications	4	5	1	4	4	6	2	0	5	3	34	3.4
Biological Sciences	13	9	15	10	12	19	38	52	46	102	316	31.6
Short Communications	0	3	1	3	1	5	1	1	4	7	26	2.6
Technological Sciences	23	46	25	14	17	24	17	15	23	42	246	24.6
Short Communications	4	6	1	2	0	1	2	0	0	1	17	1.7
Total	65	93	70	55	63	96	96	105	116	238	997	99.7

Table 7. Distribution of articles during the period of 1988 – 1997

Years	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	Total	PDA*
Physical Sciences	74	69	68	57	59	45	57	46	18	16	509	50.9
Short Communications	6	6	7	2	10	4	1	0	2	0	38	3.8
Biological Sciences	81	101	42	40	30	61	49	39	22	8	473	47.3
Short Communications	10	11	6	16	13	11	8	0	0	0	75	7.5
Technological Sciences	47	47	28	26	29	25	19	17	6	4	248	24.8
Short Communications	3	1	1	2	1	1	8	12	2	1	32	3.2
Total	221	235	152	143	142	147	142	114	50	29	1375	37.5

*PDA = per decade average.

Table 8. Distribution of articles during the period of 1998 – 2007

Years	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	Total	PDA*
Physical Sciences	30	35	39	32	29	36	32	39	40	30	342	34.2
Short Communications	2	4	3	6	11	6	5	4	4	5	50	5
Biological Sciences	24	33	28	38	43	46	46	27	23	25	333	33.3
Short Communications	1	5	4	2	5	5	6	8	6	4	46	4.6
Technological Sciences	6	11	8	6	9	6	7	7	8	6	74	7.4
Short Communications	0	2		2	1	0	1	4	3	0	13	1.4
Total	63	90	82	86	98	99	97	89	84	70	858	85.8

*PDA = per decade average.

Conclusion

Interesting results were found in this study that there are three hundred forty issues were published in 50 volumes with an average of 6.8 per issue. Total 4417 (1790; 40.5%, 1651; 37.3% and 976; 22%) articles published under the caption of Physical, Biological, and Technology out of 4929 articles. Remaining 512; 10.3% articles were short communications. There is a difference of 311; 6.3% articles published in the first decade to the second decade, instead of 517; 10.4 articles between fourth to fifth decades out of 4929 articles published in five decades. Short communications reflect the new ideas and opinions of researchers in a concise way about any discipline of study. A publisher plays a significant role to encourage researcher for contributing their knowledge, ideas, and experiments in the form of articles, to get the value that supports teaching and learning. This study found that short communication in technology gets the minimum attention of researchers, except the fourth decade 75; 51.7% out of 145 short communications in biological science during the period 1988-1997 as a maximum. The management of Pakistan Journal of Science and Industrial Research (PJSIR) try to engage and facilitate to researcher through its challenging policies.

References

Baladi, Z.H., Satti, M.H. 2018. Comparative research productivity of Pakistan Heart Journal and Journal of Saudi Heart Association. A bibliometric analysis 2012-2016. *Pakistan Heart Journal*, **51**.

Canavero, F., Franceschini, F., Maisano, D., Mastrogiacomo, L. 2014. Impact of journals and academic reputations of authors. A structured bibliometric survey of the IEEE publication galaxy. *IEEE Transactions on Professional Communication*, **57:** 17-40.

Cancino, C., Merigo, J.M., Coronado, F., Dessouky, Y., Dessouky, M. 2017. Forty years of computers and industrial engineering. A bibliometric analysis. *Computers and Industrial Engineering*, **113:** 614-629.

Fiala, D., Tutoky, G. 2017. Computer Science Papers in Web of Science. A bibliometric analysis. *Publications*, **5:** 23.

Gundes, S., Aydogan, G. 2016. Bibliometric analysis of research in international construction. *Canadian Journal of Civil Engineering*, **43:** 304-311.

Li, N., Han, R., Lu, X. 2018. Bibliometric analysis of research trends on solid waste reuse and recycling during 1992-2016. *Resources, Conservation and Recycling*, **130:** 109-117.

Mohan, B.S., Rajgoli, I.U. 2017. Mapping of Scholarly Communication in Publications of the Astronomical Society of Australia, Publications of the Astronomical Society of Japan and Publications of the Astronomical Society of the Pacific. A bibliometric approach. *Science and Technology Libraries*, **36:** 351-375.

Rawat, S., Meena, S. 2014. Publish or perish. Where are we heading?. *Journal of Research in Medical Sciences*. The official journal of Isfahan University of Medical Sciences, **19:** 87-89.

Wilson, V. 2014. Research methods. Triangulation. *Evidence Based Library and Information Practice*, **9:** 74-75.

Pak. j. sci. ind. res. Ser. B: Biol. sci. 2018 **61B**(3) 126-135

General and Specific Combining Ability Estimates for Morphological, Yield and its Attributes and Seed Traits in Sunflower (*Helianthus annuus* L.)

Muhammad Siddique Depar[a], Muhammad Jurial Baloch[a]* and Qumar-Uddin Chacher[b]
[a]Department of Plant Breeding and Genetics, Sindh Agriculture University, Tandojam, Pakistan
[b]Department of Crop Physiology, Sindh Agriculture University, Tandojam, Pakistan

(received October 28, 2016; revised May 25, 2017; accepted May 29, 2017)

Abstract: The improvement in sunflower breeding requires exploitation of combining ability of divergent male and female inbreds. Six cytoplasmic male sterile (CMS) lines and three testers were crossed in line × tester design, thus 18 F_1 hybrids were developed for evaluation of general combining ability (GCA) and specific combining ability (SCA) of inbred parents for days to 90% maturity, stem girth, head size, achenes/plant, 1000-seed weight, achene yield kg/ha, oil and protein%. The significant variances due to lines and testers both determined GCA variances revealed the predominance of additive genes whilst significance of lines × tester interactions indicated the importance of SCA variances and the involvement of non-additive genes in the expression of traits studied. The foremost role of non-additive genes was apparent when ratio σ^2 SCA/σ^2GCA was above 1.0. These results suggested the prevalence of dominant genes and possibility of hybrid crop development. The GCA effects indicated that CMS parents SF-187, 64-A-93 and ARG-0405 and tester RHP-46 were high general combiners, thus may be chosen for crossing and selection programmes, whereas F_1 hybrids SF-187 × RHP-46, 64-A-93 × RHP-46, PAC-ARG-0405 × PAC-ARG-0106, 64-A-93 × RHP-46 and PSF-025 × RHP-64 which used parents with good × good and good × poor GCA estimates revealed higher positive SCA estimates for achene yield, oil and protein traits yet manifested desirable negative effects for 90% maturity. Such results suggested that these hybrids are desirable for the exploitation of hybrid crop development or selection of desirable plants from earlier filial generations.

Keywords: combining ability estimates, line × tester analysis, sunflower

Introduction

Sunflower hybrids are cultivated on more land area than open pollinated varieties because basic advantages of hybrids over open pollinated or synthetic varieties are that hybrids are more stable, self-fertile, highly productive, even in maturity, lodging resistant with stiff stalk, shorter life cycle and wider adaptability as compared to open pollinated varieties (Jocic *et al*., 2012; Onemli, 2012; Rehman *et al*., 2012; Arshad *et al*., 2010; Kannababu and Karivaratharaju, 2000). For developing potential hybrids, sunflower breeders are constantly trying to identify new inbred lines and testers with high combining ability (Semerci, 2012; Siddiqi *et al*., 2012; Fernandez *et al*., 2004). In any hybrid development programme, a large number of crosses are to be made, yet only few of them are expected to demonstrate good performance over the standard checks or existing hybrids. This process is extremely labour extensive, time-consuming and tedious task (Andarkhor *et al*., 2012). Hence, the basic goal of sunflower breeding is the utilization of heterosis obtained by crossing potential lines and testers (Xu *et al*., 2012). The line × tester analysis developed by Kempthorne (1957) is an important biometrical approach which is used in quantitative genetics so as to obtain adequate information on combining ability of lines and testers and also inheritance of quantitative characters.

The general combining ability (GCA) and specific combining ability (SCA) are exclusively recognized in statistical genetics. Therefore, estimation of combining ability at both GCA and SCA levels for achene yield and quality characters are important genetic parameters for obtaining higher yielding hybrids (Faridi *et al*., 2015). The GCA and SCA affirm that GCA estimates are owned to additive genes whereas SCA are owned to dominant type of genes. The general and specific combining ability estimates are very meaningful biometrical techniques when plant breeders intend to produce hybrids of their own choice. Estimates of GCA and SCA studies carried-out by Leon *et al*. (1995) indicated that additive estimates were more vital for

*Author for correspondence; E-mail: j.rind58@gmail.com

seed oil percentage. The significance of additive genes for various plant characters have already been reported by several previous workers (Hlandi *et al*., 2006; Bajaj *et al*., 1997). Dominant and over-dominant gene actions were also observed for head diameter, oil%, 1000-achene weight and achene and oil production (Memon *et al*., 2015; Gangappa *et al*., 1997). Almost comparable degree of additive and non-additive gene estimates was noted for oil production (Khan *et al*., 2008). Advantageous negative GCA and SCA estimates were reported for short duration growth period by Ghaffari *et al*. (2011) and other workers like Memon *et al*. (2015); Karasu *et al*. (2010) and Khan *et al*. (2009). The development of promising hybrids can be achieved by crossing prospective male and female inbreds. Heritability estimates, specific combining ability, dominance effects and heterotic effects provide a reliable opportunity to develop hybrid varieties. Whereas additive gene action, general combining ability and genetic advance provide chance to develop potential inbreds from filial generations for higher grain and oil yields (Amin *et al*., 2014).

The present research was therefore designed to determine general and specific combining ability estimates of inbred parents and inheritance of quantitative traits for successful hybrid sunflower development.

Materials and Methods

The current studies were conducted at experimental field of Oil Seeds Section, Agriculture Research Institute, Tandojam, Pakistan. Six cytoplasmic male sterile parents such as ARG-0306, 64-A-93, PSF-025, SF-187, ARG-0405 and ARG-0505 and three testers like RHP-46, RHP-64 and ARG-0106 were hybridised in a line by tester fashion during spring 2009, hence eighteen F_1 hybrids were developed for evaluation and genetic analysis. The seeds of F_1 hybrids and parents were grown in a RCB design with four repeats during spring season 2010. The analysis of variance was determined following statistical procedures of Gomez and Gomez (1984), while mean squares for general combining ability were estimated from lines and testers and specific combining ability from lines × tester interactions by Kempthore (1957) and adopted by Singh and Choudhry (1985). The seed of F_1s and their parents were grown in 6 meter long rows keeping plant and row distances of 30.0cm and 75.0cm, respectively. The inorganic fertilizers were applied as per recommendation for sunflower. For taking the observations, 10 plants were tagged at random from each repeat per genotype for days to 90% maturity, stem girth (cm), head diameter (cm), achenes/plant, 1000-achene weight (g), seed yield (kg/ha) and oil and protein content (%).

Results and Discussion

Per se mean performance of lines, testers and F_1 hybrids. The average performance of female parents depicted in Table 1 demonstrated that SF-187 measured bigger heads, set more achenes/plant, produced higher 1000-seed weight, gave higher achene yields (kg/ha) and extracted higher oil%. The PSF-025 produced stronger stem girth and gave maximum protein content%, yet ARG-0505 matured earlier than other inbred lines evaluated (Table 1). Similarly, Khan *et al*. (2008) noted that CMS lines TS-18 followed by TS-335 performed better in many characters like yield, oil and other yield contributing characters. On the contrary to our results for phenological traits, Saleem-Uddin *et al*. (2014) observed that their four CMS lines required maximum days to maturity (106 to 125 days), yet in agreement with our results, they found bigger heads and more seed yield kg/ha. A good tester may be pure inbred line; however suitable testers are the one which correctly distinguish the performance of female parents. To ascertain the factual potential of lines, low performing testers are commonly used to determine combining ability of newly evolved inbreds. According to Hallauer and Miranda (1986) and Menz *et al*. (1999), a good tester is the one which rightly classify the comparative performance of lines, enhances the genetic gain and is easy in use. The performance of all three testers/ pollinators depicted in Table 1 indicated that tester RHP-46 measured stronger stem girth, recorded bigger heads, produced maximum achenes/plant, acquired more thousand-seed weight, gave higher achene yield (kg/ha) and produced maximum oil content%. Yet tester ARG-0106 took minimum days to 90% maturity and recorded higher protein content%. Similar to our findings, Ali *et al*. (2006) observed that tester parent KNI gave maximum oil% while Suncorn-110 recorded higher protein percentage. Inverse to our results, Saleem-Uddin *et al*. (2014) noted that tester RHP-73 was late in maturity, yet similar to our results measured bigger heads and gave seed yield kg/ha.

To develop single-cross hybrids with high genetic potential for yield and other agronomic characters, it is essential to have inbred parents (males and females)

with high combining ability. Per se performance of F_1 hybrid has not always been reflected to their parents to be considered as good or poor combiners for crossing. However, some hybrids establish similar as per se performance, good general or specific combiners with high heterotic effects (Memon *et al*., 2015b). As indicated earlier, per se mean values of the 18 F_1 hybrids were greater than the means of their parents (Table 2) and performed much better than their respective parents, which is an indication of hybrid vigour. The hybrid SF-187 × RHP-46 nevertheless recorded the maximum head diameter, set more seeds/plant, recorded higher

Table 1. Parental mean values for phenological, morphological, seed yield and its related traits, oil and protein contents of 6 female parents.

Female parent (Lines)	Days to 90% maturity	Stem girth (cm)	Head diameter (cm)	Achenes /plant	1000 seed weight	Achene yield/ha (kg)	Oil content %	Protein content %
ARG-0306	96.50	4.27	14.10	934.30	39.01	2152.0	39.70	22.17
64-A-93	92.50	4.31	14.70	946.00	41.08	2230.2	40.65	21.68
PSF-025	95.25	4.32	14.15	809.35	39.16	2143.8	38.47	22.96
SF-187	92.75	4.13	14.70	961.45	42.99	2288.0	41.32	20.53
ARG-0405	92.25	4.20	14.18	941.65	40.24	2254.3	41.17	20.73
ARG-0505	91.25	4.17	13.63	886.85	38.46	2220.0	40.22	21.99
Mean	93.42	4.24	14.24	913.27	40.15	2214.7	40.25	21.68
LSD (5%)	2.64	0.08	0.67	7.56	0.852	8.63	1.70	0.41
Male parents (Testers)	Days to 90% maturity	Stem girth (cm)	Head diameter (cm)	Achenes /plant	1000 seed weight	Achene yield/ha (kg)	Oil content %	Protein content %
RHP-46	91.25	3.97	9.45	682.10	37.44	1468.80	35.00	17.63
RHP-64	91.25	3.84	8.75	597.60	36.13	1462.20	33.57	18.17
ARG-0106	91.00	3.72	8.65	536.30	35.12	1455.30	31.71	19.38
Mean	91.17	3.84	8.95	605.33	36.23	1462.10	33.43	18.39
LSD 5%	3.15	0.15	0.12	2.73	0.58	10.07	1.07	0.65

Table 2. Per se mean performance of 18 F1 hybrids for phenological, morphological, seed yield and its related traits, oil and protein contents

Hybrids (lines and tester)	Days to 90% maturity	Stem girth (cm)	Head diameter (cm)	Achenes /plant	1000 achene weight	Achene yield/ha (kg)	Oil content %	Protein content %
ARG-0306 × RHP-46	84.75	4.64	17.98	1127.80	47.19	2640.20	43.34	28.20
ARG-0306 × RHP-64	84.50	4.97	17.40	998.40	43.72	2621.20	43.03	28.37
ARG-0306 × ARG-0106	84.75	5.39	15.30	1044.30	45.98	2631.70	42.93	29.23
64-A-93 × RHP-46	84.00	4.73	20.50	1495.70	52.91	2963.30	45.46	27.10
64-A-93 × RHP-64	84.75	5.43	18.55	1166.20	47.98	2656.00	42.35	29.31
64-A-93 × ARG-0106	85.25	5.72	17.83	1056.70	46.36	2610.20	39.80	30.92
PSF-025 × RHP-46	82.75	4.77	17.70	1016.00	45.62	2609.80	39.57	30.99
PSF-025 × RHP-64	84.00	4.90	18.18	1151.06	48.07	2645.80	42.78	29.23
PSF-025 × ARG-0106	83.50	5.24	17.98	866.70	46.60	2644.70	40.77	29.47
SF-187 × RHP-46	85.25	4.56	20.63	1500.10	53.66	3019.80	45.90	26.79
SF-187 × RHP-64	86.25	4.47	19.95	1231.80	50.66	2871.80	44.55	27.55
SF-187 × ARG-0106	84.25	4.46	18.73	1178.00	47.68	2667.80	42.11	29.33
ARG-0405 × RHP-46	81.75	4.62	19.20	1189.10	48.99	2824.80	45.00	27.47
ARG-0405 × RHP-64	86.25	4.90	19.38	1195.30	49.69	2764.20	43.83	28.03
ARG-0405 × ARG-0106	84.25	5.19	20.18	1432.50	51.44	2834.80	45.18	27.10
ARG-0505 × RHP-46	84.25	4.65	19.60	1185.70	49.48	2794.50	44.15	27.93
ARG-0505 × RHP-64	83.75	4.80	18.15	1136.50	47.30	2663.30	41.48	29.47
ARG-0505 × ARG-0106	82.75	5.35	18.58	1168.20	48.32	2629.80	40.37	30.16
Mean	84.28	4.93	18.79	1188.50	48.42	2727.40	42.92	28.70
LSD (5%)	1.90	0.15	0.58	8.16	0.86	9.11	0.72	0.23

thousand-achene weight with higher achene yield (kg/ha) and also produced higher oil percentage. Another cross 64-A-93 × RHP-46 besides seed yield provoked greater seeds per plant, gave higher thousand-seed weight and produced more oil; ARG-0405 × RHP-46 took minimum days to 90% maturity; 64-A-93 × ARG-0106 measured maximum stem girth and PSF-025 × RHP-46 produced maximum protein percentage. Usually, it is assumed, that high performing hybrids with greater SCA estimates and evolved from at least one parent with high GCA are expected to increase the number of constructive genes for hybrid crop development. Fortunately, two crosses SF-187 × RHP-46 and 64-A-93 × RHP-46 have better mean values, higher SCA estimates and possibly both parents expressed desirable GCA values; therefore performed better for almost all important yields, its related traits and oil content (Table 3-4). Large variations in parental and hybrid *per se* performance were observed for different traits. The parental performance and hybrids' performance *per se* is shown in Table 1-2. The days to 90% maturity varied from 91.00 to 96.50 and 81.75 to 86.25; stem girth from 3.72 to 4.32 cm and 4.46 to 5.72 cm; head diameter from 8.65 to 14.70 cm and 15.30 to 20.63 cm; achene/plant from 536.30 to 961.45 and 866.70 to 1500.10; 1000-seed weight from 35.12 to 42.99 g and 43.72 to 53.66 g; achene yield kg/ha from 1455.30 to 2288.00 kg and 2609.80 to 3019.80 kg; oil content% from 31.71 to 41.32% and 39.57 to 45.90% and protein percentage from 17.63 to 22.96% and 26.79 to 30.99%. These results indicated that F_1 hybrids gave significant increase in achene yield and oil characters over the parents. Similar to our results, Siddiqi *et al*. (2012) noted that some hybrids gave maximum achene yield in tonnes/ha and higher oil content over the parents.

Genetic analysis of parents and their F_1s for phenological, seed yield, oil and protein traits in sunflower. Line × tester analysis is one of the leading biometrical technique which helps in assessing enormous number of inbreds for selection of desirable parents and hybrids and endowing appropriate knowledge on GCA and SCA of inbreds. In abnormal conditions of inbred parents like self-incompatibility and male sterility where diallel crosses are not suitable mating designs, then line × tester mating design can successfully be applied to acquire the knowledge on inheritance of characters and the combining ability of inbreds. Nasreen *et al*. (2014) supported that line × tester analysis remained the major contributor in inheritance of plant characters. The mean squares indicated significant variability among the parents and their hybrids for all the traits (Table 5). Such results advocated the presence of considerable genetic variability in newly developed breeding material. These results are in consonance with Andarkhor *et al*. (2013); Ciric *et al*. (2013b); Kang *et al*. (2013) and Chandra *et al*. (2011). The significance of parent vs. hybrids

Table 3. GCA effects for seed yield and yield components and seed traits of 6 female and 3 female parents

Female parent (Lines)	Days to 90% maturity	Stem girth (cm)	Head diameter (cm)	Achenes /plant	1000 seed weight	Achene yield/ha (kg)	Oil content %	Protein content%
ARG-0306	0.39	0.17**	-1.07**	-131.63**	-2.79**	-96.43**	0.18	-0.10
64-A-93	0.39	0.36**	0.17	51.00**	0.66**	15.73**	-0.39	0.41**
PSF-025	-0.86	0.14**	-0.84**	-92.90**	-1.66**	-93.99**	-1.88**	1.20**
SF-187	0.97*	-0.43**	0.97**	114.80**	2.24**	125.73**	1.26**	-0.81**
ARG-0405	-0.19	-0.03	0.79**	83.79**	1.62**	80.51**	1.75**	-1.18**
ARG-0505	-0.69	0.02	-0.02	-25.04**	-0.06	-31.54**	-0.92**	0.49**
SE (gi.)	0.46	0.03	0.12	1.53	0.17	1.74	0.203	0.068
SE (gi-gj	0.65	0.04	0.17	2.16	0.24	2.47	0.287	0.096
Male parents (Testers)	Days to 90% maturity	Stem girth (cm)	Head diameter (cm)	Achenes /plant	1000 seed weight	Achene yield /ha(kg)	Oil content %	Protein content%
RHP-46	-0.49	-0.27**	0.47**	63.90**	1.22**	81.31**	0.98**	-0.63**
RHP-64	0.64	-0.02	-0.19**	-41.89**	-0.52**	-23.71**	0.08	-0.04
ARG-0106	-0.15	0.29**	-0.28**	-22.01**	-0.70**	-57.60**	-1.06**	0.67**
SE (gi.)	0.33	0.02	0.08	1.08	0.12	1.23	0.14	0.05
SE (gi-gj)	0.46	0.03	0.12	1.53	0.17	1.74	0.20	0.07

** = P≤ 0.01

Table 4. SCA estimates for seed yield and yield components and seed traits of eighteen sunflower crosses derived from three males and six female parents

Hybrids (lines and testers)	Days to 90% maturity	Stem girth (cm)	Head diameter (cm)	Achenes /plant	1000 achene weight	Achene yield/ha (kg)	Oil content %	Protein content %
ARG-0306 × RHP-46	0.57	-0.09	-0.22	7.10**	-1.60**	-72.15**	-0.75*	0.23*
ARG-0306 × RHP-64	-0.81	-0.03	-0.13	-16.56**	-1.39**	13.87**	-0.15	-0.19
ARG-0306 × ARG-0106	0.24	0.10*	0.36	9.47**	1.05**	58.27**	0.89*	-0.04
64-A-93 × RHP-46	-0.18	-0.29**	1.07**	192.26**	2.61**	138.84**	1.95**	-1.39**
64-A-93 × RHP-64	-0.56	0.16**	-0.22	-31.45**	-0.58*	-63.45**	-0.27	0.25*
64-A-93 × ARG-0106	0.74	0.13*	-0.85**	-160.82**	-2.03**	-75.40**	-1.68**	1.14**
PSF-025 × RHP-46	-0.18	0.07	-0.72**	-143.49**	-2.37**	-104.92**	-2.45**	1.72**
PSF-025 × RHP-64	-0.06	-0.05	0.42*	97.85**	1.83**	36.10**	1.66**	-0.63**
PSF-025 × ARG-0106	0.24	-0.02	0.31	45.63**	0.53	68.82**	0.79*	-1.09**
SF-187 × RHP-46	0.49	0.33**	0.38	132.91**	1.78**	85.35**	0.73*	-0.47**
SF-187 × RHP-64	0.36	-0.01	0.38	-29.65**	0.51	42.38**	0.29	-0.30*
SF-187 × ARG-0106	-0.85	-0.33**	-0.76**	-103.27**	-2.29**	-127.73**	-1.02**	0.77**
ARG-0405 × RHP-46	-1.85*	-0.01	-0.86**	-147.80**	-2.26**	-64.42**	-0.65	0.54**
ARG-0405 × RHP-64	1.53	0.02	-0.02	-35.14**	0.17	-20.06**	-0.92*	0.55**
ARG-0405 × ARG-0106	0.32	-0.01	0.87**	182.22**	2.09**	84.49**	1.57**	-1.09**
ARG-0505 × RHP-46	1.15	-0.02	0.35	-41.70**	-0.10	17.30**	1.17*	-0.63**
ARG-0505 × RHP-64	-0.47	-0.11*	-0.43*	14.94**	-0.55	-8.84*	-0.61	0.32*
ARG-0505 × ARG-0106	-0.68	0.13*	0.08	26.77**	0.65*	-8.46*	-0.56	0.31*
SE (si.)	0.80	0.05	0.20	2.64	0.29	3.02	0.351	0.117
SE (sij-skr)	1.13	0.07	0.29	3.74	0.41	4.27	0.497	0.166

**,* = P≤ 0.01 and 0.05 respectively.

further indicated that the data is suitable for combining ability analysis. The significance of lines and testers indicated that these findings are appropriate for estimating GCA variances and effects, especially additive types. Whereas significant mean squares for parents vs. hybrids suggested the presence of heterosis for all the characters and similar findings were reported by Mirarab and Ahmadikhah (2010). The significant mean squares due to lines by tester interactions indicated the value of knowing SCA estimates of the hybrids and obtaining information on the significance of dominant variances and effects. In consonance with our results, Ahmad *et al*. (2012) observed dominant genes for seed yield and its allied characters in sunflower and suggested the exploitation of heterosis breeding and hybrid sunflower development. Occurrence of both additive and non-additive gene effects are enough evidence for applying above approaches to improve phenological, seed yield, yield related components, oil and protein content of sunflower. Like present results, Khan *et al*. (2009) reported that additive and dominant variances were useful for agro-economic characters in sunflower.

Highly significant differences in mean values of 90% maturity, stem thickness, head size, achenes/plant, thousand-achene weight, achene yield kg/ha and oil and protein content were recorded which indicated that sunflower breeders can accomplish additional enhancement in seed yield and quality traits like oil and protein percentage. Better combining parents with desirable plant traits are regarded as superior from breeding perspective. Several workers found that SCA is more essential than GCA for seed yield in sunflower (Karasu *et al*., 2010; Mohanasundaram *et al*., 2010; Farrokhi *et al*., 2008) While Golabadi *et al*. (2015); Memon *et al*. (2015b) and Khan *et al.* (2009), affirmed that both SCA and GCA are evenly essential genetic parameters to examine the suitability of inbred parents in sunflower breeding. Ghaffari *et al*. (2011) found that achene yield was advocated by dominant genes while Chandra *et al.* (2011) noted prevalence of dominant genes for phenological and seed yield characters and additive genes controlling oil%. Contradicting to present findings that non-additive variances were greater than additive variances for achene production, head size and oil% are in agreement with the results obtained by Machikowa *et al*. (2011). Significant negative GCA and SCA effects obtained by Ghaffari *et al*. (2011) and Khan *et al*. (2008) for early maturity are in full agreement with present findings.

In the present study, the dominance variances (σ^2 D) were much greater over additive (σ^2 A) for flowering, seed yield, and oil and protein% traits (Table 5). Shinde *et al*. (2016) also observed that dominant variance was leading in the inheritance for all flowering, seed yield and quality traits in sunflower. Qamar *et al*. (2015) indicated that if variances due to σ^2 SCA are greater than due to σ^2 GCA, such conditions indicated that dominant ones were advocating those traits. Our results and the results from other researchers support the scope of heterosis breeding or the exploitation of hybrid vigour in sunflower breeding. The higher SCA variances over GCA revealed that dominant or epistasis genes are responsible for the expression of studied characters. Hence, improvement in those characters could be brought via heterosis breeding (Ciric *et al*., 2013a). Some researchers are not confirming our results like Golabadi *et al*. (2015) and Kanwal *et al.* (2015) also found that days to maturity, 100-grain weight and oil content (%) were predominantly under the additive genes. The additive variances (σ^2 A) were greater for head diameter, 1000-achene weight and seed yield per plant as compared to dominance variances (σ^2 D). However, our results indicated that genetic variances due to SCA were highly essential than GCA due to additive variances (σ^2 A). This condition also verified that non-additive genes were more capable than additive ones for all the traits studied. In accordance to our results, Karasu *et al*. (2010) found greater SCA variance for achene yield and head size and indicated that these traits were advocated by non-additive genes. Our findings also revealed the prevalence of dominant gene action as revealed by the extent of dominance being greater than 1.0, showed that over dominance genes were controlling the characters studied. Previous workers such as Mohanasundaram *et al*. (2010) and Mijic *et al.* (2008) found higher proportion of dominant variances for majority of the traits studied. Satar *et al*. (2015) also confirmed degree of dominance greater than 1.0. Memon *et al*. (2015b) also found the key function of non-additive genes because the level of dominance was much higher than 1.0 for all the traits they studied.

General combining ability (GCA) and specific combining ability (SCA) estimates. Selection of suitable parental lines is the key to achieve the objectives in sunflower breeding programmes. The proper knowledge concerning important genetic parameters like combining ability estimates, types of gene action and comparative level of genetic variances are important in improving plant traits (Khan *et al*., 2009). The presence of dominant genes are the principal validation for commencing hybrid crop production in sunflower. Exploiting the combining ability of lines with testers is decisive factor for hybrid development. Combining ability studies revealed the importance of both additive and non-additive genetic effects for seed yield, oil and

Table 5. Analysis of variance for seed yield and yield components, and seed traits in F1 hybrids developed by crossing 6 lines with 3 testers of sunflower.

Source of variation	D. F.	Days to 90% maturity	Stem girth	Head diameter	Achenes /plant	1000-achene weight	Seed yield (kg/ha)	Oil content	Protein content
Replication	3	10.99	0.02	0.06	36.65	2.97	2487.46	0.522	0.061
Genotypes	26	73.09**	1.05**	48.38**	222112.46**	109.24**	758120.49**	48.624**	68.189**
Parents	8	15.19**	0.18**	28.61**	108251.02**	23.83**	574551.16**	52.313**	12.588**
Hybrids (H)	17	5.29**	0.55**	4.23**	87183.17**	26.33**	65957.32**	15.313**	6.567**
P vs. H	1	1688.97**	16.49**	957.18**	3426801.79**	1984.71**	13993467.54**	586.788**	1560.56**
Lines (L)	5	6.02**	0.77**	8.28**	170228.09**	44.70**	100000.47**	22.143**	9.337**
Testers (T)	2	8.01**	1.92**	4.08 **	112279.35**	26.90**	25903.49**	25.062**	10.065**
L × T	10	4.38**	0.16**	2.23**	78725.31**	17.03**	36946.51**	9.948**	4.48**
Pooled error	78	2.57	0.01	0.17	27.98	0.34	36.52	0.494	0.055
				Genetic variances					
σ^2GCA		0.02	0.047	0.05	301.54	0.21	3656.06	0.21	0.05
σ^2SCA		0.45	0.04	0.52	18436.38	6.64	9227.50	2.36	1.11
σ^2SCA/ σ^2GCA		22.53	8.09	11.47	61.14	31.77	2.53	19.53	23.56
σ^2GCA = $[(1+F)/4]^2$ σ^2_A (F=0)		0.08	0.02	0.18	1206.16	0.84	14600.24	0.48	0.19
σ^2SCA = $[(1+F)/4]^2$ σ^2_D (F=0)		1.81	0.15	2.06	73745.53	26.56	36909.99	9.45	4.43

** = P≤ 0.01 and ns = non-significant.

protein traits in sunflower (Galabadi *et al*., 2015; Memon *et al*., 2015b; Khan *et al.* 2009). Kaya and Atakisi (2004) found that promising hybrids can be obtained by hybridizing cytoplasmic sterile lines and restorers with higher GCA and SCA estimates. Hence, likelihood of adopting integrated breeding strategies like diallel or line by tester design and pedigree selections would be highly rewarding by utilizing additive and dominant genes for evolving promising hybrids.

On the basis of higher GCA effects (Tables 3), three inbred CMS lines like SF-187, ARG-0405, 64-A-93 revealed higher general combining ability for almost all the traits whereas only single tester RHP-46 was identified as good restorer parents with higher GCA effects for almost all the characters. The inbred parents were ranked as high and low general combiners. All the above mentioned inbred parents (lines and testers) exhibited significantly higher GCA effects for head diameter, seeds per plant, 1000-seed weight, seed yield kg/ha, oil and protein contents. However, all the three lines and single tester manifested desirable negative GCA effects for 90% maturity suggesting their importance for breeding early maturing hybrids, synthetics, composites or open-pollinated varieties. Results further suggested that lines and a tester exhibiting higher GCA effects revealed that additive genes were important in those parents; therefore such parents are very rewarding to develop new potential inbreds or synthetic varieties. According to GCA effects, female line SF-187 was recognized as the good general combiner for seed yield/ha and related traits, head size, achenes/plant, 1000-achene weight, achene yield/ plant and oil %.

Likewise, on the basis of SCA effects, nine hybrids from eighteen crosses were documented as high specific combiners for achene yield and its associated characters, oil% and protein percentage (Table 4). However, only three hybrids were discussed in detail depending on their *per se* performance, heterotic effects, SCA estimates and GCA effects of their respective inbred parents (Tables 1-4, 6). Nonetheless, the cross 64-A-93 × RHP-46 is developed from crossing of parents with good × good GCA effects and also exhibited high positive SCA effects for seed yield (kg/ha), head size, no. of achene/plant, thousand-achene weight, achene yield/ plant, oil content%, yet desirable negative SCA effects for 90% maturity. Thus above results indicated its suitability for commercial exploitation of hybrid variety for higher seed and oil yield and also for early maturity. The high × high GCA parents involved in crosses exhibiting higher SCA also suggested that additive × additive gene interactions were involved (Table 6). These results indicated that hybrids which involve both the parents with higher GCA effects are most suitable hybrids and due to fixable additive genes may be utilized for hybridisation and selection programmes. The second cross SF-187 × RHP-46 also involved parents with good × good GCA effects also showed high positive SCA estimates for seed yield kg/ha stem girth, head size, achenes/plant, 1000-seed weight, and oil content suggesting its fitness for profitable hybrids for higher seed yields and higher protein%

Table 6. Promising specific combiners identified on the basis of higher GCA effects of parents for seed yield, oil and protein% traits in sunflower

Crosses/F1 hybrids	Status of parents for GCA	Characters
64-A-93 × RHP-46	Good × Good	Seed yield, its components and oil%.
SF-187 × RHP-46	Good × Good	Seed yield and its associated traits, stem girth and oil content.
ARG-0405×ARG-0106	Good × Poor	Seed yield and its related traits, head size and oil content.
PSF-025 × ARG-0106	Poor × Poor	Seed yield and its components, and oil content%.
ARG-0306 × ARG-0106	Poor × Poor	Seed yield and its components, maturity traits, stem thickness, head size and oil content%.
SF-187 × RHP-64	Good × Poor	Seed yield and its components and content%.
PSF-025 × RHP-64	Poor × Poor	Seed yield and its components, maturity traits, stem thickness, head size and oil content%.
PSF-025 × RHP-46	Good × Poor	Protein content%, maturity traits and stem girth
ARG-0505 × ARG-0106	Good × Good	Protein content%, maturity traits, stem thickness and head size.

(Table 6). These results suggested that selection may be rewarding in F_2 population and can be useful for transgressive breeding. Analogous results were also reported by other researchers like Binodh *et al.* (2008); Manivannan *et al.* (2005) and Sharma *et al.* (2003). The third cross combination ARG-0405 × ARG-0106 which is developed from crossing parents with high × low GCA also showed positive SCA effects for seed yield kg/ha, head size, no. of achenes/plant, thousand-seed weight, achene yield/plant and oil content%, thus demonstrated its suitability for higher seed and oil yields. These results indicated that a hybrid which involved high × low GCA parents, involved favourable genes from high parent possessing additive genes whereas recessive genes from parent possessing non-additive genes, therefore such hybrids are suitable either for hybrid crop development or single selection from later segregating generations (Peng and Virmani, 1990). The other hybrid like PSF-025 × ARG-0106 is developed by crossing parents with low × low GCA effects (Table 6), yet showed greater affirmative SCA estimates for achene yield kg/ha, head size, achenes/plant, 1000-achene weight and oil% suggesting its feasibility for high seed and oil yields. Present results suggest that above hybrid which involved parents with low GCA effects possess dominant genes which are non-fixable genetic factors; hence the best choice is to go for hybrid sunflower development for the above mentioned cross. Ciric *et al*. (2013a) found that dominant gene effects were profound for the assessed characters, which indicated that heterosis breeding may improve those characters. Likewise hybrid PSF-024 × RHP-46 for days to 90% maturity; 64-A-93 × RHP-64 and ARG-0306 × ARG-0106 for stem girth; ARG-0405 × ARG-0106 for head diameter, achenes per plant 1000-achene weight and oil content; SF-187 × RHP-64 for achene yield per plant and kg/ha; PSF-025 × RHP-46 and 64-A-93 × ARG-0106 for protein content were developed from high × low GCA parents (Table 6). Such results indicate that all these hybrids possess one parent with favorable additive genes from high parent and recessive genes from poor parent, making complementary gene interactions, hence above hybrids are equally suitable for hybrid sunflower development or single plant selections from later segregating generations (Sawargaonkar and Ghodke, 2008). The only hybrid ARG-0505 × ARG-0106 with high *per se* performance, greater heterobeltiosis and high SCA effects for protein content involved low × low GCA parents indicates that dominant × dominant genes interactions were involved in the expression of high protein content. These results reveal that the above hybrid is unique and most profitable for only hybrid sunflower development, yet not suitable for selection to improve protein percentage.

Acknowledgement

This paper is part of Ph.D thesis. The facility provided by the Oilseeds Section, Agriculture Research Institute, Tandojam for conducting experiments is highly appreciated.

References

Ahmad, M.W., Ahmed, M.S., Tahir, H.N. 2012. Combining ability analysis for achene yield and related traits in sunflower (*Helianthus annuus* L.). *Chilean Journal of Agriculture Research*, **72:** 21-26.

Amin,W., Saif-ul-Malook, Mumtaz, A., Ashraf, S., Ahmad, H.M., Hafeez, K., Sajjad, M., Bibi, A. 2014. Combining ability analysis and effect of seed priming on seedling traits in Sunflower (*Helianthus annus* L.). *Report and Opinion*, **6:** 19-30.

Andarkhor, S.A., Rameeh, V., Alitabar, R.A. 2013. Estimation of genetic parameters for yield components and seed yield in sunflower using line × tester analysis. *African Journal of Biotechnology*, **12:** 3978-3983.

Andarkhor, S.A., Mastibege, N., Rameeh, V. 2012. Combining ability of agronomic traits in sunflower (*Helianthus annuus* L.) using line x tester analysis. *International Journal of Biology*, **4:** 89-95.

Arshad, M., Khan, M.A., Jadoon, S.A., Mohmand, A.S. 2010. Factor analysis in sunflower (*Helianthus annuus* L.) to investigate desirable hybrids. *Pakistan Journal of Botany*, **42:** 4393-4402.

Awan, T.H., Mehdi, S.S. 2006. Evaluation performance and stability of sunflower genotypes against salinity stress. *Journal of Animal and Plant Sciences*, **16:** 47-51.

Bajaj, R.K., Aujla, K.K., Chahal, G.S. 1997. Combining ability studies in sunflower (*Helianthus annuus* L.). *Crop Improvement*, **34:** 141-146.

Binodh, A.K., Manivannan, N., Varman, P.V. 2008. Combining ability analysis for yield and its contributing characters in Sunflower (*Helianthus annuus* L.). *Madras Agriculture Journal*, **95:** 295-300.

Chandra, B.S., Kumar, S.S., Ranganadha, A.R.G., Dudhe, M.Y. 2011. Combining ability studies for development of new hybrids over environments in sunflower (*Helianthus annuus* L.). *Journal of Agriculture Science*, **3:** 230-237.

Ciric, M., Jocic, S., Cvejic, S., Jockovic, M., Canak, P., Marinkovic, R., Ivanovic, M. 2013a. Combining abilities of new inbred lines of sunflower. *Genetika*, **45:** 286-296.

Ciric, M.,Jocic, S., Cvejic, S., Canak, P., Jockovic, M., Marinkovic, R., Mirosavljevic, M. 2013b. Evaluation of combining abilities of new sunflower inbred lines. *Ratarstvo and Povrtarstvo*, **50:** 8-15.

Faridi, R., Khan, F.A., Malook, S., Ashraf, S., Arshad, S., Annum, N., Saleem, S. 2015. Gene action study for morphological traits in sunflower (*Helianthus annuus* L.). *American-Eurasian Journal of Agricultural & Environmental Sciences*, **15:** 769-775.

Farrokhi, E., Khodabandeh, A., Ghaffari, M. 2008. Studies on general and specific combining abilities in sunflower. Breeding and Genetics: *Procceedings of 17th International Sunflower Conference* Córdoba, Spain, pp. 561-565.

Fernandez, M.J., Velasco, L., Vich, P.B. 2004. Progress in the genetic modification of sunflower oil quality. *Proceedings of 16th International Sunflower Conference Fargo, North Dakota*, USA, pp. 1-14.

Gangappa, E., Channakrishnaiah, K.M., Ramesh, S., Harini, M.S. 1997. Exploitation of heterosis in sunflower (*Helianthus annuus* L.). *Crop Research*, **13:** 339-348.

Ghaffari, M., Farrokhi, I., Mirzapour, M. 2011. Combining ability and gene action for agronomic traits and oil content in sunflower (*Helianthus annuus* L.) using F1 hybrids. *Crop Breeding Journal*, **1:** 73-84.

Golabadi, M., Golkar, P., Shahsavari, M.R. 2015. Genetic analysis of agro-morphological traits in promising hybrids of sunflower (*Helianthus annuus* L.). *Acta Agriculturae Slovenica*, **105:** 249-260.

Gomez, K.A., Gomez, A.A. 1984. *Statistical Procedures for Agricultural Research*. 2nd edition, John Wiley & Sons, Inc., New York, USA.

Hallauer, A.R., Miranda, J.B. 1986. *Quantitative Genetics in Maize Breeding*. pp. 267-294, Iowa State University Press, Ames, IA, USA.

Hlandi, N., Skoric, D., Balalic, M.K., Sakac, Z., Jovanovic, D. 2006. Combining ability for oil content and its correlations with other yield components in sunflower (*Helianthus annuus* L.). *Helia*, **29:** 101-110.

Jocic, S., Cvejic, S., Ciric, M., Hladni, N., Miladinovic, D., Miklic, V., Radeka, I. 2012. Estimation of combining abilities in sunflower (*Helianthus annuus* L.). *Proceedings of 18th International Sunflower Conference*. Mar-Del-Plata & Balcare, Argentina.

Kang, S.A., Khan, F.A., Ahsan, M.Z., Chatha, W.S., Saeed, F. 2013. Estimation of combining ability for the development of hybrid genotypes in *Helianthus annuus* L. *Journal of Biological*, *Agriculture & Healthcare*, **3:** 68-74.

Kannababu, N., Karivaratharaju, T.V. 2000. Maternal influence of cytoplasmic genetic male sterile lines on seed quality in sunflower (*Helianthus annuus* L.). *International Journal of Plant Physiology*, **2:** 159-162.

Kanwal, N., Sadaqat, H.A., Ali, Q., Ali, F., Bibi, I., Niazi, N.K. 2015. Breeding progress for morphology and genetic pattern in *Helianthus annuus* L. *Life Science Journal*, **12:** 49-56.

Karasu, A., Oz, M., Sincik, M., Goksoy, A.T., Turan, Z.M. 2010. Combining ability and heterosis for yield and yield components in sunflower. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, **38:** 259-264.

Kaya, Y., Atakisi, I.K. 2004. Combining ability analysis of some yield characters of sunflower (*Helianthus annuus* L.). *Helia*, **27:** 75-84.

Kempthorne, O. 1957. *An Introduction to Genetical Statistics*. pp.545, John Wiley and Sons Inc., New York, USA.

Khan, H., Rahman, H.U., Ahmad, H., Ali, H., Inamullah, Alam, M. 2008. Magnitude of combining ability of sunflower genotypes in different environments. *Pakistan Journal of Botany*, **40:** 151-160.

Khan, S.A., Ahmad, H., Khan, A., Saeed, M., Khan, S.M., Ahmad, B. 2009. Using line x tester analysis for earliness and plant height traits in sunflower (Helianthus annuus L.). *Recent Research in Science and Technology*, **1:** 202-206.

Leon, A.J., Berry, S.T., Rufener, G.K., Mowers, R.P. 1995. Oil Producing Sunflowers and Production thereof. United States Patent No. US00 5476524.

Machikowa, T., Saetang, C., Funpeng, K. 2011. General and specific combining ability for quantitative characters in sunflower. *Journal of Agriculture Science*, **3:** 91-95.

Manivannan, N., Vidhyavathi, P., Murlidharan, V. 2005. Diallel analysis in sunflower. *Indian Journal of Agriculture Research*, **39:** 281-285.

Memon, S., Baloch, M.J., Baloch, G.M., Keerio, M.I. 2015a. Heterotic effects in F1s and inbreeding depression in F2 hybrids of sunflower. *Pakistan Journal of Scientific and Industrial Research*, Series-B. Biological Sciences, **58:** 1-10.

Memon, S., Baloch, M.J., Baloch, G.M., Jatoi, W.A. 2015b. Combining ability through line × tester analysis for phenological, seed yield, and oil traits in sunflower (*Helianthus annuus* L.). *Euphytica*, **204:** 199-209.

Menz, M.A., Hallauer, A.R., Russell, W.A. 1999. Comparative response of two reciprocal recurrent selection methods in BS21 and BS22 maize populations. *Crop Science*, **39:** 89-97.

Mijic, A., Kozumplik, V., Kovacevic, J., Liovic, I., Krizmanic, M., Duvnjak, T., Maric, S., Horvat, D., Simic, G., Gunjaca, J. 2008. Combining abilities and gene effects on sunflower grain yield, oil content and oil yield. *Periodicum Biologorum*, **110:** 277-284.

Mirarab, M., Ahmadikhah, A. 2010. Study on genetics of some important phenological traits in rice using line x tester. *Annals of Biological Research*, **1:** 119-125.

Mohanasundaram, K., Manivannan, N., Varman, P.V. 2010. Combining ability analysis for seed yield and its components in Sunflower (*Helianthus annuus* L.). *Electronic Journal of Plant Breeding*, **1:** 864-868.

Nasreen, S., Ishaque, M., Khan, M.A., Saleem-Uddin, Gilani, S.M. 2014. Combining ability analysis for seed proteins, oil content and fatty acids composition in sunflower (*Helianthus annuus* L.). *Pakistan Journal of Agriculture Research*, **27:** 174-187.

Onemli, F. 2012. Impact of climate changes and correlations on oil fatty acids in sunflower. *Pakistan Journal of Agriculture Sciences*, **49:** 455-458.

Peng, J.Y., Virmani, S.S. 1990. Combining ability for yield and four related traits into hybrid breeding in rice. *Oryza*, **27:** 1-10.

Qamar, R., Sadaqat, H.A., Bibi, A., Tahir, M.H.N. 2015. Estimation of combining abilities for early maturity, yield and oil related traits in sunflower (*Helianthus annuus* L.). *International Journal of Science and Nature*, **6:** 110 -114.

Rehman, R., Arshad, M., Khan, M.A., Mohmand, A.S., Shabbir, G. and Shah, M.K.N. 2012. Using multivariate analysis for selecting desirable hybrids in sunflower (*Helianthus annuus* L). *Pakistan Journal of Botany*, **44:** 1715-1720.

Saleem-Uddin, Khan, M.A., Gull, S., Usman, K., Saleem, F.Y., Sayal, O.U. 2014. Line × tester analysis of yield and yield related attributes in different sunflower genotypes. *Pakistan Journal of Botany*, **46:** 659-665.

Satar, M.A.A., Fahmy, R.M., Hassan, T.H.A. 2015. Genetic control of sunflower seed yield and its components under different edaphic and climate conditions: The 9th Plant Breeding International Conference. *Egyptian Journal of Plant Breeding*, **19:** 103-123.

Sawargaonkar, S.L., Ghodke, M.K. 2008. Heterosis in relation to combining ability in restorer lines of sunflower. *Helia*, **31:** 95-100.

Semerci, A. 2012. Productivity analysis of sunflower production in Turkey. *Pakistan Journal of Agriculture Sciences*, **49:** 577-582.

Sharma, S., Bajaj, R.K., Kaur, N., Sehgal, S.K. 2003. Combining ability studies in sunflower (Helianthus annuus L.). *Crop Improvement Journal*, **30:** 69-73.

Shinde, S.R., Sapkale, R.B. and Pawar, R.M. 2016. Combining ability analysis for yield and its components in sunflower (*Helianthus annuus* L.). *International Journal of Agricultural Sciences*, **12:** 51-55.

Siddiqi, M.H., Ali, S., Bakht, J., Khan, A., Khan, S.A., Khan, N. 2012. Evaluation of sunflower lines and their crossing combinations for morphological characters, yield and oil contents. *Pakistan Journal of Botany*, **44:** 687-690.

Singh, R.K., Choudhary, B.D. 1985. *Biometrical Methods in Quantitative Genetic Analysis*. 318 pp., Kalyani Publishers, New Dehi, India.

Skoric, D., Jocic, S., Molnar, I. 2000. General and Specific combining abilities in sunflower. In: *Proceedings of 15th International Sunflower Conference*, Toulouse, France, 12-15 June.

Xu, L., Najeeb, U., Naeem, M. S., Wan, G. L., Jin, Z. L., Khan, F., Zhou, W. J. 2012. *Technological Innovations in Major World Oil Crops*. Chapter 6, In vitro mutagenesis and genetic improvement, vol. **2,** pp. 151-173, Springer, New York, USA.

Pak. j. sci. ind. res. Ser. B: biol. sci. 2018 **61B**(3) 136-144

Genetic Variability, Heritability and Correlation Studies in F_2 Populations of Upland Cotton

Muhammad Jurial Baloch*, Rehana Lochi, Wajid Ali Jatoi, Abdul Wahid Baloch and Muhammad Ahmed Arain

Department of Plant Breeding and Genetics, Sindh Agriculture University, Tandojam, Pakistan

(received April 7, 2016; revised June 29, 2017; accepted August 7, 2017)

Abstract. A field experiment was conducted at the experimental area of the Department of Plant Breeding & Genetics, Sindh Agriculture University Tandojam, during the year 2014-2015 in order to carry-out genetic analysis in F_2 populations of upland cotton. The trial was laid-out in a Randomized Complete Block Design with four replications. The material was consisted of eight parents and ten F_2 populations. The analysis of variance revealed significant differences among the parents and F_2 populations for all the traits studied except that fibre length was non-significant in parents. The results further suggested that maximum heritability, higher genetic variances coupled with more genetic gains were expressed by the F_2 populations CRIS-134 × CRIS-508 and CRIS-134 × CIM-598 for 1st sympodial node number; CRIS-134 × Neelum-121 and CRIS-134 × CRIS-508 for sympodial branches/plant; CRIS-342 × FH-113 for boll weight; CRIS-342 × Neelum-121 for bolls/plant, seed cotton yield/plant, lint % and micronaire value and progenies CRIS-342 × MNH-886 followed by CRIS-342 × Neelum-121 for staple length. These results also suggested that a number of F_2 populations indicated their potential for various seed cotton yield and fibre traits. The phenotypic correlations revealed that most of the traits were significantly and positively associated with seed cotton yield/plant. However, higher correlations of sympodial branches/plant (r = 0.69**) and bolls/plant (r = 0.82**) with seed cotton yield indicated that both the traits are more reliable as compared to other traits for selection of higher seed cotton yields. Very interestingly, fibre traits like lint%, fibre length and micronaire were also significantly correlated with seed cotton yield, suggesting that fibre quality traits can be improved without compromising on seed cotton yield. Thus, the material under study is very promising and worthy of selection to improve many traits simultaneously.

Keywords: genetic variability, heritability, F_2 populations, correlations, cotton genotypes

Introduction

Genetic variability is referred as observed phenotypic variation which occurs in plant populations and is mainly attributable to genetic differences among them. Broad sense heritability may be defined as the ratio of genotypic variance over the phenotypic variance. In other words, it determines the magnitude of transmissibility of traits from parents to their offspring (Baloch *et al*., 2004). The additive variance, which is the variance of breeding values, is the important component of heritability. It is the chief source of measurement between the traits of parental and progenies.

The genetic potential of genotypes, genotypic and phenotypic correlations between different plant characters is available in the literature. The studies of Khan (2003) found that the seed cotton yield was positively correlated with bolls per plant and bolls weight. Further studies indicated that seed and lint indices were positively associated with seed cotton yield. Genetic variability and positive correlations were observed for yield traits in *Gossypium hirsutum* L. (Batool *et al*., 2010; Wang *et al*., 2004; Iqbal *et al*., 2003). The principal objectives in cotton breeding are higher production of seed cotton, lint yields with better fibre quality, early maturity and resistance to diseases and insect pests (Khan *et al*., 2009). For achieving these objectives, plant breeders have devoted a lot of efforts to use quantitative genetic analysis because most of the traits in cotton are considered as polygenic in nature. Quantitative traits possess continuous variation thus can be altered significantly by suitable breeding procedures (Baloch *et al*., 2010). Crop development requires breeders' ability to identify and select good performing genotypes from a population. Some difficulties may be encountered when breeders make selections for quantitative traits that are largely affected by the environmental factors (Ragsdale and Smith, 2007; Khan, 2003). Heritability of various traits and

*Author for correspondence; E-mail: j.rind58@gmail.com

the genetic potential of different populations in the form of their expression for different morpho-yield traits are urgently needed for selection of useful parental lines (Khan *et al*., 2010). Substantial genetic variances and higher heritability estimates of seed cotton yield and its component traits implied that such characters can be improved through hybridization and selection from segregating populations (Baloch *et al*., 2010; Baloch, 2004). A quantitative trait like yield being multigenic is significantly affected by environmental factors. Thus, the overall performance of a genotype may vary due to changes in the environment. It is generally stated that the higher the heritability, the simpler will be the selection process and greater will be the response to selection (Baloch *et al*., 2010). In addition, a thorough knowledge about the mean performance, extent of relationship and correlation of yield with various agronomic characters is indispensable for breeder to tackle the problems of low yield and increase the yield successfully. Ahsan *et al*. (2015); Farooq *et al*. (2015) and Rajamani *et al*. (2015) determined genotypic, phenotypic and environmental coefficient of variation and also estimated broad sense heritability and genetic advance in cotton. They estimated higher genotypic and phenotypic coefficient of variation for plant height, bolls per plant, boll weight, nodes to first fruiting branch and seed cotton yield per plant. The heritability was above 80% and the significant amount of genetic advance for these traits provided a clear picture that selection can be effective to improve these traits. Srinivas *et al*. (2015) conducted correlation analysis and observed that monopodia/plant, sympodia/plant, bolls/plant, boll weight and 2.5% span length were positively associated with seed cotton yield. While Farooq *et al*. (2015) noted that sympodia per plant expressed significant and positive correlation with bolls per plant, boll weight and yield at both genotypic and phenotypic levels. Thus, the objectives of the present research were to determine genetic variability, heritability correlations in intra-hirsutum F_2 populations for yield and fibre quality traits in upland cotton.

Materials and Methods

The present study was conducted at Experimental Field, Department of Plant Breeding and Genetics, Faculty of Crop Production, Sindh Agriculture University Tandojam so as to estimate genetic variability, heritability and correlation studies of ten intraspecific F_2 populations of upland cotton (*Gossypium hirsutum* L). Eight parents including CRIS-134, CRIS-342, Neelum-121, CIM-598, MNH-886, FH-113, IR-3701 and CRIS-508 were randomly crossed to develop ten F_2 intraspecific populations. For evaluation, the experiment was conducted in a randomized completer block design with four replications. The observations were taken on ten randomly tagged plants from each genotype per replication. Thus, forty plants were included in the study. The characters studied were; 1st sympodial node number, sympodial branches/plant, bolls/plant, boll weight, lint percentage, fibre length, micronaire value and seed cotton yield/plant. The data were analyzed according to statistical technique outlined by Gomez and Gomez (1984) through Statistix 8.1 computer software so as to calculate the differences among the genotypes for various traits. Whereas, means were compared by using the least significant difference (L.S.D.) test at 5% probability level. The genetic and phenotypic environmental variances, broad sense heritability (h^2) and expected response to selection were estimated according to Baloch *et al*. (2010). The correlations were determined according to formulae developed by Raghavrao (1983).

Results and Discussion

Analysis of variance and mean performance of parents and F_2 populations. In the present research, genetic variability, heritability and correlations were studied in F_2 populations of upland cotton (*Gossypium hirsutum* L.) so as to identify the better segregates from subsequent filial generations. The eight important traits of cotton such as 1st sympodial node number, sympodial branches/plant, boll weight, bolls/plant, lint%, micronaire, fibre length, and seed cotton yield/plant were studied.

The analysis of variance revealed significant differences among the genotypes (comprising eight parents and their ten F_2 hybrids) for all the characters. The mean squares of parents, F_2 hybrids and parents vs. F_2 hybrids were also significant for all the traits except that fibre length was non-significant for parents only (Table 1). The mean performance of parents and hybrids (Table 2) indicated that most of the F_2 hybrids gave higher average values over their parents for all the traits under test. In parental lines, CRIS-134 formed 1st sympodial node at the lowest node of 7.50 being earlier while FH-113 formed 1st sympodia at the highest node of 10.15, thus it was considered as a late maturing parent. The F_2 population developed from crosses CRIS-134 × NMH-886 and CRIS-134 × CRIS-508 formed the lowest

Table 1. Mean squares from analysis of variance of parents and their intra hirsutum F_2 populations for yield and fibre traits

Character	Source of variation					
	Replication D.F. = 3	Genotypes D.F. = 17	Parents (P) D.F. = 7	F_2 Hybrids (P) D.F. = 9	P vs. H D.F. = 1	Error D.F. = 51
1st sympodial node number	0.34	2.85**	1.84**	0.54**	30.62**	0.32
Sympodia/plant	3.91	172.01**	61.19**	88.28**	1701.29**	2.046
Bolls/plant	2.68	441.91**	224.67**	74.33**	5270.85**	5.418
Boll weight	0.04	0.22**	0.06**	0.36**	0.15**	0.057
Seed yield/plant	199.64	6101.81**	1330.43**	1304.31**	82679.20**	134.51
Lint %	3.85	63.08**	70.60**	56.00**	74.22**	11.122
Fibre length	0.45	1.450**	0.33ns	2.06**	3.80**	0.671
Micronaire value	0.005	0.033**	0.03**	0.017*	0.22**	0.008

** = significant at 1% probability level; ns = non significant

Table 2. Mean performance of parents and their intra-hirsutum F_2 populations for various yields and fibre traits

Genotypes (Parents and hybrids)	Characters							
	1st sympodial node no.	Sympodial branches/ plant	Bolls/ plant	Boll weight (g)	Yield/ plant (g)	GOT %	Staple length (mm)	Micronaire value (µg/inch)
Parents								
CRIS-134	7.50	19.45	40.90	3.17	129.65	36.13	28.45	3.94
CRIS-342	8.70	20.35	38.10	2.92	111.25	40.56	27.66	3.82
Neelam-121	8.35	20.90	36.99	3.26	120.59	42.34	28.03	3.10
CIM-598	9.95	17.85	28.85	3.05	87.99	45.52	28.47	3.91
MNH-886	9.00	18.10	35.22	3.06	107.77	50.45	28.36	3.73
FH-113	10.15	19.60	35.85	3.09	110.78	42.34	28.19	3.92
IR-3701	9.75	18.15	31.80	3.24	103.03	43.27	28.22	3.87
CRIS-508	9.60	18.30	28.65	2.96	84.80	39.98	27.86	3.85
Average	9.13	19.09	34.55	3.09	106.98	42.57	28.16	3.77
F_2 hybrids								
CRIS-134 × Neelam-121	11.40	24.25	45.35	3.54	160.54	45.10	30.14	3.92
CRIS-134 × CIM-598	10.90	21.15	42.45	3.11	132.02	45.12	28.12	4.15
CRIS-134 × IR-3701	11.25	21.50	44.70	3.18	142.15	44.14	27.95	3.96
CRIS-134 × MNH-886	10.40	22.10	44.65	3.48	155.38	50.39	29.24	4.01
CRIS-134 × FH-113	10.95	22.40	48.70	3.16	153.89	42.76	28.60	3.99
CRIS-342 × Neelum-121	10.45	24.65	52.60	3.38	177.79	44.84	27.83	3.97
CRIS-342 × IR-3701	10.45	23.60	50.35	3.10	156.09	43.32	28.74	3.97
CRIS-342 × MNH-886	11.05	22.85	48.75	2.62	127.73	40.99	29.12	3.98
CRIS-342 × FH-113	10.75	26.10	49.85	2.82	140.58	39.00	28.25	3.94
CRIS-134 × CRIS-508	10.40	25.45	38.15	3.46	132.00	48.50	28.18	4.03
Average	10.80	23.41	46.56	3.19	147.82	44.42	28.62	3.99
LSD (5%)	0.81	2.03	0.34	3.30	16.46	4.73	1.16	0.13

sympodia at node number 10.40, whereas the highest sympodia were formed by CRIS-134 × Neelum-121 at node number 11.40. The sympodial branches/plant varied from 17.85 (CIM-598) to 20.90 (Neelum-121) among the parental lines while the progenies from cross CRIS-342 × FH-113 produced maximum number of sympodial branches/plant (26.10). Similarly, in the case of boll weight, progenies CRIS-134 × Neelum-121 recorded the bigger bolls (3.54g), while the parental lines Neelum-121 showed the highest mean value (3.26g). With respect to bolls/plant, progenies from CRIS-342 × Neelum-121 formed the highest number

of bolls per plant (52.60). With respect to lint %, the parental line MNH-886 ginned higher lint (50.45%), while the progenies derived from CRIS-342 × Neelum-121 ginned maximum lint of 50.39%. The F_2 progenies gave higher seed cotton yield/plant against their parents and that ranged from 127.73g (CRIS-342 × MNH-886) to 177.79g (CRIS-342 × Neelum-121). The fibre length of parental lines ranged from 27.66mm to 28.47mm, however progenies from CRIS-342 × Neelum-121 and CRIS-134 × Neelum-121 measured the fibre ranging from 27.83mm to 30.14mm, respectively. The progenies exhibited micronaire ranging from 3.92 to 4.15µg/inches measured by progenies CRIS-134 × Neelum-121 and CRIS-134 × CIM-598, respectively.

Genetic variability analysis of F_2 progenies. The results of genetic variance (d2p), phenotypic variance (d2g), heritability percentages in broad sense (h2b.s.) and genetic gain (GA) at 10% selection intensity for eight important quantitative traits showed that most of the F_2 populations demonstrated moderate to high heritability estimates. Besides, majority of the characters were generally associated with more genetic advances indicating the presence of an appreciable amount of genetic variability mainly due to additive genes in F_2 populations. Results of 1st sympodial node number suggested that sufficient genetic variability was present in F_2 populations. Low, moderate and high heritability estimates were recorded that varied from 23.82 to 75.84% (Fig. 1). Two higher ranking progenies CRIS-134 × CRIS-508, CRIS-134 × CIM-598 however exhibited higher heritability estimates of h^2= 75.84% and h^2=75.61% with greater amount of genetic advances (4.40 and 3.98, respectively). Ahmed and Malik (1996) estimated that a one node decrease in sympodial branch matures the cotton crop by approximately 4 to 7 days earlier. Jatoi *et al*. (2012) reported that 1st sympodial branch node was significantly and positively correlated with node number to set 1st boll and sympodial branch length. For sympodial branches, about half of the progenies displayed higher genetic variability than their corresponding environmental variances resulting in low to high heritability estimates ranging from 40.35 to 76.79% (Fig. 2). The progenies, CRIS-134 × Neelum-121 and CRIS-134 × CRIS-508 nevertheless manifested higher heritability estimates of h^2= 76.79% and h^2= 76.34% and these heritability estimates were related with fair amount of genetic advances (9.94 and 3.48, respectively). Pertaining to bolls/plant (Fig. 3), it was noted that the majority of the progenies expressed higher genetic variability than environmental variances, thus moderate to high heritability percentages in broad sense and higher genetic gains were recorded. Higher heritability estimates and more genetic variances for bolls/plant were generally allied with higher genetic advances. From F_2 population, CRIS-342 × Neelum-121 exhibited higher heritability (h^2 = 91.24%) and recorded maximum genetic gains (31.15). Hussain *et al*. (2010) reported high genotypic variability, genetic

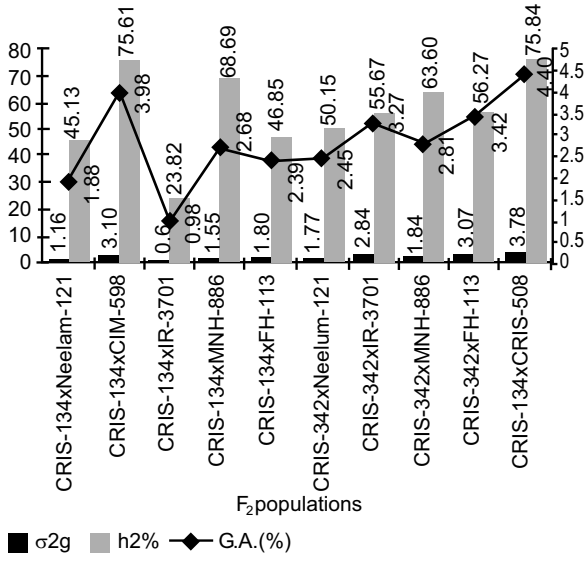


Fig. 1. Genetic components for 1st sympodial node number in F_2 populations of upland cotton.

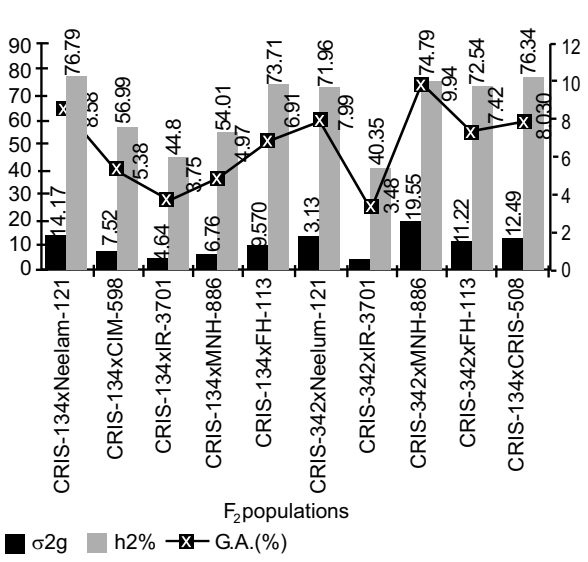


Fig. 2. Genetic components for sympodial branches/ plant in F_2 populations of upland cotton.

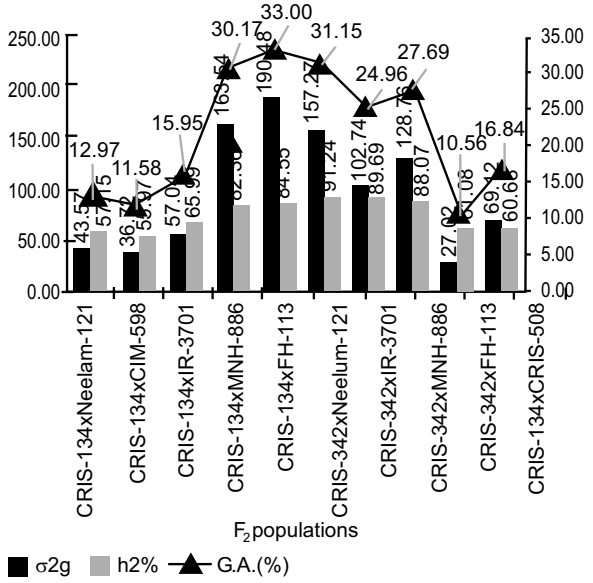


Fig. 3. Genetic components for bolls/plant in F_2 populations of upland cotton.

advance and heritability estimated for number of bolls per plant.

With respect to boll weight, greater genetic variances were displayed by less than half of the progenies as compared to their corresponding environmental variances. By and large, F_2 progenies expressed low to moderate heritability estimates ranging from 31.08 to 68.87%, while genetic gains varied from 0.38 to 1.01 (Fig. 4). The top scoring in relation to heritability percentage and genetic advance were the progenies derived from CRIS-342 × FH-113 (h^2 = 68.87%) and (GA = 1.01) followed by CRIS-342 × Neelum-121 (h^2 = 66.13%) and (GA = 0.98). Batool *et al*. (2010) and Soomro *et al*. (2010) found higher genetic variances and higher heritability estimates for boll weight in upland cotton as well and concluded that this trait is highly heritable in F_2 and F_3 generations. On the contrary, Naveed *et al*. (2004) reported low to moderate heritability estimates for boll weight which also confirm our results for some of the progenies. Regarding seed cotton yield/plant (Fig. 5), F_2 progenies exhibited fair to high genetic variability, recorded moderate to higher heritability estimates that ranged from 63.34% to 95.96% and higher genetic gains varying from 52.45 to 148.75. From the progenies evaluated, CRIS-342 × IR-3701 exhibited highest heritability (h^2 = 95.69%) and consequently demonstrated with higher genetic advance (GA = 148.75). Similar to our findings, Tabasum *et al*. (2012); Mushtaq *et al*. (2011); Batool *et al*. (2010) and Soomro *et al*. (2010) also estimated high heritability for seed cotton yield. Selection for high ginning outturn % often results in an increase in the production/plant and per unit area. The results indicated that progenies CRIS-134 × MNH-886 displayed highest heritability percentage (h^2=97.12%) coupled with greater genetic

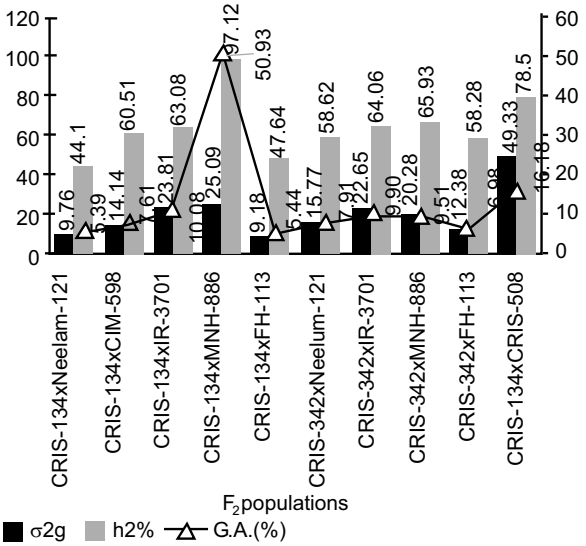


Fig. 4. Genetic components for bolls/plant in F_2 populations of upland cotton.

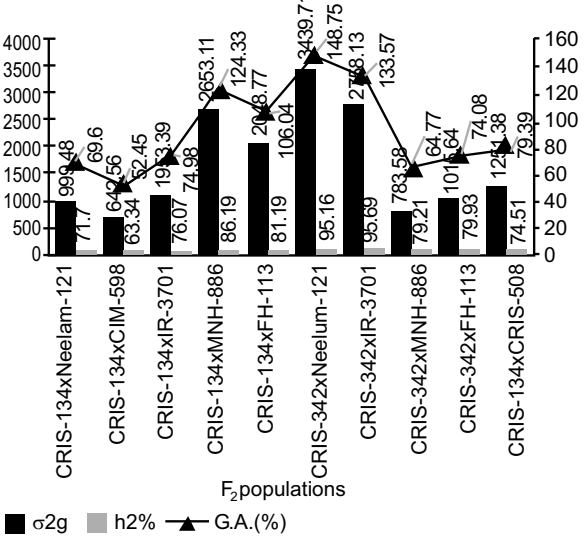


Fig. 5. Genetic components for seed cotton yield/ plant in F_2 populations of upland cotton.

advance (GA=50.93) (Fig. 6).Our results are in agreement with those obtained by Farooq *et al*. (2014). Khan *et al*. (2010) also found greater genetic variance and higher heritability percentage for lint%. Ali *et al*. (2010) noted that heritability estimates were high for lint% indicating that additive genes were controlling these traits. Regarding fibre length, majority of the F_2 populations showed higher genetic variances against their corresponding environmental variances. High heritability estimates were coupled with ample amount of genetic advances varying from 1.04 to 4.20 (Fig. 7). The progenies derived from crosses CRIS-342 × MNH-886, CRIS-342 × Neelum-121 expressed higher heritability, greater genetic variability and more genetic advances. Khan *et al*. (2009) and Memon *et al*. (2008) also recorded greater genetic variability, higher heritability associated with greater genetic advance for staple length in F_2 progenies of upland cotton. Concerning micronaire (µg/inch), flaxen amount of genetic variability from F_2 progenies were observed (Fig. 8). Higher ranking F_2 populations such as CRIS-134 × IR-3701 and CRIS-134 × CIM-598 manifested higher heritability (h^2=84.15, h^2=83.61), and maximum genetic advance (GA = 0.76 and GA = 0.92) suggesting that additive genes were involved in the expression of micronaire.

Phenotypic correlations. Correlation studies indicated that 1st sympodial node number was significantly and

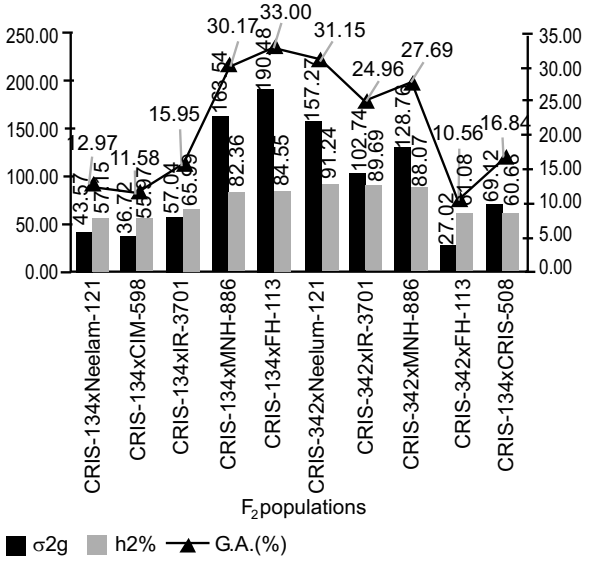


Fig. 6. Genetic components for GOT% in F_2 populations of upland cotton.

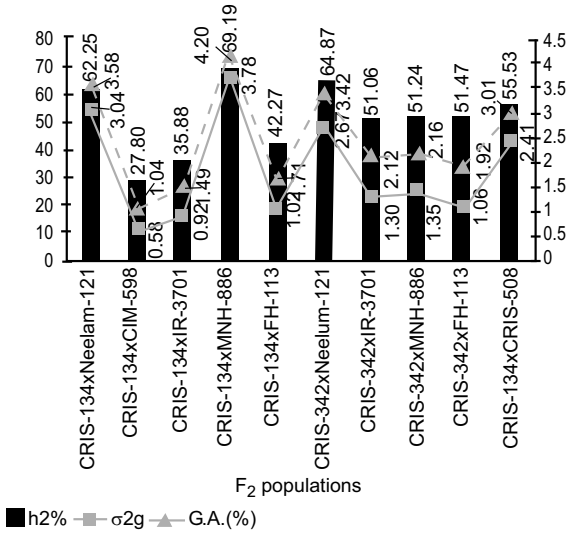


Fig. 7. Genetic components for staple length in F_2 populations of upland cotton.

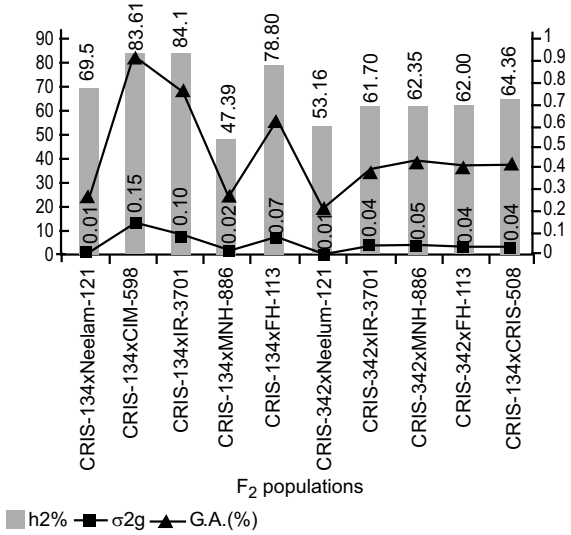


Fig. 8. Genetic components for micronaire in F_2 populations of upland cotton.

positively correlated with sympodial branches/plant, lint%, micronaire, seed cotton yield/plant, fibre length, and bolls/plant which suggested that increase in appearance of 1st sympodial node number caused a comparable increase in all the above traits. Sympodial branches/plant was significantly and positively correlated with 1st sympodial node number, micronaire, bolls per plant and seed cotton yield (Table 3). The positive relationship of sympodial branches per plant with other

traits suggested that increase in sympodial branches will cause an associated increase in 1st sympodial node number, micronaire value, bolls per plant and seed cotton yield and negative correlation with lint% revealed that increase in sympodial branches will decrease lint %. Tamilselven *et al*. (2013) and Rao and Gopinath (2013) studied correlations in upland cotton and found that sympodial branches/plant was significantly and positively correlated with bolls per plant and seed cotton yield. Significant and positive association of boll weight with lint%, micronaire, fibre length and seed cotton yield indicated that while improving boll weight, the increase in other traits like seed cotton yield, fibre length and micronaire can occur. Correlation between bolls/plant and seed cotton yield/plant demonstrated highly significant and positive relationship. Such association revealed that increase in bolls/plant will simultaneously increase seed cotton yield/plant. Results from correlation studies further revealed that lint % was positively and significantly correlated with only seed cotton yield, hence it could be inferred that significant improvement could be made in seed cotton yield along with lint% without causing adverse impact on other important traits. Significant and positive association of fibre length with bolls/plant and seed cotton yield indicated that increase in fibre length will cause a corresponding increase in both bolls/ plant and seed cotton yield. Farooq *et al*. (2014); Islam *et al*. (2013); and Rao and Gopinath (2013) assessed different hirsutum varieties for yield and other economic characters and observed significant variations for boll weight and showed its positive effect on seed cotton yield. The significantly positive correlation of micronaire with sympodial branches, bolls/plant and seed cotton yield further indicated that seed cotton yield can be improved without sacrificing fibre quality traits. Srinivas *et al*. (2015) and Baloch *et al*. (2014) conducted correlation analysis and observed that monopodia/plant, sympodia/plant, bolls/plant, boll weight and 2.5% span length were positively associated with seed cotton yield, while Farooq *et al*. (2015) noted that sympodia per plant expressed significant and positive correlation with bolls per plant, boll weight and yield at both genotypic and phenotypic levels.

Table 3. Correlation coefficients (r) between seed cotton yield and fibre traits in upland cotton

Character association	Correlation coefficient (r)
Boll weight vs. 1st sympodial node number	0.04^{ns}
Boll weight vs. sympodial branches/plant	0.05^{ns}
Boll weight vs. bolls/plant	0.08^{ns}
Boll weight vs. G.O.T.%	0.27**
Boll weight vs. staple length	0.199**
Boll weight vs. seed cotton yield/plant	0.34**
Boll weight vs. fibre fineness	0.21**
1st sympodial node number vs. sympodial branches/plant	0.37**
1st sympodial node number vs. bolls/plant	0.37**
1st sympodial node number vs. G.O.T.%	0.21**
1st sympodial node number vs. staple length	0.28**
1st sympodial node number vs seed cotton yield/plant	0.44**
1st sympodial node number vs fibre fineness	0.16*
G.O.T. % vs sympodial branches/plant	-0.002^{ns}
G.O.T. % vs bolls/plant	-0.08^{ns}
G.O.T. % vs staple length	0.05^{ns}
G.O.T. % vs seed cotton yield/plant	0.15*
G.O.T. % vs fibre fineness	0.02^{ns}
Fibre fineness vs sympodial branches/plant	0.33**
Fibre fineness vs bolls/plant	0.46**
Fibre fineness vs staple length	0.05^{ns}
Fibre fineness vs seed cotton yield/plant	0.46**
Sympodial branches/plant vs bolls/plant	0.72**
Sympodial branches/plant vs staple length	0.23^{ns}
Sympodial branches/plant vs seed cotton yield/plant	0.69**
Staple length vs bolls/plant	0.21**
Staple length vs seed cotton yield/plant	0.24**
Bolls/plant vs seed cotton yield/plant	0.82**

*,**= Significant at 0.01, 0.05 probability levels, respectively; ^{ns} = non-significant.

Conclusion

Significant differences among parents and the F_2 hybrids were recorded for all the studied traits except that fibre length was non-significant in parents. It may be concluded from the present research that based on average performance, the parents, Neelum-121, CIM-598 and FH-113 performed very well in terms of 1st sympodial node number, boll weight, fibre length, lint%, while parent CRIS-134 produced bigger bolls and higher seed cotton yield with desirable micronaire value. The estimation of genetic parameters indicated that F_2 progenies derived from the cross CRIS-134 × CRIS-508 displayed higher heritability estimates coupled with more genetic gains for 1st sympodial node number and sympodial branches; CRIS-342 × FH-113 for boll weight; CRIS-342 × Neelum-121 for bolls/plant and seed cotton yield; CRIS-134 × MNH-886 produced upper most heritability with more genetic gains for lint% and fibre length and CRIS-134 × IR-3701 for

micronaire value. Thus these populations are worth to be explored in further segregating generations so as to improve yield and fibre quality traits. The phenotypic correlations revealed that 1st sympodial node number, sympodial branches per plant, bolls/plant, boll weight, lint %, fibre length and micronaire were highly and positively associated with seed cotton yield hence these yield components can be used as reliable selection criteria to improve seed cotton yield. It further indicated that fibre traits can be improved without compromising seed cotton yield.

References

Ahmed, Z., Malik, M.N. 1996. How a short season changes physiological needs of cotton plant. pp. 16-21, *55th Plenary Meeting of the ICAC*, Tashkent, Uzbekistan.

Ahsan, M.Z., Majidano, M.S., Bhutto, H., Soomro, A.W., Panhwar, F.H., Channa, A.R., Sial, K.B. 2015. Genetic variability, coefficient of variance, heritability and genetic advance of some *Gossypium hirsutum* L. accessions. *Journal of Agriculture Science*, **7:** 147.

Ali, M.A., Bhatti, M.F., Abbas, A., Khan, I.A. 2010. Assessment of inheritance pattern of some multigenic characters in cotton (*Gossypium hirsutum* L.). *Journal of Agricultural Research*, **48:** 25-33.

Baloch, M.J., Kumar, C., Jatoi, W.A., Rind, I.H. 2014. Phenotypic correlation and regression analysis of yield and fibre traits in upland cotton (*Gossypium hirsutum* L.). *Pakistan Journal of Agriculture, Agriculture Engineering and Veterinary Sciences*, **30:** 135-146.

Baloch, M.J., Kakar, M.S., Jatoi, W.A., Veesar, N.F. 2010. Identification of potential F_2 populations from intraspecific crosses in upland cotton. *Pakistan Journal of Scientific and Industrial Research*, **53:** 151-157.

Baloch, M.J. 2004. Genetic variability and heritability estimates of some polygenic traits in upland cotton. *Pakistan Journal of Scientific and Industrial Research*, **47:** 451-454.

Batool, S., Khan, N.U., Makhdoom, K., Bibi, Z., Hassan, G., Marwat, K.B., Farhatullah, Raziuddin, F.M., Khan, I.A. 2010. Heritability and genetic potential of upland cotton genotypes for morpho-yield traits. *Pakistan Journal of Botany*, **42:** 1057-1064.

Farooq, J., Anwar, M., Rizwan, M., Riaz, M., Mahmood, K., Mahapara, S. 2015. Estimation of correlation and path coefficient analysis of various yield related parameters in cotton (*Gossypium hirsutum* L.). *Cotton Genomics and Genetics*, **6:** 1-6.

Gomez, K.A., Gomez, A. A. 1984. *Statistics for Agricultural Research*, pp. 21-25, 2nd edition, John Wiley and Sons, New York, USA.

Hussain, S., Nawab, N.N., Ali, M.A., Hussain, A., Nawaz, M.A., Malik, T.A. 2010. Evaluation of performance, genetic divergence and character association of some polygenic traits in upland cotton. *Journal of Agriculture and Social Sciences*, **4:** 79-82.

Iqbal, M., Chang, M.M., Iqbal, M.M., Hassan, M., Nasir, A., Islam, N. 2003. Correlation and path coefficient analysis of earliness and agronomic characters of upland cotton in Multan. *Journal of Agronomy*, **2:** 160-168.

Islam, M.K., Akhteruzzaman, M., Sharmin, D. 2013. Multivariate and genetic component analysis of new cotton (*Gossypium hirsutum* L.) genotypes. *Bangladesh Journal of Progressive Science and Technology*, **11:** 185-190.

Jatoi, W.A., Baloch, M.J., Panhwar, A.Q., Veesar, N.F., Panhwar, S.A. 2012. Characterization and identification of early maturing upland cotton varieties. *Sarhad Journal of Agriculture*, **28:** 53-56.

Khan, N.U., Marwat, K.B., Hassan, G., Farhatullah, Batool, S., Makhdoom, K., Ahmad, W., Khan, H.U. 2010. Genetic variation and heritability for cotton seed, fiber and oil traits in *G. hirsutum* L. *Pakistan Journal of Botany*, **42:** 615-625.

Khan, N.U., Hassan, G., Marwat, K.B., Farhatullah, Batool, S., Makhdoom, K., Khan, I., Khan, I.A., Ahmad, W. 2009. Genetic variability and heritability in upland cotton. *Pakistan Journal of Botany*, **41:** 1695-1705.

Khan, N.U. 2003. Genetic Analysis, Combining Ability and Heterotic Studies for Yield, its Components, Fiber and Oil Quality Traits in Upland Cotton (*G. hirsutum* L.). *Ph.D. Dissertation*, Sindh Agricuture. University. Tandojam, Pakistan.

Memon, S.M., Ansari, B.A., Kumbhar, Z. 2008. Inheritance studies for quantitative and qualitative traits of upland cotton (*Gossypium hirsutum* L.). *Journal of Agriculture, Agriculture Engineering and Veterinary Sciences*, **24:** 20-24.

Mushtaq, A., Khan, N.U., Fida, M.S., Iqbal, A.K., Zarina, M.B., Salma, S. 2011. Genetic potential and heritability studies for some polygenic traits

in cotton (*Gossypium hirsutum* L.). *Pakistan Journal of Botany*, **43:** 1713-1718.

Naveed, M., Azhar, F.M., Ali, A. 2004. Estimates of heritabilities and correlations among seed cotton yield and its components in *Gossypium hirsutum* L. *International Journal of Agriculture and Biology*, **4:** 712-714.

Raghavrao, D. 1983. *Design of Experiments. Statistical Techniques in Agricultural and Biological Research*. section 11.15; 271pp., Oxford and IBH Publishing Company, New Delhi, India.

Ragsdale, P.I., Smith, C.W. 2007. Diallel analysis of within-boll seed yield components. *Crop Sciences*, **47:** 1013-1017.

Rajamani, S., Sumalatha, P., Gopinath, M. 2015. Studies on genetic parameters of seed cotton yield and fibre traits in upland cotton (*Gossypium hirsutum* L.). *Journal of Cotton Research and Development*, **29:** 36-38.

Rao, P.J.M., Gopinath, M. 2013. Association analysis of yield and fiber quality characters in upland cotton (*Gossypium hirutum* L.). *Australian Journal of Basic and Applied Sciences*, **7:** 787-790.

Soomro, Z.A., Kumbhar, M.B., Larik, A.S., Imran, M., Brohi, S.A. 2010. Heritability and selection response in segregating generations of upland cotton. *Pakistan Journal of Agriculture Research*, **23:** 1-2.

Srinivas, B., Bhadru, D., Brahmeswara, M.V. 2015. Correlation and path coefficient analysis for seed cotton yield and its components in American cotton (*Gossypium hirsutum* L.). *Agricultural Science Digest*, **35:** 13-18.

Tabasum, A., Aziz, I., Asghar, M.J., Iqbal, M.Z. 2012. Inheritance of seed cotton yield and related traits in cotton (*Gossypium hirsutum* L.). *Pakistan Journal of Botany*, **44:** 2027-2031.

Tamilselvam, G., Rajendran, R., Anbarasan, K. 2013. Association and path analysis in cotton (*Gossypium hirsutum* L.). *International Journal of Research and Plant Sciences*, **3:** 36-38.

Wang, C., Isoda, A., Wang, P. 2004. Growth and yield performance of some cotton cultivars in Xinjiang, China, an arid area with short growing period. *Journal of Agronomy and Crop Sciences*, **190:** 177-183.

Pak. j. sci. ind. res. Ser. B: biol. sci. 2018 **61B**(3) 145-150

Fortification and Stability of Iodine in Bread to Mitigate Iodine Deficiency Disorder

Muhammad Sajid Manzoor[a], Imran Pasha[a]*, Shabbir Ahmad[b], Mian Kamran Sharif[a] and Muhammad Farhan Jahangir Chughtai[a]

[a]National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Pakistan

[b]Department of Food Science and Technology, Muhammad Nawaz Shareef University of Agriculture, Multan, Pakistan

(received January 2, 2017; revised October 3, 2017; accepted October 23, 2017)

Abstract. The core objective of this study was to prepare iodized bread by using iodized salt and potassium iodide (KI) to fulfill Recommended Daily Intake (RDI) of iodine. Triplicate level of each fortificant was added in separate treatments in different concentrations with an aim that 2 slices of bread provide RDI of iodine. The prepared samples were analyzed for stability of iodine by spectrophotometric method and for sensory attributes by panel of judges. Results showed good retention of iodine in bread with 15-20% loss of iodine in final product after processing. A slight level of potassium was increased in treatments in which KI was used while other minerals profile was not affected by fortificants and showed no significant behaviour after examining the results statistically.

Keywords: fortification, stability iodized bread, iodine deficiency

Introduction

A balanced diet is indispensable for optimal functioning, metabolism, growth and development of human body. Carbohydrate, protein, lipid, vitamin and inorganic micronutrients are prerequisite of balanced diet. Trace elements play vital role in human body as being structural component of hormones, vitamins and cofactors of enzymes (Freeland-Graves *et al*., 2014). Even the least deficiency of various vitamins and minerals can disturb the functioning of immune system and can cause serious diseases (Akhter *et al*., 2004). Minerals are classified as macro and micro minerals. Micro minerals also known as trace elements are inorganic constituents present in all tissues and fluids of human body. Minerals however do not produce the energy but are significant for many physiochemical processes required to carry out normal life processes (Soetan *et al.,* 2010).

Iodine, trace mineral element, present in minute fraction in human body is radiologically indispensable element. In human body, thyroid gland is the main organ in which iodine is present and it has an effectual role in the production of thyroid hormones. These hormones highly influence the functioning of nerve tissues and muscles, circulatory system, intellectual physical development and heat energy regulation of body (Zimmermann, 2008). It impedes abnormal growth of bacteria in stomach and can transmute allergic proteins entering in body to non-allergic by coating them. It also deactivates most of chemical toxins, all biological poisons and spread of cancer cells to secure body from dreadful diseases and infections (Freeland-Graves *et al*., 2014).

Iodine is a fundamental micronutrient needed at all stages of human life but the pregnancy, fetal life and early childhood are the most important stages of requirement (Zimmermann, 2008). World Health Organization (WHO), United Nations International Children's Emergency Fund (UNICEF) and International Council for Control of Iodine Deficiency Disorder (ICCIDD) recommended that preschool children should take 90 µg of iodine per day, for school children this value is 120 µg per day, 150 µg per day for 12 years and above while pregnant and lactating women should take 250 µg of iodine per day (Longvah *et al*., 2013). Pregnant women have more need of iodine because of the increased synthesis of thyroid hormone, mandatory for the normal and healthy development of fetal brain and neurological network (Elahi *et al*., 2009).

Deficiency of iodine is the public health problem all over the globe. Amongst the ten greatest challenges to global welfare, universal salt iodization is third one suggested by the participants of Copenhagen Consensus (Horton *et al*., 2008). More than 1.88 billion people in

*Author for correspondence; E-mail: ipasha2001@yahoo.com

the world have insufficient iodine level including 241 million children. People are prone to several types of risks due to iodine deficiency like neonatal hypothyroidism, endemic goiter, pregnancy loss, cretinism, infant mortality, growth retardation and intellectual impairment (Pearce *et al*., 2013; Andersson *et al*., 2012). In Pakistan, 70% people are afflicted with iodine deficiency disorder (IDD). The appalling instance of iodine deficiency in pregnant females is main reason that one third of infants in Pakistan have low birth weight (Zimmermann, 2011). During early infancy and fetal life, most drastic effect of iodine scantiness occur on the brain (IM, 2002). Hypothyroid people are less efficient and consequently, economy can be handicapped by reducing work output (Jooste and Zimmermann, 2008).

Different strategies are available and applicable to control and prevent micronutrient deficiencies including iodine deficiency disorders. Three of them are dietary diversification, supplementation and fortification for both targeted and untargeted population (Popovici *et al*., 2006). Food fortification means the addition of mandatory micronutrient in processed and treated foods. Food fortification is a worthwhile method reinforces the approaches used to diminish and restrain the problem of micronutrient malnutrition (WHO and FAO, 2006). Fortification of iodine is widespread to control and prevent the adverse effects of less iodine intake (Rasmussen *et al*., 2014). Sodium and potassium iodides and iodates are recommended additives for fortification of iodine in food. Level for their addition to salt may be equivalent to 25-65 mg iodine per kg salt (Thomson, 2009). Iodized salt also has been proven an excellent and effective mean for iodine fortification in many industrialized countries (De Benoist *et al.*, 2004). Iodized salt is used in breads, biscuits and breakfast cereals due to higher consumption of bakery products (Thomson, 2009).

Bread is an important source having vital diet components that may include iodized salt. Fortified bread not only provides nutritional minerals to our cells, but it has potential to dissolve, sanitize and clean toxic wastes from our body system. Salt is one of the few perfect fortificants for micronutrient fortification because it is among those foods that are globally consumed on regular bases at a fairly constant rate by almost all sections of a population regardless of their economic and social status (Zimmermann and Boelaert, 2015). Fortification of bread with iodine to combat iodine deficiency disorders and to determine processing effect on iodine stability were main objectives in this research.

Materials and Methods

Procurement of raw material. Wheat variety i.e. "Faisalabad 2008" for bread making was selected and procured from Ayub Agricultural Research Institute, Faisalabad. Chemicals and reagents were procured from Sigma Aldrich.

Chemical evaluation of wheat flour. Wheat flour was evaluated for chemical composition according to AACC (2000) i.e., moisture content with method No. 44-15A, crude protein with method No. 46-10, crude fat with method No. 30-10, crude fibre with method No. 32-10 and ash content with method No. 08-01. For moisture content 3-5 g sample was taken in China dish, put in hot air oven at 100±5 °C for overnight and moisture content was measured by calculating the weight loss. Wheat flour sample about 1-3 g digested with concentrated H_2SO_4 till light green colour and distilled after diluting with distilled water about 250 mL total volume. After distillation, the resultant solution of ammonium borate was titrated with 0.1N H_2SO_4. This method gives the estimation of total nitrogen which was converted to protein by multiplying with 5.7 factor.

For fat determination, 10-15 g sample was wrapped in filter paper and washed with petroleum ether in Soxhlet apparatus. At the end, fat free sample was placed in hot air oven at 100±5 °C for overnight and fat content was measured by calculating the weight loss. Fat and moisture free sample (1-3 g) was digested with 1.25% solution of H_2SO_4 and after washing with 1.25% solution of NaOH. The residues obtained after washing and filtration were charred and placed in muffle furnace at 550 °C for 5-6 h. The fibre was calculated by measuring the difference before and after placing in muffle furnace. For ash determination, 1-2 g sample was charred on direct flame and placed in muffle furnace at 550 °C for 5-6 h. The remaining residues were measured as total ash content of flour.

Minerals like iron (Fe), copper (Cu), potssium (K), zinc (Zn) and manganese (Mn) in flour was analyzed using Atomic Absorption Spectrophotomer (Varian AA240) as described in AOAC (2006). Sample (1-3 g) was digested in a mixture of HNO_3: $HCLO_4$ with 7:3 over hot plate. After digestion, sample was diluted with distilled water to make total volume 250 mL. Afterwards, sample were run on atomic absorption spectrophotometer.

Product development. For bread, a control and test runs with iodized salt and potassium iodide (source of iodine) were manufactured in the normal way in baking hall of National Institute of Food Science and Technology. Triplicate samples were prepared with each of the treatment as mentioned in Table 1. The breads were prepared according to the AACC (2000) straight dough method No 10-10B. The ingredients were mixed for 5-10 min in a Hobart A-200 mixer to form dough, afterwards the dough was molded, panned into 100 g pans, and proofed for 45 min at 95 °F (35 °C) and 85% R.H in proofer. The dough was baked at 220 °C for 22-25 min and sliced after cooling. The bread slices were packed properly in polythene bags stored under ambient conditions and were analyzed afterwards.

Analysis of product. ***Chemical composition of bread.*** Moisture, crude protein and crude fat content of bread were analyzed by AACC (2000) methods as mentioned above. Minerals like Fe, Cu and Zn of bread were analyzed using Atomic Absorption Spectrophotometer (Varian AA240) as described in AOAC (2006).

Iodine content. All bread samples were analyzed for iodine content in triplicate by a spectrophotometric method according to Moxon and Dixon (1980). Bread samples (1g) of each were taken in porcelain crucible, 2 mL of 1M potassium hydroxide solution and 1mL of 10% of zinc sulphate solution was added. Mixture was drived, completely on hot plate and the crucible was placed in muffle furnace at 450 °C for 1.5 h. The residue was dampened with zinc sulphate solution then again placed in furnace under same conditions. Subsequently, the cooled ash was transferred to centrifuge tube with 50 mLs of deionized (DI) water. The samples were then mixed with 0.4mL of 0.006M potassium thiocyanate solution and 1.6 mL of 0.2M ammonium iron (III) sulphate. At exactly 90 second intervals, 1mL of 0.3M sodium nitrite solution was added. The absorbance of mixture was measured at 450nm after 20 min incubation. Iodine content was calculated using KI as a standard.

Table 1. Treatment plan for iodine fortification in bread

Treatment	Iodized salt & potassium iodide (KI)/100g
T_o	Control
T_1	1g iodized salt
T_2	2g iodized salt
T_3	3g iodized salt
T_4	0.546mg (546µg) KI
T_5	0.818mg (818µg) KI
T_6	1.090mg (1090µg) KI

Sensory evaluation. The bread was prepared as above and subjected for sensory evaluation for appearance, colour, texture, flavor and taste by hedonic score system as described by Lawless and Heymann (2010). For sensory evaluation of bread, 20 panelists were selected covering faculties from teaching, research and extension wings. Panelists were of 24-40 years age with sound health and good sensory perceptions. The evaluation was carried out in a well-ventilated, odourless, and quiet environment.

Statistical analysis. The data obtained for each parameter was subjected to statistical analysis to determine the level of significance among the treatments and further values were finalized by applying means separation (Tukey-HSD) using SPSS (Statistical Package for the Social Sciences, version 10.0.1, 1999) according to the method described by Montgomery (2008).

Results and Discussion

Chemical composition of wheat flour. Chemical composition of wheat flour is shown in Table 2 and the proximate analysis of wheat flour shows average results. Moisture content of wheat flour was 9.73%, protein was 10.54% and fat content of flour was 1.37%. Fibre and ash contents were 2.33% and 1.67%, respectively. Flour sample contain Fe, Zn, Mn and Cu as 1.27mg/100g, 0.82mg/100g, 0.80mg/100g and 0.24mg/100g, respectively. The finding of the current study is congruent to the finding of Ahmad (2017); Pasha *et al*. (2009); and Zahoor (2003) who reported that values of moisture, protein, fat, fibre and ash were in the range of 8.92 to 11.68 %, 10 to 13.4 %, 1.09 to 2.52 % and 2.20 to 2.77 %, respectively in some Pakistani wheat varieties. Similar results regarding Fe, Zn, Mn and Cu have been obtained from the study of Khan *et al*. (2005) who reported that wheat flour contains 4.40 mg/100 g, 3.40 mg/100 g, 3.53 mg/100 g and 0.63 mg/100 g of the above mentioned minerals, respectively.

Chemical composition of bread. Bread proximate showed non-significant results revealing no effect of any treatment on the moisture, protein or fat content of bread. Baking always involves the loss of water from the 'raw' to the baked product therefore bread contains lower moisture content as compared to water added during processing (Cauvain and Young, 2000). Chemical composition of bread is presented in Table 3. In bread

treatments, moisture content shows slight decreasing trend from T_0-T_2 then increases in T_3 and T_4 again decreases slightly in T_5 and T_6. It may be due to minor deviations in processing conditions. Protein content in bread decreased than protein content of flour and shows decreasing trend in treatments. Malomo *et al.* (2011) supported the results of this research, used wheat flour of 15.47% protein content and used it for bread production. After production of bread they found decreased content of protein (11.96%) while fat increased in bread than wheat flour and shows results similar to Malomo *et al.* (2011). He used wheat flour of 2.60% fat content for bread production and after production of bread found increased trend of fat (4.29%). Best treatment T_5 contain moisture 30.31%, protein 8.72% and fat 1.88%.

Mineral analysis and their statistical behaviour showed that all of them were non-significantly affected by iodized salt and KI treatments used in bread except K content, which shows a significant result. Iron and manganese content in bread was less than previous findings of some scientists as Tuncel *et al.* (2014) observed 2.44mg/100g iron content in pan bread prepared with flour of wheat grain. Ragaee *et al*. (2006) examined 44 mg/100g of iron content in wheat flour. This was due to the reason of using wheat flour for bread making that was already low in iron and manganese content. Zinc content assessed in bread was close to the previous researches as Tuncel *et al.* (2014) investigated 0.95mg/100g zinc content in bread prepared with wheat flour. Mean values of Cu in all treatments were very near about each other with highest content of 0.23 mg/100g and lowest of 0.22 mg/100g found randomly in different treatments showed that there was no significant effect of any treatment on content of Cu in bread. Level of K slightly raised in last 3 treatments in which KI was used as fortificant but that increase in level was only of some micrograms not exceeding its recommended intake level.

Iodine content. Iodine stability is the main issue now a days that is contradicting the struggles of iodine fortification, therefore iodine stability test was done that showed only 15-20% loss of iodine and actually depicts a high stability in terms of its need to overcome its deficiency consequences. Loss of iodine level in different treatments of bread is depicted in Table 4. Three levels of iodized salt were used in bread to prepare iodine fortified bread but iodized salt through bread supply only a little portion of required iodine because the addition of salt in bread is limited for best yeast activity and acceptable sensory characteristics. Maximum of 3% iodized salt used in bread (T_3) and after loss of iodine, two slices of that bread gave 37µg of iodine. KI in this sense gave the best result and is much effective

Table 2. Chemical composition of wheat flour

Treatments	Moisture	Protein	Fat	Fibre	Ash	Fe	Zn	Mn	Cu	K
	(%)					(mg/100g)				
R_1	9.9	10.59	1.33	2.33	1.68	1.272	0.824	0.792	0.234	96.2
R_2	10.1	10.72	1.41	2.38	1.64	1.319	0.859	0.817	0.245	92.2
R_3	9.2	10.33	1.38	2.29	1.7	1.221	0.776	0.779	0.224	88.4
Mean value	9.73± 0.472	10.54± 0.199	1.37± 0.404	2.33± 0.045	1.67± 0.031	1.271± 0.049	0.82 ± 0.042	0.796± 0.019	0.234± 0.011	92.267± 3.9

Table 3. Chemical composition of bread

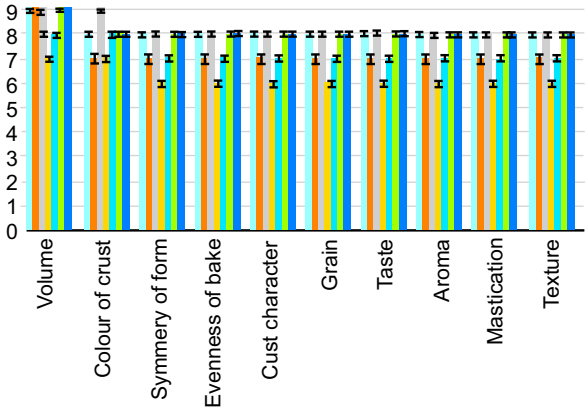
Treatments	Moisture	Protein	Fat	Iron	Zinc	Manganese	Copper	Potassium
	(%)				(mg/100 g)			
T_0	30.48 ± 0.93	8.71 ± 0.09	1.87 ± 0.09	1.26 ± 0.01	0.80 ± 0.01	0.78 ± 0.01	0.23 ± 0.01	89.27b ± 0.85
T_1	30.14 ± 0.96	8.71 ± 0.07	1.91 ± 0.07	1.25 ± 0.01	0.81 ± 0.02	0.76 ± 0.01	0.22 ± 0.01	89.47b ± 0.97
T_2	29.33 ± 0.99	8.72 ±0.11	1.87 ± 0.08	1.26 ± 0.02	0.79 ± 0.01	0.78 ±0.01	0.23 ± 0.02	90.3ab ± 0.91
T_3	29.84 ± 0.99	8.69 ± 0.09	1.87 ± 0.11	1.26 ± 0.01	0.81 ± 0.02	0.76 ± 0.01	0.22 ± 0.01	91.3ab ± 0.82
T_4	30.93 ± 0.67	8.69 ± 0.08	1.9 ± 0.08	1.25 ± 0.02	0.79 ± 0.02	0.76 ± 0.01	0.23 ± 0.01	91.3ab±0.83
T_5	30.31 ± 1.11	8.72 ± 0.07	1.88 ± 0.11	1.25 ± 0.01	0.80 ± 0.01	0.77 ± 0.02	0.23 ± 0.02	92.3a ± 0.81
T_6	29.43 ± 0.61	8.68 ± 0.09	1.89 ± 0.09	1.26 ± 0.01	0.79 ± 0.01	0.76 ± 0.01	0.22 ± 0.01	92.47a ± 0.71

Table 4. Loss of iodine level in bread

Treat-ment	Iodine	Fortificant added before processing per 100g	Iodine in bread after processing (µg/100g)
T_0	1.99g ± 0.01	_	_
T_1	30.99f ± 0.21	1g iodized salt (40µg I)	30.99
T_2	63.12e ± 0.02	2g iodized salt (80µg I)	63.13
T_3	91.02d ±0.01	3g iodized salt (120µg I)	91.02
T_4	319.20c ± 0.15	546µg KI (417µg I)	319.20
T_5	479.87b ± 0.15	818µg KI (626µg I)	479.87
T_6	651.13a ± 0.15	1090µg KI (833µg I)	651.13

to prepare iodized bread as T_5 bread treatment in which 0.818mg/100g KI was used, supplied 172 µg of iodine/ 2 slices.

Sensory analysis. In sensory evaluation of iodine fortified bread treatments volume was not altered significantly in all treatments as shown in Fig. 1. In T_2 and T_3, prepared with 2% and 3% iodized salt, volume slightly decreased due to high concentration of salt. Other external bread characteristics; crust colour, symmetry of form, character of crust and evenness of bake were non-significantly affected by different treatments. Internal bread characteristics (grain, aroma, texture and mastication of bread) are non-significantly affected by fortificants added except taste. The lowest score for taste of bread was found in T_3 (3% iodized salt). The score for taste of bread was gradually increased as the amount of KI used in bread was increased. T_6 prepared with 1.09 mg/100g KI had maximum score of taste.



To= Control; T1=1g iodized salt; T2=2g iodized salt; T3=3g iodized salt; T4=0.546mg (546µg) KI; T5=0.818mg (818µg) KI and T6=1.090mg; (1090µg) KI

Fig. 1. Sensory evaluation of bread

Conclusion

Bread fortified with iodized salt contained low level of iodine not enough to fulfill RDI but supports the projects working against IDD and its good practice to use iodized salt instead of non-iodized salt while KI fortified bread can alone achieve the goal to mitigate IDD. K content slightly increases in KI treatments. However, in sensory attributes only volume was affected in treatment in which 3% iodized salt was used, so volume of bread decreases if salt concentration is increased in bread than the recommended level. Addition of KI had positive effect on taste of bread. Stability test showed 15-20% iodine losts during processing of bread till the end of the process though it is the best means to get required iodine. Iodized salt alone would not be able to accomplish the objective but even just 2 slices of KI fortified bread provide RDI of iodine.

References

AACC, 2000. *Approved Methods of the AACC.* American Association of Cereal Chemists. vol. **1-2,** 1200 pp., St. Paul, MN, USA .

Ahmad, S., Pasha, I., Saeed, M., Shahid, M. 2017. Principal component analysis and correlation studies of spring wheats in relation to cookie making quality. *International Journal of Food Properties*, **20:** 2299-2313.

Akhter, P., Rehman, K.U., Orfi, S.D., Ahmad, N. 2004. Assessment of iodine levels in the Pakistani diet. *Journal of Nutrition*, **20:** 783-787.

Andersson, M., Karumbunathan, V., Zimmermann, M.B. 2012. Global iodine status in 2011 and trends over the past decade. *Journal of Nutrition*, **142:** 744-750.

AOAC, 2006. *Official Methods of Analysis*. 18th edition, The Association of Official Analytical Chemists. Arlington, Gaithersburgs, MD, USA.

Cauvain, S.P., Young, L.S. 2000. The contribution of water during processing, baking, cooling and freezing. In: *Bakery Food Manufacture & Quality: Water Control & Effects*, S.P. Cauvain (ed). vol. **1,** pp. 72-93, 2nd edition, Blackwell Science Limited, London, UK.

De Benoist, B., Maria, A., Ines, E., Bahi, T., Henrietta, A. 2004. Iodine status worldwide. *WHO Global Database on Iodine Deficiency*. World Health Organization, Geneva, Switzerland.

Elahi, S., Rizvi, N.B., Nagra, S.A. 2009. Iodine deficiency in pregnant women of Lahore. *Journal*

of Pakistan Medical Association, **59:** 741-743.

Freeland-Graves, J.H., Sanjeevi, N., Lee, J.J. 2015. Global perspective on trace elements requirements. *Journal of Trace Elements in Medicine and Biology*, **31:** 135-141.

Horton, S., Mannar, V., Wesley, A. 2008. *Best Practice Paper: Food Fortification with Iron and Iodine*. Copenhagen Consensus Center, Copenhagen Business School, Denmark.

Jooste, P.L., Zimmermann, M.B. 2008. Progress towards eliminating iodine deficiency in South Africa. *South African Journal of Clinical Nutrition*, **21:** 08-14.

Khan, M.I., Anjum, F.M., Hussain, S., Tariq, M.T. 2005. Effect of soy flour supplementation on mineral and phytate contents of unleavened flat bread (chapatis). *Nutrition and Food Science*, **35:** 163-168.

Longvah, T., Toteja, G.S., Upadhyay, A. 2013. Iodine content in bread, milk and the retention of inherent iodine in commonly used Indian recipes. *Food Chemistry*, **136:** 384-388.

Malomo, S.A., Eleyinmi, A.F., Fashakin, J.B. 2011. Chemical composition, rheological properties and bread making potentials of composite flours from breadfruit, breadnut and wheat. *African Journal of Food Science,* **5:** 400-410.

Montgomery, D. 2008. Experiments with a single factor: The analysis of variance. In: *Design and Analysis of Experiments*, John Wiley & Sons, Inc. USA.

Lawless, H.T., Heymann, H. 2010. *Scaling*: *Sensory Evaluation of Food Principles and Practices*, 171 pp. Springer Science Business Media, New York, USA.

Moxon, R.E.D., Dixon, E.J. 1980. Semi-automatic method for the determination of total iodine in food. *Analyst*, **105:** 344-352.

Pasha, I., Anjum, F.M., Butt, M.S. 2009. Biochemical characterization of spring wheats in relation to grain hardness. *International Journal of Food Properties,* **12:** 910-928.

Pearce, E.N., Andersson, M., Zimmermann, M.B. 2013. Global iodine nutrition: where do we stand in 2013? *Thyroid*, **23:** 523-528.

Popovici, C., Sturza, R., Deseatnicov, O. 2006. Study of the incorporation of iodine in vegetable oils. *Journal of the University of Chemical Technology and Metallurgy*, **4:** 449-456.

Ragaee, S., Abdel-Aal, E.S.M., Noaman, M. 2006. Antioxidant activity and nutrient composition of selected cereals for food use. *Food Chemistry*, **98:** 32-38.

Rasmussen, L.B., Jorgensen, T., Perrild, H., Knudsen, N., Krejbjerg, A., Laurberg, P., Pedersen, I. B., Bjerbved, L., Ovesen, L. 2014. Mandatory iodine fortification of bread and salt increases iodine excretion in adults in Denmark – A 11- year follow-up study. *Clinical Nutrition*, **33:**1033-1040.

IM, 2002. *Dietary Reference Intake of Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc*. Institute of Medicine, National Academy Press, Washington, D.C. USA.

Soetan, K.O., Olaiya, C.O., Oyewole, O.E. 2010. The importance of mineral elements for humans, domestic animals and plants: A review. *African Journal of Food Science*, **4:** 200-220.

Thomson, B.M. 2009. Stability of added iodine in processed cereal foods. *Food Additives and Contaminants*, **26:** 25-31.

Tuncel, N.B., Yilmaz, N., Kocabiyik, H., Uygur, A. 2014. The effect of infrared stabilized rice bran substitution on B vitamins, minerals and phytic acid content of pan breads: Part II. *Journal of Cereal Science*, **59:** 162-166.

WHO and FAO, 2006. *Guidelines for Food Fortification with Micronutrients*. A. Lindsay, B. de Benoist, O. Dary and R. Hurrell (eds.) WHO Library Cataloguing -in-Publication Data. World Health Organization and Food and Agriculture Organization of the United Nations. ISBN 92 4 159401 2.

Zahoor, T. 2003. High Molecular Weight Glutenin Subunit Composition and Multivariate Analysis for Quality Traits of Common Wheats Grown in Pakistan. *Ph.D. Thesis*. University of Agriculture, Faisalabad, Pakistan.

Zimmermann, M.B., Boelaert, K. 2015. Iodine deficiency and thyroid disorders. *The Lancet Diabetes & Endocrinology*, **3:** 286-295.

Zimmermann, M.B. 2011. Iodine deficiency continues to plague Pakistan. ICCIDD. *IDD Newsletter*, **39:** 1.11.2011.

Zimmermann, M.B. 2008. Iodine requirements and the risks and benefits of correcting iodine deficiency in populations. *Journal of Trace Elements in Medicine and Biology,* **22:** 81-92.

Pak. j. sci. ind. res. Ser. B: biol. sci. 2018 **61B**(3) 151-157

Spatial Patterns and Trends of *Escherichia coli* in Public Water Supply System of Lahore, Pakistan

Isma Younes[a], Abdul Ghaffar[a], Rakhshanda Sadaf[b]*, Naheeda Naseem[a] and Muhammad Shafiq[c]

[a]Department of Geography, University of the Punjab, Lahore, Pakistan

[b]Department of Geology, Federal Urdu University of Arts, Science and Technology, Karachi, Pakistan

[c]Directorate of Earth Sciences, SUPARCO, Karachi, Pakistan

(received May 30, 2017; revised November 11, 2017; accepted December 29, 2017)

Abstract. Most of the Northern and Central part of Lahore district is supplied with ground water by Water and Sanitation Government Authority. The quality of public water supply is getting deteriorating due to increasing population of Lahore city. Moreover, the effects of microbial water pollutants on health of population of study area have been addressed. In this research, presence of *Escherichia coli* in pre & post monsoon seasons has been focused. The water samples were taken from tube wells as well as adjacent houses. A questionnaire survey was also conducted to find out the responses of people in study area. It was clear from the results of the study that *E. coli* were detected in all the water samples of public water supply system. An increase in growth of pathogens was also noticed in post monsoon season. It was also proved from study that many people were suffering from diarrhoea at sample places where *E. coli* were identified. The mixing of sewage with drinking water was the major cause of presence of pathogens in water samples of houses although they were less or absent in water samples of tube wells.

Keywords: spatial patterns, *Escherichia coli*, water supply system, sewage contamination

Introduction

An adequate supply of safe drinking water is a major prerequisite for the health. Gray (2010) analyzed that water borne diseases are a major cause of deaths around the world. According to WHO, 1.8 million people die due to diarrhoea and cholera throughout the world (Hamid *et al*., 2013, Poff and Aregai, 2002) and nine out of ten deaths are among children and nearly all of these deaths occur in the developing countries (Nicholas, 2004). Drinking water needs to be free from all the pathogens, all solids, animal and human waste (Goel, 2011). It is assigned to be polluted when anthropogenic pollutants spoil it and it does not remain any more useful for human beings as potable water (EPA, 2015). There are many natural sources that lead to change in ecological status of water and quality such as algal bloom, earthquakes, volcanoes, floods and storms (Paul, 2011; Ghosh, 2008).

Drinking water is borrowed from two major sources which are surface water and groundwater. All water contains natural impurities, particularly inorganic impurities that emanate from geological bed through which the ground water flows and, to some extent, man-made pollution caused by both chemicals and microorganisms. In general, surface waters are more vulnerable to pollution than groundwater. There are many sources of anthropogenic contaminants, some of which are more critical than others. These are point and non-point sources. Discharges from industrial areas and sewage treatment works are point sources and these sources are more immediately detectable and controlled such as urban and agricultural runoff (Prica, 2010). Such sources can give rise to a significant variation in the contaminant load over time.

Quality of ground water depends on many natural factors such as nature of rocks, nature of rainfall and nature of already existing ground water (Ahmad *et al*., 2011). It is also affected by a number of anthropogenic factors such as explicit release of industrial waste in water bodies, urban sewage and agricultural runoff (Agarwal, 2009). Pollutants in groundwater are very less as compared to surface water. As soil acts as a filter for many pollutants. Water pollutants also infiltrate in ground water from septic and underground tanks that are used for management of sewage treatment (Goel, 2011).

World Health Organization asserted that water quality is not up to the mark in many parts of Lahore, Pakistan. First of all, Water and sanitation Authority (WASA) was not following the drinking water quality standards set by WHO (WHO, 2011; 2000; 1997) at many locations (WASA Tubewells). Secondly, although the water quality

*Author for correspondence; E-mail: r.sadaf@fuuast.edu.pk

is better in water samples of tubewells but its quality becomes poor as it travels to end points (houses) of the water supplying system. The deteriorated conditions of old water supply pipe lines and sewage collecting pipe lines allow sporadic mixing of water from both pipe lines. As a result of mixing of sewage in drinking water, many water-borne diseases are common among people of the study area.

This study has focused on Lahore district. WASA is the only public water supplying authority of Lahore district but it does not supply water to the whole district rather it supplies water to only a small part of the North and Centre of the district. This research was conducted to identify the patterns of *Escherichia coli* in public water supply system and to study its effects on human population. The pathogen selected for this study was *Escherichia coli* as it is one of the major causes of diarrhoea among people.

Materials and Methods

A primary data collection was done for measuring microbial quality of water samples as well as for obtaining information about responses of population. The water samples were collected from sources (tubewells installed by WASA) of public water supply system. One hundred and twenty-two tubewells were taken as sample sites for taking water samples to analyze microbial parameters. The same number of water samples was also taken from houses. As the district is having nine towns and one cantonment area, water samples were collected from all the towns of the Lahore district. Water samples for analysis were also collected from nearby houses. The use of public water supply by target population was confirmed. Water samples were collected and analyzed both in pre and post monsoon seasons to study the changing pattern of growth of microbes in different temperatures and other climatic conditions. WHO Standards were used to measure, analyze and detect different bacteria in water samples. Data about water sources, quality of public water supply, socio-economic conditions of people and disease prevalence among people was collected using questionnaires. The data about number of patients of diarrhoea were collected through questionnaire survey.

Inverse Distance Weighting (IDW) method of interpolation was applied for mapping of *E. coli*. Wong applied the Standard Deviation (Wong and Lee, 2005) using tool of directional distribution for measuring the geographic distribution. This technique focuses on the trend of spatial distribution of *E.coli* in public water supply system in study area. It also reflects that the water of sample locations was polluted in both seasons. Scott also calculated the center of data using spatial distribution technique (Scott and Janikas, 2010).

Figure 1 shows the sample locations of water samples for analyzing microbial parameter in the study area. Blue dots highlight the tubewells and red dots focus the sample houses. A boundary was marked around these sample locations so that patterns of microbial pollution can be shown accurately.

Results and Discussion

The colour hues are applied in Fig. 2-3 to display patterns of *E.coli*. Dark brown hue was calling attention to the sample locations where *E.coli* was not detected. Yellow to green hues were used for showing the locations where it was detected but number of colonies was less. Besides this the light blue to dark blue colour shows the sites where quality of drinking water was worst due to presence of large number of colonies of *E.coli*.

Figure 2 highlights the patterns of *E.coli* in pre-monsoon water samples of tubewells. Table 1 shows that *E.coli* was detected in both seasons in water samples at many places. Figure 1 shows that *E.coli* was detected in water samples of Baghechi, Belal Park, Lahori Gate, Sheranwala Gate, Yaki Gate, Paniwala Talab, Akbari Gate, Masti Gate, Texali Gate, Taj Pura, Paki Thatti, Akbar Shaheed road, Kot Khwaja Saeed, Begumpura,

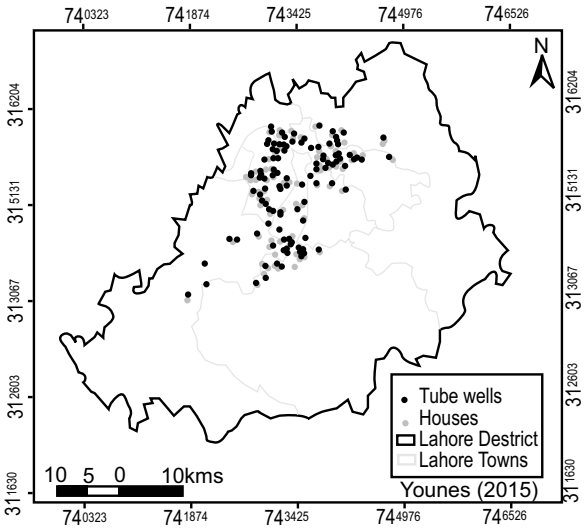


Fig. 1. Sample locations in study area.

Table 1. *E.coli* in water samples at different sample locations

Locations	Houses-Pre	Houses-Post	TW-Pre	TW-Post
Loction93	0	1	0	0
16B-1 block phatniwala	2	17	1	4
2C-II township	4	15	0	2
3D-II township	10	15	1	7
3D-1 township	7	15	0	5
6A II township	32	36	1	13
Ahmed block	4	6	2	5
Akbari gate	16	31	3	5
Al fasial town burji	0	1	0	1
Amer sidu phatak	18	36	0	0
Askari	0	0	0	0
Baba farid colony	0	0	0	0
Badami bagh	30	41	0	0
Bagh gul begum	1	1	0	1
Bagh munshi ladha	9	11	1	2
Baghich	27	45	3	4
Baghrian	2	7	0	0
Bata pur	0	1	0	0
Begumpura	6	17	2	1
Bhatti colony	0	1	0	1
Bilal park	2	5	2	5
Cblock faisal town	11	17	6	14
Chah miran	2	13	1	1
Chandni chowk town ship	2	5	1	4
CMH	0	0	0	0
D block greentown	12	30	2	2
Daroghawala	6	8	3	7
Dars bary mian	17	24	8	12
Data nagar	11	21	0	7
Dhobi ghat	11	15	0	0
Dhobi mandi	3	7	1	1
Dry port	11	17	0	0
F and v market	0	0	0	0
Fate h garh	1	1	0	0
G-4johar town 1	4	12	0	1
Garison golf	0	0	0	1
General hospital	0	1	0	1
Ghaziabad	10	15	0	1
Girls hostel pu 7	0	0	1	1
Green town	24	37	0	0
Gulshan colony mustafabad	1	1	1	0
Gulshan block	7	9	0	0
Gulshan ravi b block	4	7	0	3
Gulshan colony	0	0	3	1
Gulshan ravi G block	3	22	2	2
Gurumanget	3	5	0	1
Gwala colony harbanspura	14	17	1	1
Huma block flats	12	34	11	14
lchhramor	2	2	0	2
Iftikhar park	2	5	0	0
Islampura	11	17	2	7
Jafaria colony	2	7	0	1
Jahanzeb block	3	6	2	6
Jallo Park	0	0	0	0
J-block johar town	2	5	0	0
Jnaz gah	5	12	0	0
Jubli town	0	0	0	0
Karim park	1	1	0	1
Khwaja ahmed sadiq	0	0	0	0
Kot khwaja saeed	2	5	0	0
Kot muhammad	1	4	0	0
Lahore college	0	0	0	0
Lall Pull	7	15	4	9
Liberty marker	4	18	0	1
Lahari gate	4	12	0	4
Masti gate	4	12	2	5
Mehmood booti	12	23	3	11
Mian fazal haq colony	0	1	0	0
Model colony	3	1	0	0
Mohalla islam Khan	2	2	0	0
Moman pura	2	4	0	1
Moon market gulshen	9	1	1	0
Mohari gate	22	26	1	2
Muslimabad Fateh gar	0	12	1	2
N block model town	1	4	0	2
Nabi bakish park	2	5	1	3
Naisar bagh	0	0	0	0
Nawankot samanabad	2	3	0	1
Nehru park	11	13	2	1
New jail road near PEC	0	0	0	0
Nishter colony	1	3	1	0
Nishter colony 2	0	0	0	1
Old anarkali	4	12	2	5
Old ravi well centre	01	0	2	7
Pak block	3	21	2	2
Pak mint	12	29	0	1
Pakki thatti	5	12	2	5
Pani walatalab	2	5	1	12
Patiala ground	0	1	0	1
Pindi Rajputan	7	13	1	3
Punjab cooperative	0	1	0	0
Q block	1	1	1	4
Quaid e millat collony	2	2	3	5
Race cource road	11	14	0	0
Residential colony pu	0	0	0	0
Salam atpura no 5	5	9	0	1
Salam atpura tkia	2	3	0	1
S-Block bahar colony	2	2	2	1
Shadman market	2	6	1	1
Shadman nala	1	14	1	7
Shah gohar pir	2	5	0	0
Shah kamal	0	1	0	1
Shahuwari	11	23	0	0
Shalimar garden	17	23	5	9
Shahuwari gate	22	42	0	1
Shish mehal	1	1	0	1
Sirajpura	3	5	2	3
Taj bagh scheme	0	12	0	1
Tajpura ground	1	1	4	1
Tariq colony maqbol	0	1	0	0
Taxali gate	5	11	0	1
Thokar niaz beg	1	1	0	1
Usman block	3	6	0	1
Vc office pu	0	0	0	0
WASA colony nawan k	2	3	0	0
Windsor park	1	4	0	0
Yakki gate	24	35	2	2
Zafar ali road	6	7	4	6

TW= Tube wells

Contiued in column 2

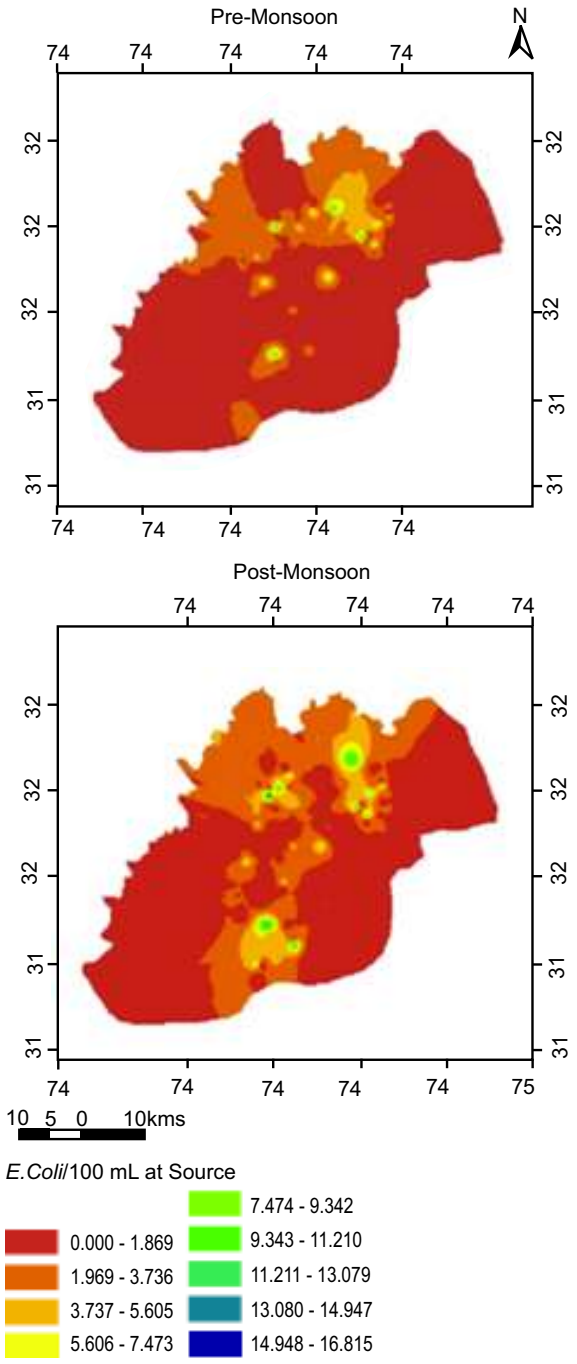


Fig. 2. Pre & post monsoon patterns of *E.coli* at source (2016).

Shadman ChahMiran, Gulshen Ravi, BaghMunshi Ledha, Ghaziabad, Taj Bagh, Lal Pull, Dars Bary Mian, Shalimar Garden, Mehmood Booti, Nala, Pindi Rajputen, Ahmed Block, KotLakhpat, Sirajpura, Pak Mint, S-Block Bahar Colony, Green Town, 6-AII Township, Chandni Chowk, C-Block Faisal Town, Nishter Colony, Daroghawala, Gwala Colony Harbanspura, 3-DII Township and Muslimabad.

Figure 2 also reflects the post-monsoon patterns of *E.coli*. which was detected in all samples of water except water samples of some areas like Badami Bagh, Dhobi Ghat, Lahore College, Q-Block Model Town, Momen Pura, Windsor Park, KotKhwaja Saeed, Iftihkhar Park, Jubli Town, Nasir Bagh, Modal Colony, Wasa Colony Nawaankot, Janazgah, F-n-V Market, J-Block Johar Town, Green Town, Patiala Ground, Gulshen Colony, Khwaja Ahmad Sadeq, Bagrian, Quaid-i-Millat Colony and Mian Fazal-e-Haq Colony. It was present in rest of all water samples of all locations. It was detected at many locations in post monsoon season where it was not detected in pre-monsoon season.

Figure 3 highlights patterns of *E.coli* at the end points in pre-monsoon season. *E.coli* was detected in water samples of those sites where it was also detected in water samples of tube wells such as Baghechi, Lahori Gate, Bilal Park, Yaki Gate Sheranwala Gate, Paniwala Talab, Akbari Gate, Masti Gate, Begumpura,Texali Gate, Paki Thati, Taj Pura, KotKhwaja Saeed, ChahMiran, Gulshen-e-Ravi, Bagh Munshi Ladha, Ahmad Block, Ghaziabad, TajBagh, Muslimabad, Mehmood Booti, Sirajpura, Shadman Nala, Green Town, Pak Mint, Kot Lakhpat, Lal Pul, Pindi Rajputan, Dars Bary Mian, Nishter Colony, S-Block Bahar Colony, 6-AII Township, Akbar Shaheed road, C Block Faisal Town, Daroghawala, Gwala Colony Harbenspura, Shalimar Garden, Chandni Chowk and 3 DII Township. *E.coli* was also detected in water samples of 16-B-1 Block Phatniwala, D Block Green Town, Begrian, Quaid-i-Millat Colony, Amer Sidhu Phatak, Race Course Road,Gulestan Colony Mustafabad, Gwala Colony, Model Colony, Zafer Ali Road , Gwala Colony Harbenspura, Sahuwari, Ichhra Mor, Janazgah, Nawankot, Shah Gohar Pir, Pak Block, Gulshen Block, Jahanzeb Block, Paki Thati, Shadman Market, Windsor Park, G-4 Johar Town, Momanpura, Salametpura No-5, Tajpura, Data Negar, Mohala Islam Khan, Badami Bagh, Karim Park, Nehru Park and Shalimar Garden. An increase in number of *E.coli* can be observed at sample locations of tube wells as well as end points in pre-monsoon seasons. It is quite clear at many tube well sites where *E.coli* was not detected but it was detected at end points. It highlights that water pipes that supply water were either not clean and were having sporadic mixing of sewage in drinking water. It is because of corrosion of both pipes.

A depression cone that is present in Centre of the district has also been reason of inclusion of salinity from the southern part of the district as well as addition of microbial and chemical pollution from River Ravi. Therefore, the sewage that mixes with groundwater and easily seeps down to aquifer and becomes part of groundwater. Afterwards, this water is extracted for drinking purposes.

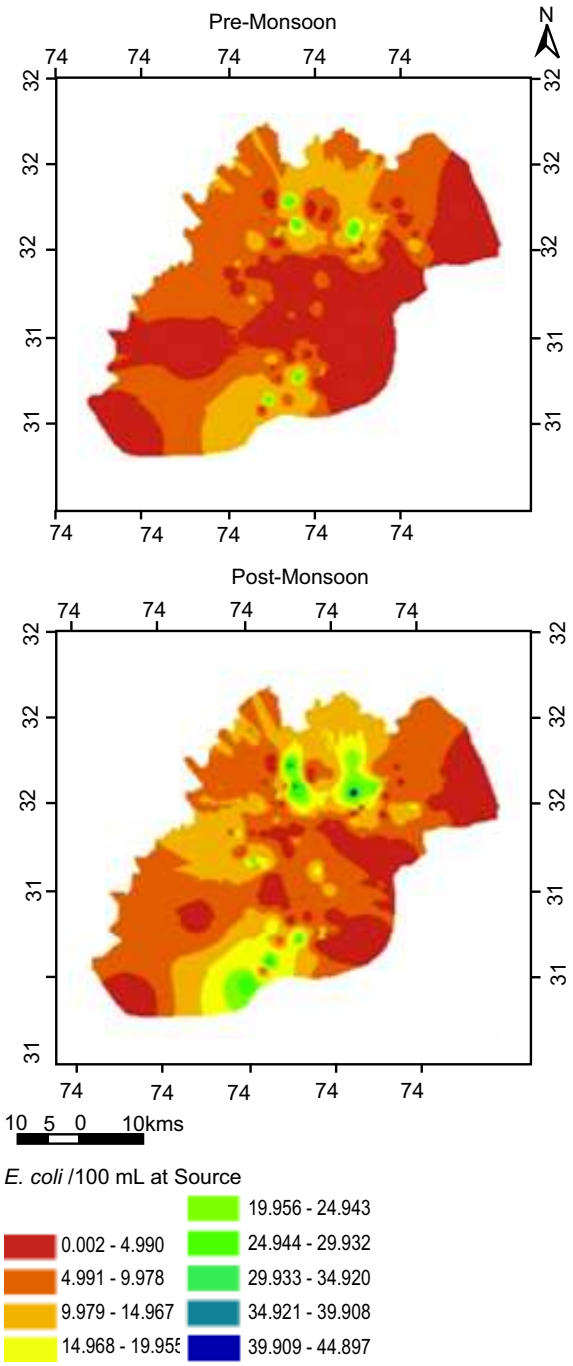


Fig. 3. Pre & post monsoon patterns of *E. coli* at end points (2016).

A few locations such as Lal Pull, Mughal Pura, Sheranwala Gate, Dars Bary Mian, Paniwala Talab, Ichra, , Liberty Market, Ahmed Block, Pindi Rajputan, Akbari Gate, General Hospital, Dhobi Ghat, Fatah Garh, Paki Thati Salametpura Takia, Baghichi, Bilal Park, Mori Gate, Zafar Ali Road, Dhobi Mandi, Bagh Munshi Ladha, Al-Faisal Town Burji, Fateh Garh, Lahore College, Tajpura Scheme, Dry Port, Tajpura Ground, Karem Park, Old Ravi Well Centre, Janazgah, Shadman Nala, Shadman Market, Shalimar Garden, Bagh-Gul Begum, Windsor Park, Salamatpura No-5, F-and-V Market, Gulshan-i-Ravi B block, Akbar Shaheed Road, Pak Mint, Gwala-Colony Harbanspura, Green Town, Township, Nishter Colony, Gulshen Colony, Beghrian, D-Block Green Town and Chandni-Chowk Township where water was much more polluted at sample end points than water of sample tube wells. The prime cause of pollution at those sites was the mixing of waste water from sewage collecting pipelines to water supplying pipelines. There were also a few locations where *E.coli* was not detected in water samples of tube wells but it was detected in water samples of end points.

Figure 3 also highlights the post-monsoon patterns of *E.coli* in water of sample houses. An increase in *E.coli* colonies was also noticed in post-monsoon period due to favorable climatic conditions for their growth at all the above mentioned end points during pre-monsoon season. It was also identified that *E.coli* was not detected at many places in water samples of tube wells in post-monsoon season but it was detected in water samples of end points. The major reason of prevalence of *E.coli* in water samples of houses was the mixing of sewage and public water supply due to old pipe lines.

Figures 4-5 show the trend of *E.coli* in different seasons. The trend was shown by using ellipses of standard deviation. It is a common way for measuring direction of a data to compute standard distance in three dimensions.

It creates a new class of features that is containing an elliptical polygon which is centered on the mean centre for all the features of the field. Figures 4-5 with standard deviation ellipses are showing two standard deviation ellipses in each figure. The ellipse in yellow hue reflects the distributional trend of *E.coli* in pre-monsoon season. The ellipse with red outline reflects trend of *E.coli* in post-monsoon season.

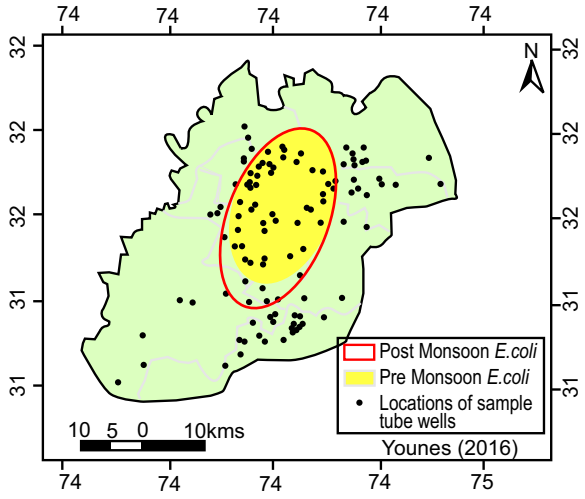


Fig. 4. Distributional trend of *E.coli* at tubewells

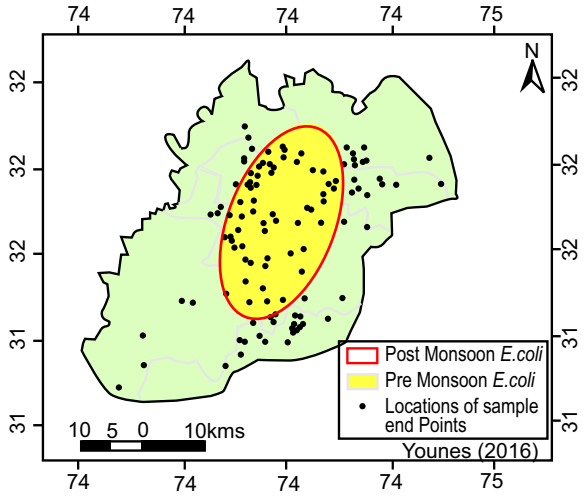


Fig. 5. Distributional trend of *E.coli* at end points

Figures 4-5 highlight the difference in distribution of *E.coli* in pre and post monsoon seasons in water samples of tube wells and end points. It is evident from Fig. 4 that *E.coli* were found in the centre of study area in pre-monsoon season in water of tube wells but in post-monsoon season its spread can be seen in southward direction.

Table 2 shows the correlation among diarrhoea and *E.coli* in pre and post monsoon season. It shows that there is a very strong and significant correlation among *E.coli* and diarrhoea during pre-monsoon season. It is also clear from Table 2 that the correlation between *E.coli* in post-monsoon season and diarrhoea is highly significant and shows the stronger relationship. Figure

Table 2: Correlation: diarrhoea, *E.coli*-Pre-monsoon, *E.coli*-Post monsoon

	Diarrhoea	E.coli-Pre-Monsoon		
E.coli-Pre-monsoon	0.863		0.000	
E.coli-Post monsoon	0.890	0.967	0.000	0.000

Cell Contents: Pearson correlation
P-Value

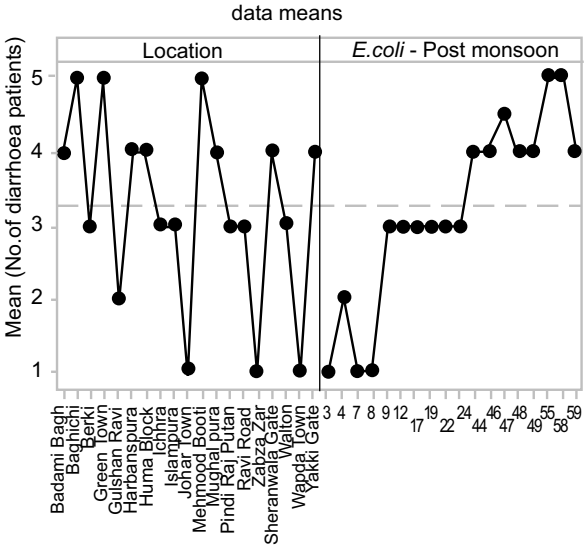


Fig. 6. Presence of *E.coli* and patients of diarrhoea

6 shows the presence of colonies of *E.coli* and patients of diarrhoea at different sample sites in study area. It is quite clear from the figure that the of patients of diarrhoea were large at those locations where numbers of colonies of *E.coli* were more.

Conclusion

Lahore is the second largest populated city of Pakistan. Most of the population of the city depends on public water supply system for drinking purpose. In this research it has been identified that *Escherichia coli* was detected in drinking water that is supplied by Public water supply system (WASA). It has also been pointed out that inhabitants of study area are suffering from diarrhoea caused by the presence of *E. coli* in drinking water. There were a few locations of end points where public water supply was more polluted with *E.coli* than water of tube wells. The major reason was the direct

addition of sewage from sewage pipes into water supplying pipes as both the pipes were very old and rusted. The regulatory authority WASA maintains and monitors the supply of public water in different parts of the city, yet it lacks proper implementation of rules as far as regular testing of water quality is concerned. It has also failed in changing pipes after regular interval through which public water is supplied.

Recommendations

- Industries need to treat their sewage on regular basis before disposing it off into River Ravi.
- In future, the depth of tube wells needs to be more than 700 feet to the east of the District.
- Regular testing of quality of water and water supplying lines need to be done by responsible authority.
- A broad maze of unlined waste water drains is found in Lahore that cause leakage of water from these drains into shallow unconfined aquifer which needs to be addressed at priority.

References

Agarwal, S.K. 2009. *Water Pollution*, APH Publishing Corporation, New Delhi, India.

Ahmad, N., Ahmad, M., Rafiq, M., Iqbal, N., Ali, M., Sajjad, M.I. 2011. Hydrological modeling of the Lahore aquifer using isotopic chemical and numerical techniques. *Science Vision*, **17:** 3-4.

EPA, 2015. Ground water contamination: Getting up to speed. Envoirmental Protection Agency, Ministry of Environment, Government of Pakistan.

Ghosh, G.K. 2008. *Environmental Pollution: A Scientific Dimension*, 260pp., APH Publishing Corporation, Darya Ganj, New Delhi, India.

Goel, P.K. 2011. Water Pollution: *Causes, Effects and Control*, 418pp., 2nd edition, New Age International Publications, India. ISBN 10: 8122418392

Gray, N.F. 2010. *Water Technology. An Introduction for Environmental Scientists and Engineers*, 3rd edition, Elsevier, London. UK.

Hamid, A., Yaqub, G., Sadiq, Z., Tahir, A., Noorulain. 2013. Intensive report on total analysis of drinking water quality in Lahor. *International Journal of Environmental Sciences*, **3:** 2161-2171

Nicholas, J. A. 2004. Microbial contamination of drinking water and disease outcomes in developing regions. *Toxicology*, **198:** 229-238.

Paul, B.K. 2011. Environmental Hazards and Disasters: Contexts, Perspectives and Management, 334pp., Wiley Publications, N.J., USA. ISBN: 978-0-470-66001-0.

Poff, B., Aregai, T. 2002. Bacteriological water quality analysis in Oak Creek Canyon, Arizona. *Water Research*, **3:** 431-436.

Prica, M., Dalmacija, B., Dalmacija, M., Agbaba, J., Krèmar, D., Trickoviæ J., Karlovic, E. 2010. Changes in metal availability during sediment oxidation and the correlation with immobilization potential. *Ecotoxicology and Environmental Safety*, **73:** 1370-1377.

Scott, L.M., Janikas, M.V. 2010. Spatial statistics in arc GIS, *Handbook of Applied Spatial Analysis*. pp. 27-41, Springer, Germany.

WHO, 1997. *Guidelines for Drinking Water Quality: Surveillance and Control of Community Supplies*, World Health Organization (WHO). vol. **3,** 2nd edition, Geneva, Switzerland.

WHO, 2000. *Global Water Supply and Sanitation Assessment 2000 Report*, 80 pp., World Health Organization, USA.

WHO, 2011. *Guidelines for Drinking-Water Quality*, 564 pp., 4th edition, World Health Organization, Geneva. Switzerland. ISBN 978 924 1548151

WHO, 2011. Facts and figures on water quality and health, *Water Sanitation and Health Publication* WHO, Geneva, Switzerland.

Wong, W.S.D., Lee, J. 2005. Statistical analysis of geographic information with ArcView GIS, 446 pp., Hoboken, Wiley, NJ, USA. ISBN 978-0471468998.

Pak. j. sci. ind. res. Ser. B: biol. sci. 2018 **61B**(3) 158-164

Population Status and Distribution of Himalayan Brown Bear (*Ursus arctos isabellinus*) in Musk Deer National Park Neelum, Azad Jammu and Kashmir (Pakistan)

Liaqat Ali Khan[a], Riaz Aziz Minhas[a], Muhammad Siddique Awan[a], Khawaja Basharat Ahmad[a], Nuzhat Shafi[a], Usman Ali[b]* and Naeem Iftikhar[c]

[a]Department of Zoology, University of Azad Jammu and Kashmir, Muzaffarabad, Pakistan
[b]Department of Zoology Mirpur University of Science and Technology (MUST), Mirpur, 10250 Azad Jammu and Kashmir, Pakistan
[c]Department of Wildlife and Fisheries, Government of Azad Jammu and Kashmir, Muzaffarabad, Pakistan

(received October 3, 2016; revised May 19, 2017; accepted June 22, 2017)

Abstract. The Himalayan brown bear (*Ursus arctos isabellinus*) is considered as 'Endangered' in Pakistan. However, a small population of this species still exists in northern Pakistan including Azad Jammu and Kashmir (AJK). A study was conducted to determine population status and distribution of Himalayan brown bear in Musk Deer National Park (MDNP), from April 2011 to September 2012. MDNP, covering an area of 528.16 km^2, is situated in the extreme north of AJ&K (upper Neelum Valley) about 155 km away from Muzaffarabad. Study area was divided into three zones (Phulawai, Sardari and Loser) and searched for brown bear signs and evidences. A total of 17 transect surveys were carried out to collect the data on current population status and distribution of Himalayan brown bear in the study area. In addition, questionnaires based surveys were carried out in the area to gather maximum information about this species. Based on direct and indirect signs collected, a total population of about 12 individuals with a population density of 0.42 bear/km^2 was estimated in the MDNP with maximum (0.45 bear/km^2) in Loser and minimum (0.37 bear/km^2) in Phulawai zone. Altitudinal preference was recorded highest (0.46 bear/km^2) at the elevation level of >3000 m asl. For the proper management and conservation of Himalayan brown bear, more comprehensive study should be carried out throughout its potential habitat.

Keywords: population, distribution, Himalayan brown bear, Musk Deer National Park, Azad Jammu and Kashmir

Introduction

The brown bear (*Ursus arctos isabellinus*) is distributed in most of the Europe, Asia, North America, Middle East, and some parts of North Africa (Swenson *et al*., 2000; Servheen *et al*., 1999). In Asia, brown bear founds along Himalayas (from Pakistan to Bhutan), Afghanistan, Turkey, Iran, Central Asian mountains, Mongolia to Russia, and northern China, (Fig. 1) (Nawaz, 2007; Sathyakumar, 2001). Himalayan brown bears distributed in Jammu and Kashmir, northern Indian states including Uttaranchal and Himachal Pradesh (Sathyakumar, 2006), while in China, poorly defined populations are scattered in the northeast and west regions (Gong and Harris, 2006). Comparatively, Japan has a dense population though reliable data are lacking (Mano, 2006). In Pakistan, brown bear found in 7 healthy populations in the mountain ranges of Himalaya, Karakoram, and Hindu Kush including Gilgit Baltistan, Azad Jammu and Kashmir, and Khyber Pakhtunkhwa (KPK) (Nawaz, 2007). Bear population confined in patchy distribution in alpine meadows and sub-alpine zone of Deosai Plateau, Khunjrab National Park, Nanga Parbat, and Astore (Virk *et al*., 2003), nevertheless in western Himalaya, only Deosai Plateau has the stable population (Nawaz, 2007). In KPK, this species is distributed in the Kalam (Kohistan), Pallas Valley (Indus Kohistan) Kaghan Valley, and Chitral (Nawaz, 2007; Akbar, 2003; Arshad, 2003; Roberts, 1997).

In Azad Kashmir, brown bear is restricted to northern region including Machiara National Park, Gumote National Park, Shonther Valley, and Kel areas (Nawaz, 2007; Iftikhar, 2006). The Gurez Valley, mainly Musk Deer National Park, has good habitat conditions and likewise dense bear population (Nawaz, 2007). Besides Neelum Valley, they may also be found in the Leepa Valley (Jhelum Valley) and Haji Pir (district Bagh) areas (Iftikhar, 2006). Although, there is a large area with a potential habitat in Neelum Valley, but brown bears are

*Author for correspondence;
E-mail: usman.zoology@must.edu.pk

restricted in certain pockets of the Valley, mainly in protected areas. Hunting pressure (Qamar *et al*., 2005), conflict with humans, habitat fragmentation (Nielsen *et al*., 2006; 2004) are important forces that reduce the bear population and thus distribution.

Various protected areas are established around the world that aim to support a viable population of brown bears, but only some of them are large enough to achieve this goal. Therefore, brown bear conservation must be integrated with many other human land-uses (Nielsen *et al*., 2006; Can and Togan, 2004; Herrero, 1994). Various countries established management guidelines intended to decrease human impacts on brown bears and their habitat, however many countries have limited or no bear management protocols and regulations (Zedrosser *et al*., 2001; Servheen *et al*., 1999). In Himalayan region, brown bear exist in low densities, their potential habitat range in India is estimated at 4,300 km^2 and very little of this range is protected (Sathyakumar, 2001).

Although, brown bears are globally considered as Least Concerned (McLellan *et al*., 2008), however, they face many threats in Pakistan which cause its population to decline continuously and considered as 'endangered' (Sheikh and Molur, 2004). The largest population in Pakistan is estimated at 43 individuals existed in Deosai National Park, other six populations have less than 20 bears separately (Nawaz, 2007). A total of 20-25 bears were estimated in the north-eastern part of Neelum Valley (Nawaz, 2007), which is connected to Deosai National Park via Dudgai Top.

Main threats to the brown bear are increase in human population and thus increase in livestock, fuel wood and ethno-plant extraction, illegal trade of pelt and fat of bear, and climate change (Nawaz, 2007; Sheikh and Molur, 2004). Present study was designed to investigate the current status and distribution of brown bear in Musk Deer National Park (upper Neelum Valley) of Azad Jammu and Kashmir, Pakistan.

Materials and Methods

Study area: Musk Deer National Park (MDNP) is situated in the extreme northern part of AJ&K (upper Neelum Valley) about 155 km away from Muzaffarabad. The area of Gurez Valley was declared as Musk Deer National Park in 2007 covering an area of 528.16 km^2 (Sharda Forest Division) from Macchal to Kamri top. The park is bounded to the east and north east by occupied Kashmir through Line of Control (LoC) to the west and north west by Gilgit Baltistan (GB). Study area is geographically linked with Deosai National Park in GB (Fig. 1). Study area was divided in three zones i.e. Phulawai, Sardari and Loser based on geographic division in sub-valleys. Study zone Phulawai was further divided in study localities including Doga (34° 48.70N, 74° 29.16E), Saonarr (34° 46.59N, 74° 36.85E) and Hanthi (34° 47.46N, 74° 29.12E). Sardari zone, was sub-divided in three localities, Helmet (34° 45.86N, 74° 31.75E), Taobut (34° 43.27N, 74° 53.27E) and Karimabad (34° 44.21N, 74° 55.49E), while Loser zone has four study localities such as Gagai (34° 43.58N, 74° 52.26E), Rata Pani (34° 43.29N, 74° 56.49E), Dudhegai (34° 42.69N, 74° 46.95E) and Qamri (34° 40.66N, 74° 50.12E) (Fig. 1).

The study was conducted in MDNP, Gurez Valley, District Neelum, Azad Jammu and Kashmir (AJK) from April 2011 to December 2012. Line transect walks of varying length and width were carried out in 22 stands covering about 28.75 km^2 area following Nawaz (2007) to gather data on distribution and population status of brown bear. 2-3 members of survey team traversed area parallel to each other by keeping a distance of 10 to 100 m apart, depending upon the terrain of the study area. Length of transect was measured using Garmin etrex (30x) GPS device, while average width was measured using Laser Range Finder Monocular Scan (TAC Vactor Optics; 8x30; 1200 m). Population in a particular locality was estimated through measuring direct signs (fecal dropping, foot print, food remains, and sites of livestock depredation) and indirect reports of local peoples, hunters and shepherds. Based on the average daily home range/traveling distance (average 2.3 km for the daytime and 1.7 km for night) and daily activeness (6 h 40 min for day; 4 h 20 min; 4 h 30 min for night) of the animal as given by the Gavrilov (2015), all evidences including direct and indirect within ~4 km area (average 24 h travelling range) were transformed into one animal (the least count), with exceptions where based on different foot prints (e.g., one small one large etc.) two animals were considered.

Results and Discussion

Distribution. Data revealed that Himalayan brown bear was distributed in different localities of Musk Deer National Park (MDNP). The direct and indirect evidences of bear were frequently found in all three zones of the study area (Phulawai, Sardari and Loser) (Table 1).

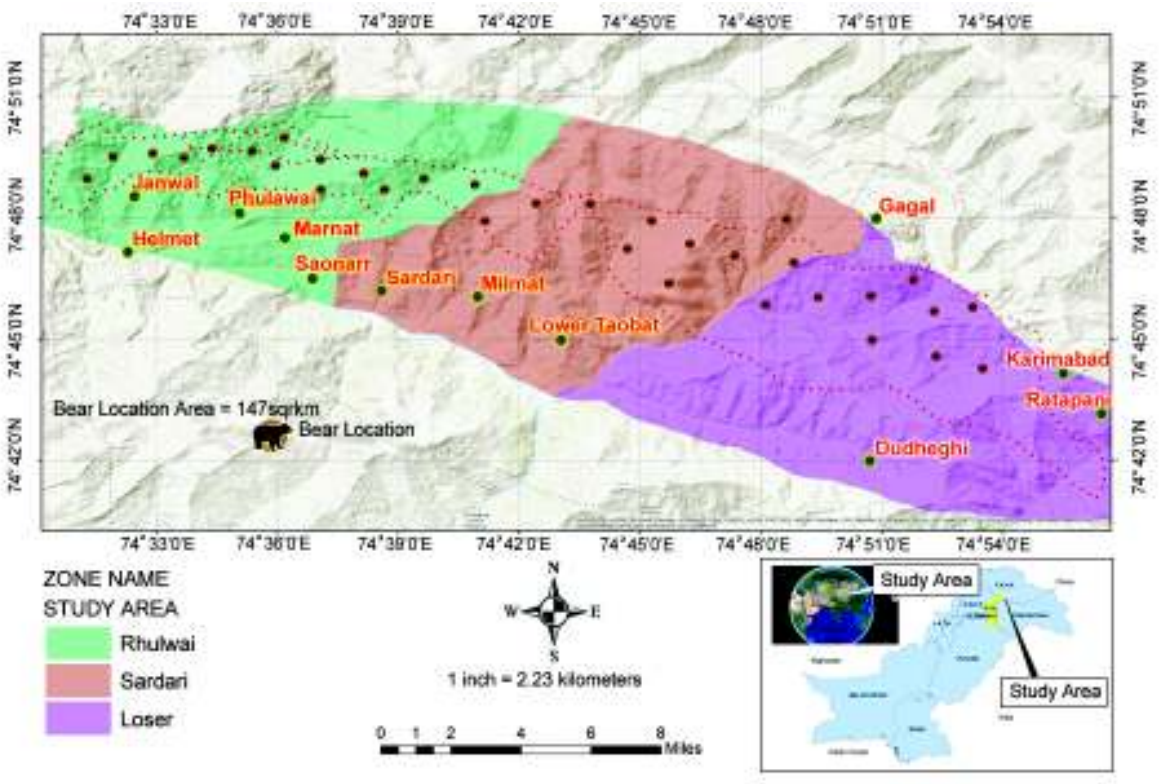


Fig. 1. Location map of study area (MDNP) showing different study zones.

Maximum bear population (n=5) was recorded in Losar zone, that is connected to Deosai National Park. Among three Zones, the maximum bear evidences (n=20) were found at Loser followed by Sardari (n=18) and Phulawai (n=12) (Table 1). Earlier studies conducted in Neelum Valley confirmed the distribution of brown bear in Gumote, Shonther, Gurez and valleys (Nawaz, 2007; Iftikhar, 2006; Qamar *et al*., 2005). The Gurez Valley branching off at Kel, having MDNP on the both sides of River Neelum, particularly has potential habitat of brown bear (Nawaz, 2007; Qamar *et al*., 2005), though, poaching and other human activities confined brown bear to certain area of the Valley (Qamar *et al*., 2005).

Population density. A total population of about 12 animals estimated in 17 field surveys was distributed over an area of 28.60 km^2 in different localities of MDNP. Population density of the study area was recorded as 0.42 bear/km^2 (Table 2), maximum (0.45 bear/km^2) at Loser zone followed by Sardari (0.43 bear/km^2) while least (0.37 bear/km^2) population density noted at Phulawai zone (Table 1).

Loser Zone has high population due to its potential habitat features. Most of the area consists upon a mixture of steep and gentle slopes, covered with a plenty of vegetation and far from human settlement. Vegetation cover not only provide hide to the bear but also has a plenty of food. Highest population density (0.63 bear/km^2) was recorded at Qamri locality of Loser area. This locality has direct connection to Deosai National Park through Qamri Top and there are strong evidences that brown bear is frequently visiting both sides of the Qamri Top. Fecal droppings (n=6), foot prints (n=5) and ground scratching (n=5) were recorded in this locality (Table 1). Population density estimated based on direct evidences, indirect evidences were asked to confirm field observations. Direct evidences confined to different localities provide a complete picture on population estimation because brown bear remain active more than 40% in a day period, traveling 4 km per day in average. Speed is varying in different habitats and maximum speed is up to 6.5 km/h in the grasslands to 0.3 km/h in scrubland (Gavrilov, 2015). In forests, the average speed of bear recorded as 0.7 km/h (Gavrilov, 2015).

Altitudinal variation was also noted in bear population in the study area. Altitude of the study area was ranged between 2200 m and 3800 m asl, which was divided in three classes. Class I has altitude of less than 2500 m, Class II ranged between 2500 m to 3000 m while Class III has an altitude of >3000 m asl. Overall highest population density (0.46 bear/km^2) was recorded in Class III, followed by Class I (0.40 bear/km^2) while least (0.39 bear/km^2) was noted in Class II (Fig. 2). In neighboring region i.e., India, the potential distribution range of Himalayan brown bear is about 36,800 km^2 in the sub-alpine and alpine regions between 3000-5000 m in the Himalayas and Trans-Himalayan regions (Rathore, 2008). Himalayan brown bear preferred alpine

Table 1. Comparison of Himalayan brown bear population in different localities of MDNP during study period

Zone	Locality	Mean elevation	Area surveyed (km^2)	Habitat conditions	Evidence		Estimated population	Population density (bear/km^2)
					Direct	Indirect		
Phulawai	Doga	2480	2.9	Gentle slopes usually thickly vegetated with *Pinus* spp., *Taxus wallichiana*, *Arnebia benthamii*, *Valeriana jatamansi*, *Berberis lycium*. Agricultural activities, including grass cutting, grazing etc are observed in this locality.	FD=4, FP=1, GS=2	WW=2 Sh=2, LP=1	1	0.34
	Saonarr	2490	2.1	Thick vegetation of *Pinus wallichiana*, *Pinus roxburghii*, *Cedrus deodara*, *Saussurea lappa* at lower altitude while *Berberis lycium* and *Betula utilis* at higher gentle slopes. Grazing is common along with terrace cultivation.	FD=2	WW=2. Sh=2	1	0.48
	Hanthi	2540	3.2	Herbal blend includes *Aconitum heterophyllum*, *Angelica cyclocarpa*, *Podophyllum hexandrum* at higher slopes while frequent terrace cultivation noted on lower areas. *Betula utilis*, *Pinus wallichiana*, and *Pinus roxburghii* are important tree species.	FD=4	WW=3, Sh=2	1	0.31
	Total		8.2				3	0.37
Sardari	Helmet	2590	3.9	Gentle slopes covered with evergreen forests of *Pinus wallichiana* and *Pinus roxburghii*. Cultivation observed on both sides of river Neelum, upper altitudes has grazing impacts.	FD=8	WW=3, Sh=2, LP=1	2	0.51
	Karim-abad	2530	2.6	Gentle and steep slopes covered with *Pinus* spp. Along with *Betula utilis*, *Saussurea lappa*, *Aconitum heterophyllum* and *Berberis lycium* at upper reaches.	FD=1	WW=3 Sh=4, LP=2	1	0.38
	Taobut	3280	2.8	Dense Pinus vegetation covered gentle slopes of area where grazing and cultivation activities are prominent.	FD=4, GS=2	WW=3, Sh=5, LP=3	1	0.36
	Total		9.3				4	0.43
Loser	Gagai	3330	2.1	Area having blend of Alpine and Sub alpine, *Taxus wallichiana*, *Arnebia benthamii*, *Valeriana jatamansi*, *Berberis lycium* and *Betula utilis* are important species. Upper reaches are under heavy livestock grazing pressure, both by local and nomads.	FD=6, FP=10, FR=3, GS=6	WW=2, Sh, =5, LP=3	1	0.48
	Ratta Pani	2990	3	Steep slopes having main vegetation of *Taxus wallichiana*, *Valeriana jatamansi*, *Arnebia benthamii*, *Aconitum heterophyllum*, *Berberis lycium* and *Betula utilis*	FD=9, FP=7, GS=4	WW=4, Sh=5, LP=4	1	0.33
	Dudhegai	3450	2.8	Gentle slopes charactrized by dense covering of *Taxus wallichiana*, *Arnebia benthamii*, *Valeriana jatamansi*, *Berberis lycium* and *Betula utilis*. Seasonal heavy livestock grazing pressure is noted.	FD=5, FP=2, GS=2	WW=5, Sh=7, LP=4	1	0.36
	Qamri	3380	3.2	Both steep and gentle slopes covered with mixed alpine grasses and sub-alpine scrub. Area grazed heavily by nomad livestock at spring and autumn season during their travel to MDNP and vice versa.	FD=6, FP=5, GS=5, FR=6	WW=6, Sh=8, LP=4	2	0.63
	Total		11.1				5	0.45

FD = fecal droppings; FP = foot prints; FR = food remains; GS = ground scratching; WW = wildlife watcher; Sh = shepherd; LP = local people.

meadows and subalpine scrub mostly. However, it may descend down in search of food and shelter in different seasons and circumstances. Results of present study corresponded with previous literature, such as Nawaz (2007), who reported 10-15 individuals in the same area. Most of this population was found distributed in the extreme northern portion of the study area, which is in the proximity of the Deosai National Park. Based on the findings of the present study and information collected earlier, it is inferred that these bears are still present and their population is almost stable in the area.

Among the three zones of the study area, the majority (42%) of the bear population was found in Loser Zone (n=5) followed by Sardari Zone (n=4, 33%) and Phulawai Zone (n=3, 25%). Losar and Sardari Zones are located on the northern part of the national park, bordering the Northern areas and the Indian-held Kashmir. These areas are potential habitats of brown bear outside the study area and bear movements are usually reported between the study area and these adjoining areas. Besides, these zones of the park have sparsely populated human settlements, with less disturbance for bear population. Population density of these bears varies with respect to habitats and favorable habitats have high population density (Seryodkin, 2006; Miller *et al*., 1997). They face high hunting pressure because of the medicinal value of body parts and fat (Qamar *et al*., 2005). Fragmentation enhances highly risk of mortality of brown bears because low home range and food coerce bear toward human settlements and intensify its conflict (Nielsen *et al*., 2006; 2004).

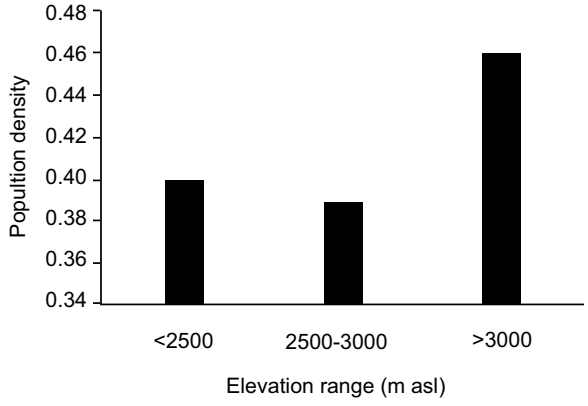


Fig. 2. Estimated populations of Brown bear at differen t elevation ranges in MDNP during 2011-2012.

Population growth of such isolated and undersized populations are adversely affected even small numbers of members eliminated (Wakkinen and Kasworm, 2004); on the contrary, avoiding just a few deaths possibly halts a population decline (Garshelis *et al*., 2005; Wiegand *et al*., 1998).

Habitat fragmentation roots a great demographic and genetic risk to isolated populations (Proctor *et al*., 2004). Increasing human populations multiply this risk and speeds up the rate of habitat degradation in their vicinities (Nawaz, 2007; Can and Togan, 2004). However, if protection is provided, small populations could successfully be recuperated (USFWS, 2005). Reintroduction is an important tool that has restored numbers and geographic range in numerous locations in the U.S. and Western Europe (Clark *et al*., 2002; Servheen *et al*., 1994).

The reasons that the population of brown bears in the area could not increase in the area is more probably due to the huge human settlements. Rapid increase in human population, number of settlements and utilization of natural resources are the major contributing factors which adversely affect the brown bear population (Nawaz, 2007). These activities such as grazing, agriculture, fuel wood collection, hydroelectric developments etc. are well reported in literature (Waller and Servheen, 2005; Proctor *et al*., 2005; 2004). Various studies including Ali *et al*. (2016); Qamar *et al*. (2012); Qamar *et al*. (2010) and Ali *et al*. (2007) reported that potential habitats are disturbed by human being and over grazing, timber and fuel wood extraction, illegal collection of medicinal plants and illegal hunting are the major issues of the Neelum Valley. High demand of its fat for medicinal use is the severe risk to these animals in the area (Qamar *et al*., 2005).

Conclusion

In conclusion, Musk Deer National Parks harbors 12 Himalayan brown bears with a mean population density of 0.42 bear/km^2. Study area has potential bear habitat characterizing different vegetation cover and ecological attributes. Bear population preferred higher altitudes (>3000 m asl). North-eastern boundary of MDNP is connected with Deosai National Park. Effective conservation efforts could support bear population up to self-sustaining level hence human interference should be reduced to maximum level in MDNP. This is an incipient study, and many of the scientific aspects of this precious threatened species are yet to be explored in the Neelum

Valley. It is recommended that genetic diversity of brown bear could be investigated, that gives us the roots of Neelum Valley bear population. It is also suggested that the effects of illegal hunting (particularly intensity in chronological order) and habitat fragmentation may be studied.

References

Akbar, G. 2003. Zonification for the Valley Catchments of Bar Palas with Zone Specific Management Prescriptions. Palas Conservation and Development Project. WWF-Pakistan.

Ali, U., Minhas, R.A., Awan, M.S., Ahmed, K.B., Qamar, Z.Q., Dar, N.I. 2016. Human-grey wolf (Canis lupus Linnaeus, 1758) conflict in Shounther Valley, District Neelum, Azad Jammu and Kashmir, Pakistan. *Pakistan Journal of Zoology*, **48:** 861-868.

Ali, U., Ahmed, K.B. Awan, M.S., Ashraf, S., Basher, M., Awan, M.N. 2007. Current distribution and status of Himalayan ibex in upper Neelum Valley, District Neelum, Azad Jammu and Kashmir, Pakistan. *Pakistan Journal of Biological Science*, **10:** 3150-3153.

Arshad, M. 2003. Review of approaches to species conservation in Pakistan. Palas Conservation and Development Project. WWF-Pakistan.

Can, Ö.E., Togan, I. 2004. Status and management of brown bears in Turkey. *Ursus*, **15:** 48-53.

Clark, J.D., Huber, D., Servheen, C. 2002. Reintroducing bears: lessons and challenges. *Ursus*, **13:** 153-163.

Garshelis, D.L., Gibeau, M.L., Herrero, S. 2005. Grizzly bear demographics in and around Banff National Park and Kananaskis Country, Alberta. *Journal of Wildlife Management*, **69:** 277-297.

Gavrilov, G. 2015. Movement and activity pattern of a brown bear (*Ursus arctos* L.) tracked in Central Balkan Mountain, Bulgaria. *ZooNotes*, **70:** 1-4.

Gong, J., Harris, R. 2006. The status of bears in China. In: *Understanding Asian Bears to Secure their Future*. pp. 96-101, Japan Bear Network, Ibaraki, Japan.

Herrero, S. 1994. The Canadian national parks and grizzly bear ecosystems: the need for interagency management. *International Conference on Bear Research and Management*, **9:** 7-21.

Iftikhar, N. 2006. *Wildlife of Azad Jammu and Kashmir.* 69 pp., Al-Sheikh Press, Muzaffarabad, Azad Jammu and Kashmir, Pakistan.

Mano, T. 2006. The status of brown bears in Japan. In: *Understanding Asian Bears to Secure their Future*. Mano, T. (ed.), pp. 111-121, Japan Bear Network, Ibaraki, Japan.

McLellan, B.N., Servheen, C., Huber, D. 2008. Ursus arctos. In: *IUCN Red List of Threatened Species*, IUCN 2011, Version 2011.2. <www.iucnredlist.org>. Downloaded on 13 June 2015.

Miller, S.D., White, G.C., Sellers, R.A., Reynolds, H.V., Schoen, J.W., Titus, K., Barnes, V.G., Smith, J.R.B., Nelson, R.R., Ballard, W., Schwartz, C.C. 1997. Brown and black bear density estimation in Alaska using radio telemetry and replicated marker sight techniques. *Wildlife Monographs*, 133 pp.

Nawaz, M.A. 2007. Status of the brown bear in Pakistan. *Ursus*, **18:** 89-100.

Nielsen, S.E., Stenhouse, G.B., Boyce, M.S. 2006. A habitat-based framework for grizzly bear conservation in Alberta. *Biological Conservation*, **130:** 217-229.

Nielsen, S.E., Herrero, S., Boyce, M.S., Mace, R.D., Benn, B., Gibeau, M.L., Jevons, S. 2004. Modeling the spatial distribution of human-caused grizzly bear 12 mortalities in the Central Rockies ecosystem of Canada. *Biological Conservation*, **120:** 101-113.

Proctor, M.F., McLellan, B.N., Strobeck, C., Barclay, R.M.R. 2005. Genetic analysis reveals demographic fragmentation of grizzly bears yielding vulnerably small populations. *Proceedings of the Royal Society*, **272:** 2409-2416.

Proctor, M.F., Servheen, C., Miller, S.D., Kasworm, W.F., Wakkinen, W. L. 2004. A comparative analysis of management options for grizzly bear conservation in the U.S.-Canada trans-border area. *Ursus*, **15:** 145-160.

Qamar, Z.Q., Ali, U., Minhas, R.A., Dar, N.I., Anwar, M. 2012. New distribution information on woolly flying squirrel (*Eupetaurus cinereus* Thomas, 1888) in Neelum Valley of Azad Jammu and Kashmir, Pakistan. *Pakistan Journal of Zoology*, **44:** 1333-1342.

Qamar, Z.Q., Anwar, M., Dar, N.I., Ali, U. 2010. Ethnobotanical study of wild medicinal plants of Neelum Valley, Azad Jammu and Kashmir, Pakistan. *Pakistan Journal of Wildlife*, **1:** 25-30.

Qamar, Z.Q., Awan, M.S., Maqsood, A., Shahid, M. 2005. Status of wild species and their management in Ghomat Game Reserve, District Muzaffarabad. *Journal of Natural Science*, **3-4:** 100-108.

Rathore, B.C. 2008. Ecology of brown bear (*Ursus arctos*) with Special Reference to Assessment of Human-brown bear Conflicts in Kugti Wildlife Sanctuary, Himachal Pradesh and Mitigation Strategies. *Ph.D. Thesis*, Saurashtra University.

Roberts, T.J. 1997. *The Mammals of Pakistan* (Revised edition), pp. 1-525, Oxford University Press Karachi, Pakistan.

Sathyakumar, S. 2006. The status of brown bears in India. In: *Understanding Asian Bears to Secure their Future*. pp. 7-11, Japan Bear Network, Ibaraki, Japan.

Sathyakumar, S. 2001. Status and management of Asiatic black bear and Himalayan brown bear in India. *Ursus*, **12:** 21-30.

Servheen, C., Herrero, S., Peyton, B. 1999. Bears. Status survey and conservation action plan. IUCN/SSC Bear and Polar Bear Specialist Groups. IUCN, Gland, Switzerland and Cambridge, UK.

Servheen, C.W., Kasworm, W.F., Their, T. 1994. Transplanting grizzly bears as a management tool: Results from the Cabinet Mountains, Montana. *Biological Conservation*, **71:** 261-268.

Seryodkin, I. 2006. The biology and conservation status of brown bears in the Russian Far East. In: *Understanding Asian Bears to Secure their Future*. pp. 79-85, Japan Bear Network, Ibaraki, Japan.

Sheikh, K.M., Molur, S. (eds.). 2004. *Status and Red List of Pakistan's Mammals. Based on the Conservation Assessment and Management Plan*. IUCN Pakistan, 312 pp.

Swenson, J.N., Gerstl, B.D., Zedrosser, A. 2000. *Action Plan for the Conservation of the Brown Bear in Europe (Ursus arctos)*. Council of Europe, Strasbourg, France.

USFWS, 2005. Endangered and threatened wildlife and plants; Designating the greater Yellowstone ecosystem population of grizzly bears as a distinct population segment; removing the Yellowstone distinct population segment of grizzly 13 bears from the federal list of endangered and threatened wildlife. U.S. Fish and Wildlife Service. *United States Federal Register*, **70:** 69854-69884.

Virk, D.A.T., Sheikh, D.M.K., Marwat, A.H. 2003. *NASSD Background Paper: Biodiversity*. IUCN Pakistan, Northern Areas Progamme, Gilgit. x+74 pp.

Wakkinen, W.L., Kasworm, W.F. 2004. Demographics and population trends of grizzly bears in the Cabinet–Yaak and Selkirk Ecosystems of British Columbia, Idaho, Montana, and Washington. *Ursus*, **15:** 65-75.

Waller, J.S., Servheen, C. 2005. Effects of transportation infrastructure on grizzly bears in Northwestern Montana. *Journal of Wildlife Management*, **69:** 985-1000.

Wiegand, T., Naves, J., Stephan, T., Fernandez, A. 1998. Assessing the risk of extinction for the brown bear (*Ursus arctos*) in the Cordillera Cantabrica, Spain. *Ecological Monographs*, **68:** 539-571.

Zedrosser, A.B., Dahle, J.E., Swenson, J.N., Gerstl, N. 2001. Status and management of the brown bear in Europe. *Ursus*, **12:** 9-20.

Pak. j. sci. ind. res. Ser. B: biol. sci. 2018 **61B**(3) 165-171

Repellent Responses of Maize Weevil, *Sitophilus zeamais* Motsch (Coleoptera:Curculionidae) towards Entomocidal Plant Products

Riaz-ud-din[a], Muhammad Mamoon-ur-Rashid[a] and Muhammad Mansoor[b]*
[a]Department of Entomology, Gomal University, Dera Ismail Khan, Pakistan
[b]Arid Zone Research Centre (PARC), Dera Ismail Khan, Pakistan

(received August 31, 2016; revised. March 30, 2017; accepted August 24, 2017)

Abstract. Laboratory studies were conducted to investigate the repellency effect of six plant species (*Azadirachta indica, Caralluma fimbriata, Allium sativum, Curcuma longa, Citrullus colocynthis* and *Calotropis procera*) against *Sitophilus zeamais* reared on maize grains (Cv. Azam White) in the Laboratory of Entomology Department, Gomal University, Dera Ismail Khan, Pakistan. Six concentrations viz. 5000, 10000, 15000, 20000, 25000 and 30000 ppm of each plant powder were applied to 20 g of sterilized maize grains under constant conditions of 27 ± 1°C and 65 ± 5% relative humidity. Twenty newly emerged adult maize weevil were introduced into glass petri dishes and percent repellency of plant powders was determined. *A. indica* seed powder at 30,000 concentration showed 100% repellency against maize weevil followed by *C. longa* (76%) after 72 h exposure period whereas *C. procera* was found least effective showing only 39% repellency of the test insects compared to control. The powders of *A. sativum*, *C. fimbriata* were found moderately repellent against the test insects. During the observations, it was also noted that repellency of the tested plant powders was dose dependent, the higher the concentration of the tested powders, the higher was the repellency and vice versa. The findings of this study indicated that the selected plant products could be used for a safer control of maize weevil.

Keywords: plant powders, insect repellency, *Sitophilus zeamais,* maize grain

Introduction

Maize weevil (*Sitophilus zeamais*) (Motschulsky) (Coleoptera:Curculionidae), is one of the most damaging pests of stored cereals (Nakakita *et al.,* 1991). Whole grains are attacked by weevil adults and larvae inscrutably feed and develop within the grains (Ileleji *et al.,* 2007). Infestation by this weevil commences in the field (Ileke *et al.*, 2014.) but mostly damage occurs during storage. Damaged grains resulted reduction in germination, weight, nutritional and commercial values (Yuya *et al.,* 2009).

In recent decades, the use of synthetic insecticides has gained paramount importance as a means of controlling such insect pests. Nevertheless, its promiscuous usage as preservative is being discouraged due to a range of adverse effects. Various biological, environmental, and economic consequences associated with its usage are bringing them into disrepute (Park *et al*., 2003). According to Nishi *et al.* (2004), methyl bromide fumigation has been officially forbidden in developed countries since 2005 and was banned in developing countries in 2015 because it encounters human health and destroys ozone. The need for an alternative and effective preservative during storage is now essential. The use of botanical base insecticides is one of the several methods being given attention. Plant materials have played a major role in search towards controlling insect pest of the farm.

Stored product insects like most phytophagous insects use chemical cues (semiochemical) to find hosts. However, certain plants have evolved counter strategies as part of their defensive mechanism against insects. One such defense is the emission of repellent or deterrent volatile organic compounds. Natural chemicals that are easily bio-degradable, effective and non-toxic to human could be exploited for protection of stored grains from insect damage (Donald *et al.,* 2010). The use of natural products is more prevalent in the control of insect pests in storage systems. Farmers can grow them and they can also be locally available, cheaper and easier to use than the synthetic insecticides (Govindann *et al.,* 2010). Udo (2005) pointed that poor farmers in developing countries use different plant materials to protect grains against pest infestation by mixing grains with protectants made up of plant products. Many plant powders were evaluated and found effective in the management of *Sitophilus zeamais* attacking maize grains in the stores (Suleiman *et al.,* 2011; Danjumma *et al.,* 2009). This

*Author for correspondence; E-mail: joyadkpk@goolemail.com

study describes laboratory investigations to evaluate the efficacies of local plant powders viz. *Azadirachta indica, Caralluma fimbriata, Allium sativum, Curcuma longa, Citrullus colocynthis* and *Calotropis procera* in modifying the behavior of maize weevil in storage condition. The outcome of this study can prove to be a mile stone for development of environmental friendly, sustainable and economically viable option for control of this obnoxious insect.

Materials and Methods

Insect cultures. Maize seeds (Cv. Azam White), plastic jars (5L), muslin cloth, funnel and mesh sieves used to culture corn weevils were thoroughly cleaned. Maize grains having 12-14% moisture content (MC) were used to culture the insects. Five hundred grams maize grains were placed in each glass jar. Initial culture of *S. zeamais* was obtained from the laboratory of Entomology Section, Agricultural Research Institute, Dera Ismail Khan, Pakistan for further multiplication. The maize grains were sterilized by using a Gallenkamp oven at 60 °C for three hours to remove the chances of previous infestation in the grains (Isah *et al*., 2012). Insect culture was raised in the laboratory maintained at controlled temperature of 27 ± 3 °C and 65 ± 5% relative humidity with 12:12 hour day length (L:D). Mixture of two hundred, one week old male and female, adult maize weevils were introduced in each jar. After introduction of the insects, the top of the jars was covered with muslin cloth and tighten by rubber band in order to prevent the insects from escaping and to allow exchange of gases in and out of the jars. The jars were then placed in an incubator at controlled temperature for ten days. After ten days the parent insects were removed through sieving and introduced to another jars in order to multiply the culture of the insects. The jars containing infested maize grains were left undisturbed for twenty days. Emerging adult insects were collected and were kept in separate jars according to their age. Adults that emerged on same day were considered of the same age and were used for the experimental purpose.

Plant powder preparation. The plant materials were collected from the local farmers and brought to laboratory. The collected plant materials were thoroughly washed with tap water to clean the dust and dirt. The plant material of *Azadirachta indica, Caralluma fimbriata, Allium sativum, Curcuma longa, Citrullus colocynthis* and *Calotropis procera*, was dried in an oven at 45 °C temperature until reaching constant weight. Later, it was pulverized in an electric mill and was sieved to have a fine and homogenous powder (Table 1). Powders were then stored at room temperature in nylon bags after due tagging (Sayonara *et al.,* 2009).

Repellent response of plant materials against maize weevil. The repellent effect of all the plant materials used against maize weevil was evaluated using the area preference method. Six concentrations 5000ppm, 10000ppm, 15000ppm, 20000ppm, 25000ppm and 30000ppm of powder were prepared from the stock solution by using calculated amount of distilled water following the standard method described by Musabyimana *et al.* (2001). Whatman No.1 filter paper was cut into two equal halves (8 cm), one half of each filter paper was treated with plant materials as uniform as possible by using micropipette and the other half of the filter paper was treated with distilled water and used as a control. The plant material treated and water treated filter paper halves were then air dried for 30 min to get solvent evaporated completely. Then these two halves (plant materials treated and water treated) were attached length wise, edge-to-edge with adhesive tape and were placed at the bottom in glass petri dish having 16 cm diameter. Ten pairs of newly emerged weevils were released at the centre of the glass petri dishes and were offered with a choice of dispersing on to either treated or untreated maize grains. The petri dishes were then subsequently covered and kept in an incubator at 27± 1 °C and 65±5% relative humidity. The experiment was laid out in a completely randomized design having 5 repeats. The number of insects settled on treated and untreated halves were counted after 1, 2, 3, 6, 24, 48 and 72 h, respectively.

Percent repellency (PR) was calculated as follows:
PR = [(Nc-Nt)/Nc] 100%

Table 1. Detail of plant materials evaluated for insecticidal activities against *S. zeamais*.

Common names	Technical names	Parts used
Neem	*Azadirachta indica*	Seed
Succulent cactus	*Caralluma fimbriata*	Fruits
Garlic	*Allium sativum*	Bulbs
Turmeric	*Curcuma longa*	Rhizomes
Bitter Apple	*Citrullus colocynthis*	Fruit
Aak	*Calotropis procera*	Leaves

where:

Nc= Number of insects present in control
Nt= Number of insects present in treated filter paper

Statistical analysis. The recorded data were subjected to analysis of variance (ANOVA) and means were separated by applying the Least Significant Difference (LSD) test at 5% probability level. All statistical analyses were carried out using computer software STATISTIX version 8.1.

Results and Discussion

Repellent response of plant materials against maize weevil. The settling response of *S. zeamais* differed significantly (P<0.05) under different treatments (Table 2-8). The adults of *S. zeamais* preferred the untreated maize grains as compared to treated grains and settled significantly more on untreated grains. The settling response of the test insect decreased significantly (P<0.05) with the increase in the concentrations of tested plant powders.

Among the tested powders, *Azadirachta indica* seed powder was found more effective as compared to other treatments, whereas; *Calotropis procera* and *Citrullus colocynthis* were found least effective (Table 2-8). *A. indica* seed powder at highest concentration repelled 100% test insects followed by *C. longa* (76%) after 72 h exposure period from the treated grains whereas; *C. procera* was found least effective showing only 39% repellency of the test insect. The extracts of *A. sativum* also produced good results at all the evaluated concentrations compared to control. From the results obtained it is clear that all the tested plant powders have significant (p<0.05) effect on the repellency of adult *S. zeamais*. Arannilewa *et al.* (2006) reported that 1.5 g of *A. sativum* applied to 25 g of maize grains caused mortality of 85% in adult *S. Zeamais,* 14 days after application. Similarly, Sayonara *et al.* (2009) concluded that the powders from the stalks, seeds, and leaves of the plant 49-1-XIV, applied as powders at 1% concentration have anti-insect effects against *S. zeamais* showing mortality, decrease of the emergence of adults,

Table 2. Mean percent (± SE) repellency of *S. zeamais* on maize grains treated with different concentrations of plant powders after 1 hour exposure period

	Concentrations (ppm)					
Treatments	5000	10000	15000	20000	25000	30000
Azadirachta indica	64.00 ± 4.18 a	66.00 ± 6.52 a	65.00 ± 4.18 a	67.00 ± 5.70 a	76.00 ± 2.23 a	80.00 ± 3.54 a
Caralluma fimbriata	25.12 ± 5.70 d	31.11 ± 6.52 d	32.45 ± 6.52 c	27.00 ± 6.52 c	32.00 ± 7.58 d	32.00 ± 4.18 d
Allium sativum	39.00 ± 5.70 c	44.00 ± 4.47 c	51.00 ± 3.54 b	52.00 ± 7.91 b	56.00 ± 6.52 c	57.00 ± 5.00 c
Curcuma longa	51.00 ± 2.24 b	56.00 ± 2.24 b	60.00 ± 2.74 a	63.00 ± 2.74 a	66.00 ± 2.24 b	68.00 ± 2.74 b
Citrullus colocynthis	25.00 ± 5.00 d	23.00 ± 2.74 e	29.00 ± 2.24 c	33.00 ± 2.74 c	33.00 ± 2.74 d	35.00 ± 3.54 d
Calotropis procera	17.00 ± 2.74 e	15.00 ± 3.54 f	20.00 ± 2.74 d	21.00 ± 2.24 d	26.00 ± 2.24 e	31.00 ± 4.18 d
LSD Value	5.83	5.77	8.08	6.68	5.89	5.46

Each value is a mean ± standard error of five replications. Means followed by the same letters along the column are not significantly different at (P>0.05) using LSD Test.

Table 3. Mean percent (± SE) repellency of *S. zeamais* on maize grains treated with different concentrations of plant powders after 2 hours exposure period

	Concentrations (ppm)					
Treatments	5000	10000	15000	20000	25000	30000
Azadirachta indica	65.00 ± 5.00 a	69.00 ± 6.52 a	68.00 ± 3.54 a	69.00 ± 2.23 a	80.00 ± 3.54 a	80.00 ±3.54 a
~~*Caralluma fimbriata*~~	~~29.00 ± 3.54 cd~~	~~30.00 ± 4.18 d~~	~~33.00 ± 7.07 c~~	~~35.00 ± 4.18 c~~	~~35.00 ± 2.24 d~~	~~37.00 ± 5.70 d~~
Allium sativum	40.00 ± 4.47 c	44.00 ± 5.70 c	51.00 ± 7.91 b	52.00 ± 5.70 b	58.00 ± 5.70 c	59.00 ± 5.70 c
Curcuma longa	51.00 ± 4.18 b	58.00 ± 5.70 b	63.00 ± 7.91 a	63.00 ± 7.58 a	68.00 ± 9.75 b	69.00 ± 6.52 b
Citrullus colocynthis	26.00 ± 7.42 de	28.00 ± 5.70 d	28.00 ± 4.18 cd	33.00 ± 5.70 c	38.00 ± 5.70 d	36.00 ± 4.18 d
Calotropis procera	18.00 ± 5.70 e	17.00 ± 5.70 e	23.00 ± 6.12 d	21.00 ± 6.52 d	27.00 ± 5.70 e	33.00 ± 2.74 d
LSD Value	10.04	5.77	6.47	9.23	7.72	6.13

Each value is a mean ± standard error of five replications. Means followed by the same letters along the column are not significantly different at (P>0.05) using LSD Test.

Table 4. Mean percent (± SE) repellency of *S. zeamais* on maize grains treated with different concentrations of plant powders after 3 hours exposure period

	Concentrations (ppm)					
Treatments	5000	10000	15000	20000	25000	30000
Azadirachta indica	65.00 ± 7.91 a	67.00 ± 5.70 a	70.00 ± 5.70 a	71.00 ± 6.52 a	78.00 ± 5.7 a	90.000 ± 3.54 a
Caralluma fimbriata	31.00 ± 2.73 d	34.00 ± 2.23 d	34.50 ± 5.48 c	36.00 ± 2.74 c	37.00 ± 2.74 d	37.50 ± 5.70 d
Allium sativum	43.00 ± 10.36 c	50.00 ± 6.51 c	52.00 ± 7.91 b	49.00 ± 7.91 b	55.00 ± 4.18 c	56.00 ± 7.42 c
Curcuma longa	56.00 ± 2.23 b	58.00 ± 2.74 b	65.00 ± 2.74 a	70.00 ± 3.54 a	70.00 ± 5.00 b	73.00 ± 2.74 b
Citrullus colocynthis	25.00 ± 5.00 de	26.00 ± 5.48 e	33.00 ± 2.74 cd	35.00 ± 5.00 c	40.00 ± 3.54 d	36.00 ±2.24 d
Calotropis procera	18.00 ± 2.73 e	20.00 ± 2.24 f	27.00 ± 2.74 d	28.00 ± 2.74 c	29.00 ± 4.18 e	35.00 ± 3.54 d
LSD Value	7.81	5.77	7.48	9.15	5.95	5.32

Each value is a mean ± standard error of five replications. Means followed by the same letters along the column are not Significantly different at (P>0.05) using LSD Test.

Table 5. Mean percent (± SE) repellency of *S. zeamais* on maize grains treated with different concentrations of plant powders after 6 hours exposure period

	Concentrations (ppm)					
Treatments	5000	10000	15000	20000	25000	30000
Azadirachta indica	68.00 ± 4.47 a	76.00 ± 6.52 a	70.00 ± 5.00 a	73.00 ± 8.37 a	85.00 ± 7.91 a	96.00 ± 4.18 a
Caralluma fimbriata	31.00 ± 2.74 d	33.00 ± 8.94 d	35.00 ± 7.91 c	37.00 ± 9.62 c	39.00 ± 4.18 d	38.00 ± 6.52 d
Allium sativum	43.00 ± 5.70 c	49.00 ± 9.62 c	53.00 ± 4.47 b	56.00 ± 7.42 b	56.00 ± 8.94 c	59.00 ± 6.52 c
Curcuma longa	62.00 ± 2.74 b	65.00 ± 3.54 b	66.00 ± 3.54 a	67.00 ± 5.70 a	68.00 ± 5.70 b	68.00 ± 5.70 b
Citrullus colocynthis	25.00 ± 5.00 e	30.00 ± 2.24 d	27.00 ± 2.74 d	40.00 ± 3.54 c	43.00 ± 2.74 d	40.00 ± 3.54 d
Calotropis procera	16.00 ± 2.24 f	20.00 ± 1.03 e	25.00 ± 2.74 d	26.00 ± 2.24 d	31.00 ± 2.24 e	36.00 ± 2.24 d
LSD Value	5.26	7.81	7.29	8.50	7.62	6.30

Each value is a mean ± standard error of five replications. Means followed by the same letters along the column are not significantly different at (P>0.05) using LSD Test.

Table 6. Mean percent (± SE) repellency of *S. zeamais* on maize grains treated with different concentrations of plant powders after 24 hours exposure period

	Concentrations (ppm)					
Treatments	5000	10000	15000	20000	25000	30000
Azadirachta indica	68.00 ± 5.70 a	77.00 ± 2.74 a	74.00 ± 7.91 a	78.00 ± 7.58 a	91.00 ± 6.52 a	97.00 ± 4.47 a
Caralluma fimbriata	29.00 ± 6.52 d	34.50 ± 7.91 d	36.00 ± 6.52 c	37.00 ± 5.70 d	39.00 ± 6.12 d	44.00 ± 7.91 d
Allium sativum	46.00 ± 2.74 c	48.00 ± 6.52 c	56.00 ± 5.71 b	59.00 ± 5.70 c	60.00 ± 6.519 c	61.00 ± 7.91 c
Curcuma longa	61.00 ± 5.48 b	65.00 ± 3.54 b	66.00 ± 2.24 a	68.00 ± 6.71 b	68.00 ± 5.70 b	72.00 ± 4.47 b
Citrullus colocynthis	25.00 ± 5.00 d	28.00 ± 2.74 e	32.00 ± 6.71 cd	38.00 ± 2.74 d	43.00 ± 2.74 d	42.00 ± 6.71de
Calotropis procera	18.00 ± 2.74 e	18.00 ± 2.74 f	27.00 ± 2.23 d	28.00 ± 2.74 e	31.00 ± 2.24 e	36.00 ± 2.24 e
LSD Value	6.41	6.01	8.08	7.90	7.62	7.81

Each value is a mean ± standard error of five replications. Means followed by the same letters along the column are not significantly different at (P>0.05) using LSD Test.

negative effect on the settling response, lower weight loss of the grain, and had no effect on the germination of the treated seeds. In our study the use of *A. indica* seed powder might have exerted a toxic effect by disrupting normal respiratory process of the weevils as already reported by Fekadu *et al.* (2012). The ability of tested plant powders to cause repellency against *S. zeamais* adults on the maize grains might be attributed to the contact toxicity of powders on the weevil. The findings of this study also revealed that the selected plant products applied at varying amounts were effective in reducing maize grain damage caused by *S. zeamais*.

Table 7. Mean percent (± SE) repellency of *S. zeamais* on maize grains treated with different concentrations of plant powders after 48 hours exposure period

	Concentrations (ppm)					
Treatments	5000	10000	15000	20000	25000	30000
Azadirachta indica	68.00 ± 5.70 a	79.00 ± 6.52 a	79.00 ± 8.22 a	82.00 ± 5.70 a	98.00 ± 4.47 a	100.00 ± 00 A
Caralluma fimbriata	30.00 ± 4.18 c	34.00 ± 4.18 d	39.00 ± 4.18 d	39.00 ± 6.12 d	40.00 ± 4.18 de	43.00 ± 5.70 de
Allium sativum	47.00 ± 5.70 b	51.00 ± 7.91c	56.00 ± 9.35 c	59.00 ± 6.52 c	63.00 ± 7.58 c	65.00 ± 6.52 c
Curcuma longa	63.00 ± 2.74 a	66.00 ± 7.42 b	68. 00 ± 2.24 b	69.00 ± 6.52 b	74.00 ± 4.18 b	76.00 ± 4.18 b
Citrullus colocynthis	25.00 ± 6.12 cd	29.00 ± 7.42 d	36.00 ± 4.47 de	38.00 ± 5.70 de	44.00 ± 5.48 d	47.00 ± 5.70 d
Calotropis procera	18.00 ± 5.70 d	20.00 ± 6.12 e	30.00 ± 5.70 e	31.00 ± 4.18 e	35.00 ± 7.91 e	38.00 ± 2.74 e
LSD Value	8.16	8.75	8.99	7.62	7.62	6.13

Each value is a mean ± standard error of five replications. Means followed by the same letters along the column are not significantly different at (P>0.05) using LSD Test.

Table 8. Mean percent (± SE) repellency of *S. zeamais* on maize grains treated with different concentrations of plant powders after 72 hours exposure period

	Concentrations (ppm)					
Treatments	5000	10000	15000	20000	25000	30000
Azadirachta indica	68.00 ± 5.70 a	81.00 ±4.18 a	81.00 ± 5.48 a	83.00 ± 5.71 a	100.00 ± 0.00 a	100.00 ± 0.00 a
Caralluma fimbriata	32.00 ± 2.74 c	35.00 ± 4.18 d	36.00 ± 4.18 d	43.00 ± 2.24 d	44.00 ± 2.24 d	47.00 ± 2.24 d
Allium sativum	49.00 ± 2.74 b	51.00 ± 7.91 c	50.00 ± 9.35 c	62.00 ± 7.07 c	66.00 ± 7.91 c	67.00 ± 7.91 c
Curcuma longa	65.00 ± 7.91 a	67.00 ± 5.70b	67.00 ± 5.70 b	73.00 ± 2.74 b	77.00 ± 5.70 b	76.00 ± 4.18 b
Citrullus colocynthis	26.00 ± 9.62 c	31.00 ± 6.52 d	31.00 ± 7.42 d	38.00 ± 5.70 d	45.00 ± 5.00 d	50.00 ± 7.91 d
Calotropis proceran	24.00 ± 9.62 c	22.00 ± 8.37 e	22.00 ± 7.91 e	28.00 ± 5.70 e	36.00 ± 4.18 e	39.00 ± 4.18 e
LSD Value	9.15	8.29	8.29	6.74	7.14	6.84

Each value is a mean ± standard error of five replications. Means followed by the same letters along the column are not significantly different at (P>0.05) using LSD Test.

This agrees with the findings of Arienilmar *et al.* (2005) who reported 2.81% grain damage of maize when 1.5% of *A. sativum* was applied. This is due to the strong aroma of the powder which might have served as feeding deterrent to the weevils. The reduction in grain damage was observed to be directly proportional to the amount of the applied plant materials. Similar results on the efficacy of *A. indica* derivatives against various insect pests have been reported by various scientists (Ashamo *et al*., 2013; Cosmas *et al.,* 2012; Edelduok *et al.,* 2012; Mamoon-ur-Rashid *et al*., 2012; Abiodun *et al.,* 2010). The results from our laboratory based experiment suggest that *A. indica*, and *C. longa*, powders can be used as insect repellent for the safer management of *S. zeamais* on stored maize.

Conclusion

Overall, *Azadirachta indica* and *Cureuma longa* powders were found more effective to control maize weevil *Sitophilus zeamais* at all the post treatment intervals whereas; *Calotropis procera* powder was found least effective.

Acknowledgement

The authors thank Pakistan Science Foundation (PSF), Islamabad, Pakistan for providing financial support for this study under Grant No. PSF/NSLP/KP-GU (424). Dr. Imran Bodlah, Assistant Professor, Department of Entomology, PMAS Arid Agriculture University, Rawalpindi is greatly acknowledged for identification of maize weevil.

References

Abiodun, A.D., Makanjuola, W.A., Teslim, O.K., Alafia, O.A., Kasali, A.A., Eshilokun, A.O. 2010. Toxicity of *Chenopodium ambrosioides* L. (Chenopodiaceae) products from Nigeria against three storage insects. *Journal of Plant Protection Reasearch*, **50:** 379-384.

Arannilewa, S. T., EkrAakene, T., Aakinneye, J. O.

2006. Laboratory evaluation of four medicinal plants as protection against the maize weevil, *Sitophilus zeamais*. *African Journal of Biotechnology,* **5**: 2032-2036.

Arienilmar, A.L., Da Silva, L.R., Faroni, D.A., Guedes, N.C., Martins, J.H., Pimentel, A.G. 2005. Modelos analíticos do crescimento populacional de *Sitophilus zeamais* emtrigoarmazenado. *Engenharia Agrícola e Ambiental,* **10**: 55.

Ashamo, M.O., Odeyemi, O.O., Ogungbite, O.C. 2013. Protection of cowpea, *Vigna unguiculata* L. (Walp.) with *Newbouldia laevis* (Seem.) extracts against infestation by *Callosobruchus maculatus* (Fabricius). *Archives of Phytopathology and Plant Protection,* **46:** 1295-1306.

Cosmas, P., Christopher, G., Charles, K., Friday, K., Ronald, M., Belta, M. 2012. *Tagetes minuta* formulation effect *Sitophilus zeamais* (Weevils) control in stored maize grain. *International Journal of Plant Research*, **2:** 65-68.

Danjuumma, B.J., Majeed, Q., Manga, S.B., Yahaya, A., Dike, M.C., Bamaiyi, L. 2009. Effect of some plant powders in the control of *Sitophilus zeamais* Motcsch (Coleoptera: Curculionidae) infestation on maize grains. *American Eurasian Journal of Scientific Research,* **4**: 313-316.

Edelduok, E., Aakpabio, E., Eyo, J., Ekpe, E. 2012. Bio-insecticidal potentials of testa powder of melon, *Citrullus vulgaris* Schrad for reducing infestation of maize grains by the maize weevil, *Sitophilus zeamais* Motsch. *Journal of Biology, Agriculture and Healthcare,* **2:** 13-17.

Fekadu, G., WAaktole, S., Santiago, D.R. 2012. Evaluation of plant powders and cooking oils against maize weevil, *Sitophilus zeamais* M. (Coleopteran: Curculionidae) under laboratory conditions. *Molecular Entomology,* **3:** 4-14.

Govindan, K., Nelson, S.J., David, P.M.M. 2010. Fly-ash excellent filler for black pepper, *Piper nigrum* dust formulation against *Callosobruchus maculatus*. *Journal of Biopesticides*, (1 special Issue) **3:** 320-324.

Ileke, K.D., Ogungbite, O.C., Olayinka-Olagunju, J.O. 2014. Powders and extracts of *Syzygium aromaticum* and *Anacardium occidentale* as entomocides against the infestation of *Sitophilus oryzae* (l.) [Coleoptera: Curculionidae] on stored sorghum grains. *African Crop Science Journal,* **22**: 267-273.

Ileleji, K.E., Maier, D.E., Woloshuk, C.P. 2007. Evaluation of different temperature management strategies for suppression of *Sitophilus zeamais* (Motschulsky) in stored maize. *The Journal of Stored Products Research,* **43:** 480-488.

Isah, M.D., Abdullahi, G., Sastawa, B.M. 2012. Distribution patterns of insect pests infesting some field and stored commodities in Maiduguri, North-Eastern Nigeria: Implications for their management. *Journal of Applied Research and Technology,***1:** 227.

Mamoon-ur-Rashid, M., Khattak, M.K., Abdullah, K. 2012. Residual toxicity and biological effects of *Azadirachta indica* (*Azadirachta indica*) oil against cotton mealybug, *Phenacoccus solenopsis* Tinsley (Sternorrhyncha: Pseudococcidae). *Pakistan Journal of Zoology,* **44:** 837-843.

Musabyimana, T., Saxena, R.C., Kairuew, C., Khan, Z.R. 2001. Effects of *Azadirachta indica* derivatives on behavioral and physiological responses of the *Cosmopolites sordidus* (Coleoptera: Curculionidae). *Journal of Economic Entomology,* **94:** 449-454.

Nakakita, H., Sittisuang, P., Visarathanonth, P., Kuwahara, M., Urairong, P., Sinchisri, P. 1991. *Studies on Quality Preservation of Rice Grains by the Prevention of Infestation by Stored-product Insects in Thailand*. Report for the collaborative work between the Tropical Agricultural Research Center, Japan and Department of Agriculture, Thailand, pp 25-33.

Nishi, A., Imamura, T., Miyanoshita, A., Morimoto, S., Takahashi, K., Visarathanonth, P., Kengkanpanich, R., Shazali, M.H., Sato, K. 2004. Predatory abilities of *Amphibolus venator* (Klug) (Hemiptera: Reduviidae), a predator of stored product insect pest. *Journal of Applied Entomology and Zoology*, **39:** 1407-1408.

Park, C., Kim, S., Ahn, Y.J. 2003, Insecticidal activity of asarones identified in *Acorus gramineus* rhizome against three coleopteran stored-product insects. *Journal of Stored Products Research,* **39:** 333-342.

Sayonara, G., Pino, O., Herrera, R.S., Valenciaga, N., Fortes, D., Sánchez, Y. 2009. Control of *Sitophilus zeamais* with plant powder from one species of the

Fabacea family (49-1-XIV). *Cuban Journal of Agricultural Science,* **43:** 311.

Suleiman, M., Majeed, Q., Abdulkarim, B. 2011. Toxicity of three plant powders as biopesticides against *Sitophilus zeamais* Mots on stored guinea corn grains. *Biological and Environmental Sciences Journal for the Tropics,* **8:** 273-277.

Udo, I.O. 2005. Evaluation of the potential of some local spices as protectants against the maize weevil *Sitophilus zeamais* Mots. (Coleoptera: Curculionidae). *Journal of Applied Sciences and Environmental Management,* **9:**165-168.

Ukeh, D.A., Birkett, M.A., Bruce, T.J., Allan, E.J., Pickett, J.A., Luntz, A.J. 2010. Behavioural responses of the maize weevil, Sitophilus zeamais, to host (stored grain) and non-host plant volatiles. *Pest Management Science*, **33:** 44-50

Yuya, A.I., Tadesse, A., Tefera, T. 2009. Efficacy of combining Niger seed oil with malathion 5% dust formulation on maize against the maize weevil, *Sitophilus zeamais* (Coleoptera: Curculionidae). *Journal of Stored Products Research,* **45:** 67-70.

Pak. j. sci. ind. res. Ser. B: biol. sci. 2018 **61B**(3) 172-181

Review

Epigenetic Alterations and their Dietary Backgrounds

Rani Saira Saleem[a]* and Rani Wafa Shear[b]
[a]Institute of Biochemistry and Biotechnology, University of Punjab, Lahore, Pakistan
[b]Biotechnology Department, University of Gujrat, Pakistan

(received June 27, 2016; revised April 25, 2017; accepted May 10, 2017)

Abstract. The key intention of this review is to summarize the different studies which relate the genome-diet interactions with future perspectives of exploring an insight into the well defined functions of diverse micronutrients and other dietary components that play a vital task in defining the early developmental patterns of an organism. Human fetus development is a complex process that is totally dependent on the dietary components which interact with the genes to regulate the different proliferation and differentiation stages. We want to explore those complex interactions that lay hidden between micro-nutrients and gene expressions but are means of the apparent changes of a phenotype of an individual. Along with this the review will also perceive some basics of development of certain diseases as well, due to these complex genome-diet interactions thus leading to refine the dietary outlines for maternal and prenatal developmental stages in future. Research has also shown that genome-diet interactions are very complex as without proper nutrients the end result is the genome instability which may lead to chronic diseases, developmental defects and certain types of cancer.

Keywords: epigenetics, genome diet, methylation, DNA sequence, eukaryotes

Introduction

The definition of the term 'Epigenetics' was given by Waddington in 1942 while, discussing the modulation of development by gene regulation. He presented the basic idea for the interaction of genes with their environment thus concluding a particular phenotype (Waddington, 1953). Epigenetics is the branch of biology which deals with the study of heritable cellular and physiological traits, which are not characterized by alterations in the DNA sequence. In the most widely studied epigenetic changes include the DNA structural changes during the cell differentiation process or morphogenesis at early developmental stages. They may include methylation of DNA or modifications of histones which might be responsible for changing the expression of certain genes playing their role in the right development of a life form. Epigenetic modifications actually translate the relationship between heredity and environment and amongst the environmental factors the dietary exposure serves as the most important clue. Experimental procedures and statistical data obtained from different studies relate that the maternal and fetus dietary exposure in the first 1000 days of life is very important. This is also called as 'primary dietary exposure'. This primary dietary exposure has been found responsible for bringing up and carrying the epigenetic changes that might last for the whole life of an individual or might affect the individual's generation after generation by regulating chromatin remodeling. The early micronutrient status of fetus holds enough strength to alter the metabolism rate, organ growth, and regulate the genes responsible for cell differentiation and organogenesis process. It refers to such external modifications responsible for turning the genes on and off which are other than involving the actual DNA sequence (Waddington, 1953).

In this case the actual coding sequence of DNA is not affected but DNA structure itself gets modulated in such a way that certain genes are turned on or off as a result. Interestingly nowadays, Epigenetic marks are recognized as mechanistic links between environment, nutrition and genes. DNA modifications or epigenetic alterations serve as the basis of changes in the metabolism rate, cell differentiation pattern, brain development and organogenesis of the fetus by manipulating the expression of developmental genes and a network of transcriptional factors. These epigenetic changes are under the strong influence of environmental interactions which affect the developmental stages in one way or the other. With the increasing understanding about the

*Author for correspondence;
E-mail: ranisairasaleem@hotmail.com

field of epigenetics, it becomes clearer that epigenetic incidents or inherited changes in the gene expression that take place without any alteration in the genomic DNA sequence are important thus leading towards cancer. The epigenetic mechanisms actually decide the fate of the cell and also build up certain barriers to prevent the reversion to preceding cellular states (Reik, 2007). The development of a cell starts from a pluripotent stage from which it changes into many cell types and then to tissues and organs, respectively. The factors which direct the translation of this pluripotent staged cell to such a complex form also include the dietary factors or various necessary micronutrients like iron, calcium, vitamin A & D, zinc and magnesium etc. The gene expression programme becomes more effective, well established and potentially efficient in the right direction, well driven by the force of these micronutrients which are needed in small amounts but are indispensible for the development of fetus by all means.

The study of micro-nutrients and their pathways in the body may lead us to a clear understanding that they might play a significant role by interacting with the epigenetic mechanisms. The chromatin modifications which are resulted as environmental response can be reversed, are temporary and allow adaptations. In some situations these might keep on moving throughout the existence of an individual. This is also called as metabolic imprinting which is regarded as the result of motherly nourishment that is given during development and the suckling period on DNA patterns and gene regulation patterns that operate lifetime risk of chronic diseases (Waterland and Garza, 1999).

Chromatin conformation and regulation of epigenetic changes. The packaging of huge amount of DNA into their nucleus is a tedious task which a eukaryotic cell has to go through. The eukaryotic DNA consists of transcriptionally active DNA called Euchromatin and transcriptionaly incompetent DNA that is Heterochromatin. As result of this complex packaging process a basic unit nucleosome of about 146bp comes into play which is laterally epigenetically modified under the effect of various factors to allow the cell to control transcriptional activity and genetic expression (Rountree *et al*., 2001). The organization of eukaryotic chromatin is still partially understood. The technique like X-ray crystallography has enabled us to have a deep insight into the detailed structure of the essential repeating element of chromatin that is nucleosome.

There are more than about 30,000 genes in our genome functional in various body parts of an organism. This varied expression of genes depends on variety of transcription factors. Scientists have shown via *in vitro* and *in vivo* experiments that although cells of body are identical yet they are different in their expression profiles. The remarkable chromatin modifications that are well studied in eukaryotes include methylation of DNA patterns, modifications taking place in histones and functioning of small RNAs both considerately and independently to constitute the epigenome which transmit gene expression outline via the process of mitosis/meiosis (Surani, 2013). Such epigenetic modifications together have emerged as critically important for the regulation roles in the transcriptional rheostat.

DNA methylation. It is the characteristic variation in eukaryotic DNA which involves methylation of cytosines at the carbon 5 position of the CpG dinucleotides. CpG sites are the regions of DNA in which cytosine nucleotide is followed by Guanine. CpG is shorthand for 5'—C—phosphate—G—3'. These regions often serve as promoters or transcription start sites. Cytosines in CpG dinucleotides can be methylated to form 5-methylcytosine (Jabbari and Bernardi, 2004). 5 methylcytosine performs similar functions as that of normal cytosine. Methylation of DNA can also be continued directly from the germ line silencing any one of the parental chromosome also known as genomic imprinting. The eukaryotic genome can be categorized into two groups, with methylated and non methylated DNA. In mammals about 70% methylated CpG's content and only 1-2% DNA consists of non methylated CpG.

The initial information and identification of the methylated DNA was stated by experiments performed by Razin and Cedar, 1977 (Ball *et al*., 1983). It was almost after a decade that micro-injection experimentation confirmed that DNA methylation via *vivo* and *in vitro* methylated DNA sequences exposed that it results to inactivate the chromatin material (Keshet *et al*., 1986). The genomic methylation patterns in mammals that take place during embryogenesis involve overall three DNA methyltranferases DNMT1, DNMT3a, DNMT3b. These are also known as *de novo* methyltransferases which are meant to ascertain the methylation outline during developmental stages (Okano *et al*., 1999). Later on the identification of two methyl binding proteins MeCP1and MeCP 2 assisted to understand that DNA methylation has its role to play

with DNA structure and gene expression (Lewis *et al*., 1992; Meehan *et al*., 1989). Thus, concluding that DNA methylation is accountable for the transcriptionally inactive DNA. Any change in the methylation patterns at any stage of development of an organism may lead to abnormalities. Imprinting and methylation patterns in germ cells are shown in Fig. 1a (adapted from Rountree *et al*., 2001). The primary methyl donating specie for this process is SAM (S-Adenosyl metheonine) that is regarded as a molecule of cyclic cellular process called as one carbon metabolism.

This methylation of DNA is liable for the regulation of thousands of genes present in the genome of an individual. Methylation is involved in the processes of:

- Inactivation of an X-chromosomes in females
- Genetically imprinted genes
- Silencing of certain genes

Histone modifications. At molecular level specific chromatin domains can be recognized by the help of histone modifications. The different type of histone modifications include methylation, phosphorylation, acetylation, sumoylation and ubiqutination of histones residues. Such different changes in histones might affect the transcriptional patterns of genomic DNA (Schneider *et al*., 2004; Offield *et al*., 1996). The methylation of histone which takes place at specific residues like lysine and arginine straightly influences the action and functions of DBP (DNA binding proteins) thereby regulating the expression of genes and thus affecting the strength of the genome (Mosammaparast and Shi, 2010). The acetylation of histones is one of the main frequent transcriptional controls adapted by most of the eukaryotes (Grunstein, 1997). The mechanism involved in this control includes acetylation of NH_2 terminal of H3 and H4 by histone acetyltransferases (HATs) which will facilitate the transcriptional activity of the genes. However, this transcriptional activity is stopped by removing the acetyl group by histone deacetylases (HDACs). The HATs and HDACs are used as the co activators and repressors of the transcriptional machinery of the cell, respectively (Rountree *et al*., 2001).

There have been several different proteins that have been identified for their roles in the transcription repression (MBD1, MBD2, MBD3, and MeCP2) (Wade, 2001). They are supposed to act for the repression of transcription during the function of HDACs. This relationship was indicated earliest for MeCP2 protein (Nan *et al*., 1998; Jones *et al*., 1998). MeCP2 has also its role in anchoring a multiprotein repressory complex to the DNA which is responsible for deacetylation of histones and alterations of chromatin by recruiting deactylases HDAC1 and HDAC2 also shown in Fig. 1b. Experiments were performed to show that transcriptional inactivation which is sourced by the deacetylation might be lessened by HDAC inhibitor Trichostatin A (TSA) (Eden *et al*., 1998; Jones *et al*., 1998; Nan *et al*., 1998). Such explanations and studies resolve the supposed pathway of gene silencing by relating finally the long three method suspected correlation between DNA methylation, chromatin remodeling and expression of genes (Razin, 1998).

One carbon metabolism pathway and SAM. Both the above types of DNA modifications are interlinked by their common dependence on S-Adenosyl Methionine

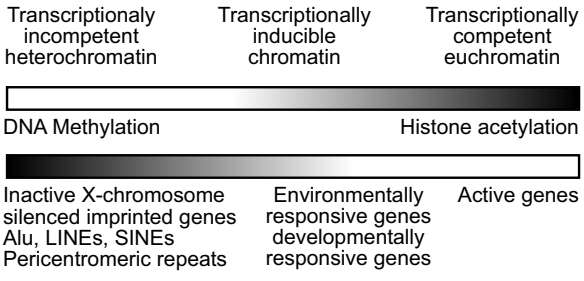


Fig. 1a. The transcriptional rheostat. DNA methylation and histone acetylation help to establish chromatin states that either foster or inhibit transcription (adapted from Rountree *et al*., 2001).

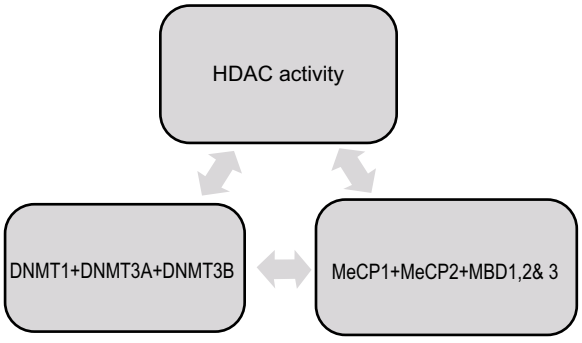


Fig. 1b. The interrelationship between DNA methylation and Histones modification Transcriptional Regulatory Machinery.

which plays central role as methyl donor. It is important to remember here that almost all the DNA and histone methyltranferases require this cofactor to transfer methyl group to cytosine, lysine or arginine residues. This is an important co enzyme that carries the transmethylation reactions that takes place in liver. SAMC (S-Adenosyl-metheonine Mitochondrial Carrier protein) belongs to solute carrier family 25 members 26, which consists of 274 amino acid proteins that transports SAM, as well as metabolites, nucleotides and cofactors, through the mitochondrial inner membrane. The one carbon metabolism involves TP driven reactions in which methionine is converted into S-adenosylmetheonine, a universal methyl donor. DNMTs help to put together methyl group from SAM to carbon 5 position of cytosine therefore, forming 5-methyl cytosine and resulting in methylation.

S-adenosylmetheonine was for the first time discovered by Giulio Cantoni also known as AdoMet. It is conjugate of methionine and ATP. The formation of SAM is catalyzed by MAT i.e. Methyl Adenosyltransferase also known as SAM Synthase. Regeneration of SAM can take place by the demethylation of SAM via the methionine cycle taking place in liver. This methionine cycle also called one carbon metabolism is folate dependent as shown in Fig. 2. Any irregulation of this methionine cycle is the result of the folate deficiency via dietary insufficiency or lack of balance diet which may result in dictating various hereditary changes (Loenen, 2006). One other important function that has been demonstrated for SAM is that it also serves as cofactor for the activity of nucleases EcoK1 which can not otherwise restrict DNA in the absence of SAM. MTR enzyme of methionine synthase is an essential enzyme for catalyzing the 5-methyl THF induced remethylation of Homocysteine to Methionine (Stover, 2011). This MTR is a cobalamine (Vit B-12) reliant enzyme which performs its function well provided the presence of sufficient amount of this vitamin in the cell.

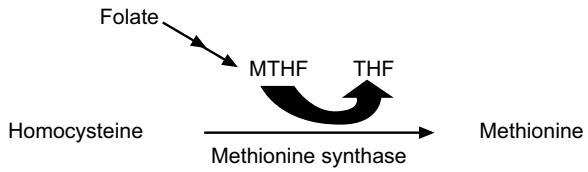


Fig. 2. Folate mediated induction of 1C metabolism.

One carbon metabolism is a mechanism that is present in the mitochondria, cytoplasm and nucleus as well and this pathway is induced by the presence of dietary micronutrients as cofactors, like folate and also known as folate mediated 1-C metabolism. This pathway has been studied for the *de novo* synthesis of purines, thymidylate and methionine. This is because these entire pathways involve Tetrahydrofolate (THF) as cofactor which is the precursor of folate (Stover and Fox, 2008).

In one carbon metabolism mitochondria utilizes the cofactor THF to generate formate from the amino acids serine, glycine, and from sarcosine and dimethylglycine which are the result of choline degradation. The formate is then transported to cytoplasm where its condensation with THF in an ATP dependent environment takes place and 10 Formyl THF is resulted (Caudill and Stover, 2008). The formate generation is critical for the AdoMet-dependent methylation reactions, including chromatin methylation and also it serves as the primary source for the *de novo* biosynthesis of purines and for homocysteine remethylation to methionine (Appling, 1991). It is important to consider here that a mammalian cell has many different AdoMet dependent methyltransferases which are involved in different cell processes like chromatin remodeling, regulatory functions for gene transcription (Miranda and Jones, 2007), protein compartmentalization (Winter-Vann *et al*., 2003), and the biosynthesis and catabolism of small molecules including neurotransmitters. S adenosylehomocysteine is the product that is resulted from the AdoMet depenedent transmethylation reactions.

One carbon metabolism is highly sensitive to the dietary components i.e. vitamins (Herbig, 2002). Certain dietary components are significant to show genomic interactions and folate is one of them as shown in Fig. 3 adapted from Jing (1997). A follow up study by health professionals indicated that the use of alcohol significantly overcome the effects of genetic mutations responsible for colorectal cancer because ethanol has the cleavage ability for folate and it also inhibits the absorption and utilization of folate and increases its excretion. Furthermore, alcohol acts like a methyl group antagonist thereby, causing imbalanced DNA methylation (Jing, 1997).

The purines and thymidylate are required for DNA synthesis whereas; SAM is required as a part of DNA methylation factory. This folate dependent synthesis of DNA and SAM precursors for genome programming

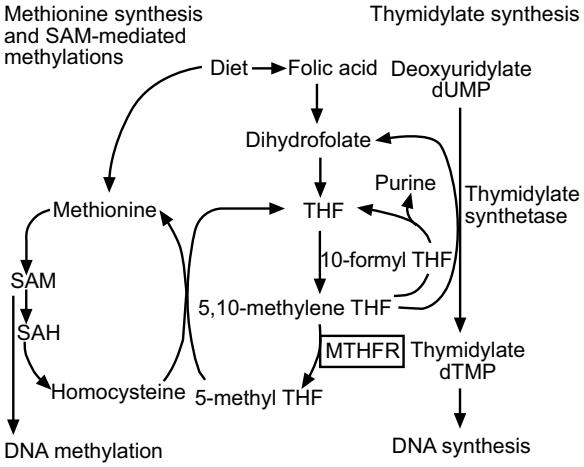
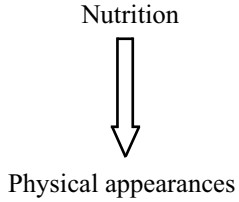


Fig. 3. An overview of one carbon metabolism and dietary factor involved (Adapted from Jing Ma, 1997).

is largely dependent on the availability of many vitamins and minerals at the same time. Thus we can say that the folate dependent one carbon metabolism serves as a central relationship between nutrient status and genomic programming during the fetal development and this nutritional pool is developed by the maternal dietary background (Stover, 2007). Any sort of disturbances in this folate mediated 1-C metabolism increases the risk of certain cancers, cardiovascular diseases, neurological disorders and developmental anomalies such as spina bifida, cleft palate and spontaneous abortions (Stover, 2004). However, the folate supplementation reduces the risks of diseases and developmental disorders in the population (DeMarco, 2000).

The role of micronutrients in DNA modifications. The intake of micronutrients and balance diets offers the biggest challenge in regulation of the metabolic and differentiation processes taking place at the time of development of fetus. As the growing fetus is largely dependent on the micronutrient status of the mother therefore, pregnant woman is at high risk of micronutrient deficiencies (Doyle *et al*., 1992). For understanding the interaction between nutrients and epigenetic mechanisms one need to have a complete insight into the physiological roles of micronutrients for maternal and prenatal health. The pregnancy outcomes can be affected by the micronutrients by resulting alterations in maternal and fetal metabolism by changing their role, enzyme activities, signal transduction and transcription pathways (Berti *et al*., 2011). Micronutrients distort the metabolism and genetic regulations in a number of complicated pathways. The folate dependent biosynthesis of chromatin material for DNA synthesis and methylation is dependent on the supply of many vitamins including B-12, B-6, niacin, riboflavin and minerals which are actually responsible for DNA synthesis and modifications.



The embryonic stages are the most sensitive stages that immediately respond to the nutrient induced adaptations in the gene expression. This phenomenon is labeled as metabolic imprinting or metabolic programming (Waterland and Garza, 1999). However these adaptations come into play within the early critical windows of development and lasts for adulthood. The above explained relationship between maternal nutrients status and fetal epigenetic programming actually serves as the basis for fetal origin of adult disease hypothesis which proposes that nutrition acts very early in life to predict the risks for adverse outcomes of the coming adult life (Barker, 1997). On the other hand genetic variations in the epigenetic programming can also impair the nutrition absorption and utilization which may result in the differences in the nutrient tolerances such as iron tolerance and thus lead to the variations in the nutrient requirements of the body. Iron deficiency is responsible for causing the reduced motor skill development of the growing children along with reduced power of learning and memorization (Carter *et al*., 2010).

The global methylation effects of maternal folate status have been followed in different studies till now. One of them clearly indicates that there was a considerable decrease in the small intestinal tissue as adults when an offspring was exposed to low folate dietary intake during the gestation and lactation period (McKay *et al*., 2011). Another study also related that the intake of folate according to the recommended dose in US women of child bearing age reduced the global methylation levels in murine colorectal tissue which was calculated by the liquid chromatography mass spectrometry (Sie *et al*., 2011). A number of studies have been done for studying the impacts of global methylation patterns on the colorectal cancer in post menopause women. These

studies actually analyzed the global DNA methylation patterns by the indirect incorporation of ^{3}H methyl SAM (Pufulete *et al*., 2005a). The patients which were diagnosed with colorectal cancer when given an increased folate supplementation they showed an increase in the methylation patterns thus showing a direct correlation between folate supplementation and colorectal cancer prognosis (Pufulete *et al*., 2005b).

Gene specific studies have enabled the researchers to explore the impact of folate mediated DNA methylation patterns on various genes responsible for anomalies like cancer and diabetes. This has enabled us to explore the window for designing the therapeutics and preventive strategies from these anomalies by the help of dietary supplementation and hence targeting the epigenetic mechanisms. Till now the methylation patterns of different tumor related genes like NAT1, p53, several loci of CRC, and PPAR-α, an insulin receptor gene have been related with the high or low folate mediated methylation pattern changes thus resulting in wide variety of expression changes (McKay *et al*., 2011; Wakefield *et al*., 2010; Burdge *et al*., 2009).

In addition to folate certain other micro nutrients have also been analyzed for their dietary influence on the methylation patterns. Choline is one of them which also serves as an indirect methyl donor in one carbon metabolism pathway. The effect of maternal choline deficient diet has been studied on three candidate genes cdkn3, cdkn2b and calb2. It was concluded that cdkn3 gene of mouse fetal brain of embryonic stage day 17 displayed a state of hypomethylation for its promoter region as exposed to choline deficient diet which proved the role of choline in the methylation mechanisms (Niculescu *et al*., 2006). Studies have also shown that early deficiency of choline in diet may cause the malfunctioning of methylation machinery.

The role of vitamin A has been studied in detail and it has been shown that vitamin A is critical for the regulation of alveolorization and septation in the lung development (Massaro and Massaro, 2010; 2006). This may relate to the hidden cause of some genes might get switch off and resulted in malformation of lung and its functions. However, the excess of vitamin A has also been related with the teratogenicity in humans (Biesalski 1989). Two studies conducted by Barreto *et al*. (1994) and Pearson *et al*. (1992) on the premature babies showed that the chances of chronic lung prematurity can be reduced to a larger extent by the administration of vitamin A. It is hypothesized that some genetic and metabolic pathways are correlated via this critical vitamin which hamper the development of the lungs in the premature babies (Biesalski, 2003). Vitamin A is converted to all trans retinoic acid (RA) which is transported to nucleus where it is interacted as an activating agent to the RA nuclear receptors. The induction of differentiation with retinoic acid and vitamin D3 was successfully conducted in myelodysplastic syndromes (Blaszek *et al*., 1990). The role of retinoic acid in preventing the tumor progression in central nervous system has been studies but the mechanism has still to be elucidated (Ross and Stephensen, 1996).

It is therefore, suggested that it may be the result of some regulatory role of certain transcriptional regulators or repressors to the thyroid hormone receptors. Vitamin A deficiency produces a number of malformation and differentiation abnormalities in the growing embryo. Figure 4 indicates all relationships that can be predicted among one carbon metabolic pathway and associated epigenetic regulations with the fetal nutritional status.

This review article illustrates the severe consequences and genetic diseases that are resulted from improper genetic programming and imprinting at the early developmental stages of the fetus. Above all, it focuses that the genetic diseases can be reduced to a larger extent by managing and monitoring the dietary needs of the fetus and maternal environment. Studies adapted from human epidemiological contexts and experimentation of the animal models clearly depicts that maternal nutrients can programme the gene expression and patterns of embryonic methylation that persist throughout the adult life and may contribute to many diseases (McMillen and Robinson, 2005). The importance of DNA methylation is clearly reflected by the study of large number of genetic diseases including cancer and summarized in Fig. 5.

The dietary vitamins and minerals continue to play an important role in lateral life and help in the management of cardiovascular, renal, and pulmonary functions. Every individual vitamin and mineral has its own characteristic role in managing a certain metabolic pathway for example calcium intake may help to keep an eye on blood pressure in adults (Van Mierlo *et al*., 2006). Zinc and magnesium have their well defined roles in insulin pathways and zinc metabolism has been studied in type 2 diabetes mellitus (Taylor, 2005; Chausmer, 1998;

Garland, 1992). Therefore, it is concluded that the development of micronutrient deficiencies is although a slow process but may lead to severe consequences at the later stages of life as shown in Fig. 6 adapted from Shenkin (2004). The folate dependent 1-Carbon metabolism serves as a channel that links the cellular metabolism mechanisms to the epigenetic machinery of the cell. The common molecule that translates the information in this relationship is AdoMet. The cellular methylation patterns can be read AdoMet/AdoHcy ratio. The folate induced changes in the cell can influence the activity of DNA and histone methyl transferases thus controlling the chromatin methyl patterns. Yet it is still to elucidate the respective effects of these dietary dependent methlaytion patterns of different developmental genes like Hox clusters, shh signaling and FG factors. The future research on these gene clusters, their transcriptional factors and domains, their protein products might give us a detailed insight of the type of the effects produced by these folate dependent mechanisms on development of the fetus. Furthermore, it is also important to emphasize on exploring the roles of other associated minerals on the one carbon metabolic pathways and on the methylation patterns. There is a need to explore the methylating patterns of individual genomic loci of some above mentioned candidate genes for developmental anomalies and then to study the regulating mechanisms of these candidate genes by following dose dependent micronutrients exposures. This will surely help us to elucidate the developmental roles played by these genes as well as their expression dependency on dietary components.

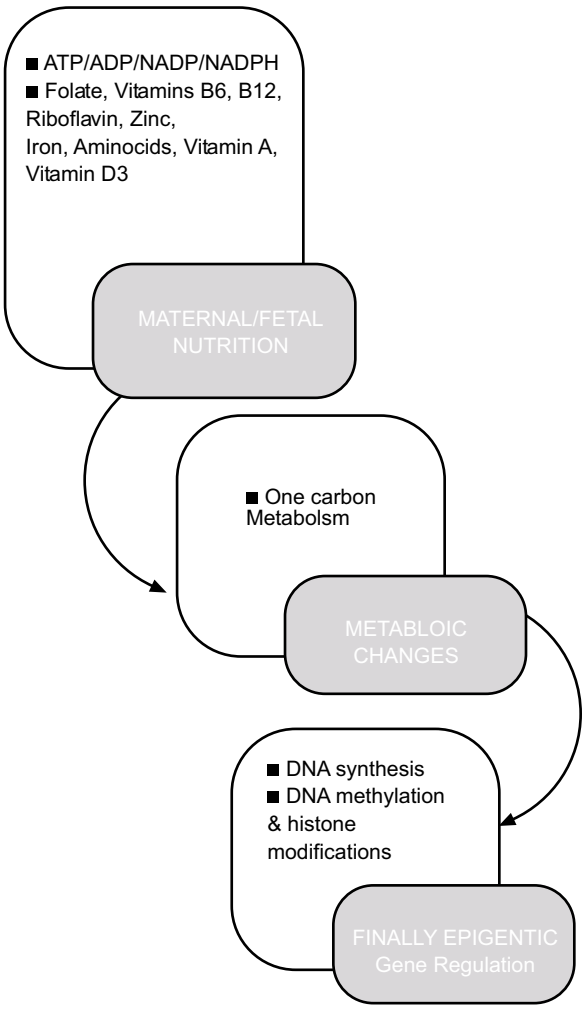


Fig. 4. Relationship between maternal/fetus nutrition and epigenetic changes.

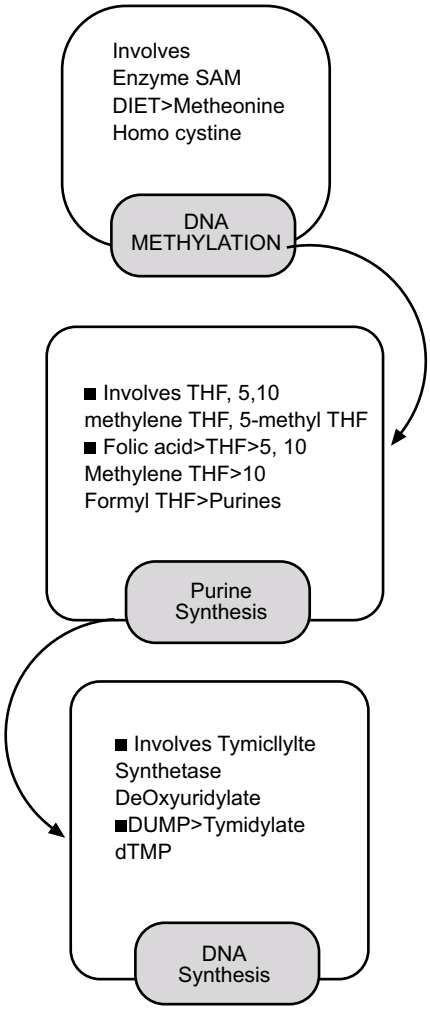


Fig. 5. Important cellular metabolic pathways and their relationship.

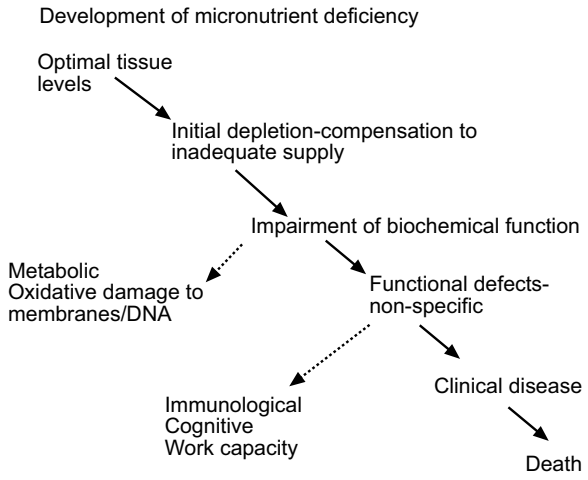


Fig. 6. Pathways for micronutrient deficiencies (Adapted from Shenkin, 2004).

References

Appling, D.R. 1991. Compartmentation of folate-mediated one-carbon metabolism in eukaryotes. *FASEB Journal*, **5:** 2645-2651.

Ball, D.J., Gross, D.S., Garrard, W.T. 1983. *Proceedings of National Academy of Sciences*, USA, **80:** 5490-5494.

Barker, D.J. 1997. Intrauterine programming of Coronary heart disease and stroke. *Acta Paediatrica Supplementum*, **423:** 178-182.

Barreto, M.I., Santos, I.M.P., Assis, A.M.O. 1994. Effect of vitamin A supplementation on diarrhoea and acute lower respiratory-tract infections in young children in Brazil. *Lancet*, **344:** 228-231.

Berti, C., Biesalski, H.K., Bartner, R., Lapillone, A., Pietizik, K., Poston, L., Redman, C., Koletzko, B., Cetin, I. 2011. Micronutrients in pregnancy: Current knowledge and unresolved questions. *Clinical Nutrition*, **30:** 689-701.

Biesalski, H.K. 2003. The significance of Vitamin A for the development and function of lung. *Forum Nutrition*, **56:** 37-40.

Biesalski, H.K. 1989. Comparative assessment of toxicology of Vitamin A and Retinoid in man. *Toxicology*, **57:** 117-161.

Blaszek, I., Mathè, G. 1990. Pathobiology of myelodysplastic syndromes. *Biomedicine and Pharmacotherapy*, **44:** 69-83.

Burdge, G., Lillycrop, K.A., Phillips, E.S., Slater-Jefferies, J.L., Jackson, A.A., Hanson, M.A. 2009. Follic acid supplementation during Juvenile-pubertal period in rats modifies the phenotype and epigenotype induced by prenatal nutrition. *The Journal of Nutrition*, **139:** 1054-1060.

Carter, R.C., Jacobson, J.L., Burden, M.J., Armony-Sivan, R., Dodge, N.C., Angelilli, M.L. 2010. Iron deficiency Anemia and cognitive function in infacncy. *Pediatrics*, **126:** 427-432.

Caudill, S. 2008. Genetic and epigenetic contributions to human nutrition and health: managing genome-diet interactions. *Journal of American Diet Association*, **108:** 1480-1487.

Chausmer, A.B. 1998. Zinc, insulin and diabetes. *Journal of American College of Nutrition*, **17:** 109-115.

DeMarco, P.M.A. 2000. Folate pathway gene alterations in patients with neural tube defects. *American Journal of Medical Genetics*, **95:** 216-223.

Doyle, W., Wynn, A.H.A., Crawford, M.A. 1992. Nutritional counseling and supplementation in the second and third trimester of pregnancy, a study in a London population. *Journal of Nutrition and Medicine*, **3:** 249-256.

Eden, S., Hashimshony, T., Keshet, I., Cedar, H., Thorne, A.W. 1998. DNA methylation models histone acetylation. *Nature*, **394:** 842-843.

Garland, H.O. 1992. New experimental data on the reltionship between diabetes mellitus and magnesium. *Magnesium Research*, **5:** 193-202.

Grunstein, M. 1997. Histone acetylation in chromatin structure and transcription. *Nature*, **389:** 349-352.

Herbig, K.C.E. 2002. Cytoplasmic serine hydroxymethyltransferase mediates competition between folate-dependent deoxyribonucleotide and S-adenosylmethionine biosyntheses. *Journal of Biological Chemistry*, **277:** 38381-38389.

Jabbari, K., Bernardi, G. 2004. Cytosine methylation and CpG, TpG(CpA) and TpA frequencies. *Gene*, **333:** 143-149.

Jing, M.A. 1997. Methylenetetrahydrofolate Reductase Polymorphism, Dietary Interactions, and Risk of Colorectal Cancer1. *Cancer Research*, **57:** 1098-1102.

Jones, P.L., Veenstra, G.J.C., Wade, P.A., Vermaak, D., Kass, S.U., Landsberg, N., Strouboulis, J., Wolffe, A.P. 1998. Methylated DNA and MeCP2 recruit histone deacetylase to repress transcription. *Nature Genetics*, **19:** 187-191.

Keshet, I., Lieman-Hurwitz, J., Cedar, H. 1986. DNA methylation affects the formation of active chromatin. *Cell*, **44:** 535-543.

Lewis, J.D., Meehan, R.R., Henzel, W.J., Maurer-Fogy, I., Jeppesen, P., Klein, F., Bird, A.P. 1992. Purification, sequence and cellular localisation of a novel chromosomal protein that binds to methylated DNA. *Cell*, **69:** 905-914.

Loenen, W. 2006. S-Adenosylmethionine: jack of all trades and master of everything. *Biochemical Society Transactions*, **34:** 330-333.

Massaro, D., Massaro, G.D. 2010. Lung development, lung functions and retinoids. *New England Journal of Medicine*, **362:** 1829-1831.

Massaro, D., Massaro, G.D. 2006. Towards therapeutic pulmonary alveloar regeneration in humans. *Proceedings of American Thoracic Society*, **3:** 709-712.

McKay, J.A., Waltham, K.J., Williams, E.A., Mathers, J.C. 2011. Folate depletion during pregnancy and lactation reduces genomic DNA methylation in murine adult offspring. *Genes and Nutrition*, **6:** 189-196.

McMillen, I.C., Robinson, J. 2005. Developmental origins of the metabolic syndrome, prediction, plasticity and programming. *Physiological Reviews*, **61:** 571-633.

Meehan, R.R., Lewis, J.D., McKay, S., Kleiner, E.L., Bird, A.P. 1989. Identification of a mammalian protein that binds specifically to DNA that contains methylated CpGs. *Cell*, **58:** 499-507.

Miranda, T.B., Jones, P.A. 2007. DNA methylation: the nuts and bolts of repression. *Journal of Cell Physiology*, **213:** 384-390.

Mosammaparast, N., Shi, Y. 2010. Reversal of histone methylation: biochemical and molecular mechanisms of histone demethylases. *Annual Review of Biochemistry*, **79:** 155-179.

Nan, X., Huck-Hui, N.G., Colin, A., Johnson, Carol, D.L., Bryan, M.T., Robert, N.E., Adrian, B. 1998. Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. *Nature*, **393:** 386-389.

Niculescu, M.D., Crciunescu, C.N., Zeisel, S.H. 2006. Dietary choline deficiency alters global and gene specific DNA methylation in developing hippocampus of mouse fetal brains. *FASEB Journal*, **20:** 43-49.

Offield, M.J., Jetton, T.L., Labosky, P.A., Ray, M., Stein, R.W., Margnuson, M.A., Hogan, B.L., Wright, C.V. 1996. PDX-I is required for pancreatic outgrowth and differentiation of rostral duodenum. *Development*, **122:** 983-995.

Okano, M., Bell, D.W., Haber, D.A., Li, E. 1999. DNA methyltransferases Dnmt3a and Dnmt3b are essential for *de novo* methylation and mammalian development. *Cell*, **99:** 247-257.

Pearson, E., Bose, C., Snidow, T. 1992. Trial of vitamin A supplementation in very low birth weight infants at risk for bronchopulmonary dysplasia. *Journal of Pediatrics*, **121:** 420-427.

Pufulete, M., Al-Ghnaniem, R., Khushal, A., Appleby, P., Harris, N., Gout, S., Emery, P.W., Sanders, T.A. 2005a. Effect of Folic acid supplementation on genomic DNA methylation in patients with colorectal adenoma. *Gut*, **54:** 648-653.

Pufulete, M., Al-Ghnaniem, R., Rennie, J.A., Appleby, P., Harris, N., Gout, S., Emery, P.W., Sanders, T.A. 2005b. Influence of folate status on genomic DNA methylation in colonic mucosa of subjects without colorectal adenoma or Cancer. *British Journal of Cancer*, **92:** 838-842.

Razin, A. 1998. CpG methylation, chromatin structure and gene silencing—a three-way connection. *EMBO Journal*, **17:** 4905-4908.

Razin, A., Cedar, H. 1977. Distribution of 5-methylcytosine in chromatin. *Proceedings of the National Academy of Sciences*, **74:** 2725-2728.

Reik, W. 2007. Stability and flexibility of epigenetic gene regulation in mammalian development. *Nature*, **447:** 425-432.

Ross, A.C., Stephensen, C.B. 1996. Vitamin A and Retinoids in antiviral responses. *FASEB Journal*, **10:** 979-985.

Rountree, M.R., Bachmann, K.E., Baylin, J.G. 2001. DNA methylation chromatin inheritance and cancer. *Oncogene*, **20:** 3156-3165.

Schneider, R.B., Bannister, A.J., Myers, F.A., Thorne, A.W., Crane-Robinson, C., Kouzarides, T. 2004. Histone H3 lysine 4 methylation patterns in higher eukaryotic genes. *Nature Cell Biology*, **6:** 73-78.

Shenkin, A. 2004. Basics in clinical nutrition: Trace elements and vitamins in parenteral and enteral nutrition. eSPEN, *the European e-Journal of Clinical Nutrition and Metabolism*, **3:** e293-297.

Sie, K.K., Medline, A., vanWeel, J., Sohn, K.J., Choi, S.W., Croxford, R., Kim, Y.I. 2011. Effect of maternal and post weaning folic acid supplementation on colorectal cancer risk in offspring. *Gut*, **60:** 1687-1694.

Stover, F. 2008. Folate-mediated one-carbon metabolism. *Vitamins and Hormones*, **79:** 1-44.

Stover, P.J. 2011. Polymorphisms in 1-Carbon, meta-

bolism, epigenetics and folate-related pathologies. *Journal of Nutrigenetics and Nutrigenomics*, **4:** 293-305.

Stover, P.J. 2007. Humn nutrition and genetic variation. *Food and Nutrition Bulletin*, **28:** 101-115.

Stover, P.J. 2004. Physiology of folate and vitamin B-12 in helth and disease. *Nutrition Reviews*, **64:** 2-12.

Surani, J.A. 2013. DNA methylation dynamics during the mammalian life cycle. *Philosophical Transactions of Royal Society of London Biological Sciences*, 368.

Taylor, C.G. 2005. Zinc, the pancreas and diabetes: Insights from rodent studies and future directions. *Biometallics*, **18:** 305-312.

Van Mierlo, L.A. 2006. Blood pressure response to calcium supplementation;a meta-analysis of randomized controlled trials. *Journal of Human Hypertension*, **20:** 571-580.

Wade, P.A. 2001. Methyl CpG binding proteins and transcriptional repression. *Bioessays*, **23:** 1131-1137.

Waddington, C.H. 1953. Epigenetics and evolution. *Proceedings of the Society of Experimental Biology*, **7:** 186-199.

Wakefield, L., Boukouvala, S., Sim, E. 2010. Characteriztion of CpG methyltion in the upstream control region of mouse Nat 2: Evidence for a gene environment interaction in the polymorphic gene implicated in folate metabolism. *Gene*, **452:** 16-21.

Waterland, R., Garza, C. 1999. Potential mechanisms of metabolic imprinting that lead to chronic disease. *American Journal of Clinical Nutrition*, **69:** 179-197.

Winter-Vann, A.M., Kanen, B.A., Bergo, M.O., Young, S.G., Melnyk, S., James, S.J., Casey, P.J. 2003. Targeting Ras signaling through inhibition of carboxyl methylation: An unexpected property of methotrexate. *Proceedings of National Academy of Sciences* USA, **100:** 6529-6534.

Pak. j. sci. ind. res. Ser. B: biol. sci. 2018 **61B**(3) 182-186

Review

Probable Ingredients for *Trans* Free Margarine with Omega-3 Fatty Acids

Muhammad Ajmal, Muhammad Nadeem*, Maryam Batool and Imran Taj Khan
Department of Dairy Technology, University of Veterinary and Animal Sciences, Lahore, Pakistan

(received October 20, 2015; revised July 25, 2017; accepted July 31, 2017)

Abstract. Margarine is widely used as table spread, in cooking and bakery products. Awareness of consumers regarding the intake of omega fatty acids has led the food industry to develop foods which are rich sources of omega fatty acids. Harmful effects of *trans* on the development of cardiovascular diseases have steered the researchers to find out wide range of *trans* free options, without compromising on functional and physical properties of fats. Nutritionists recommend margarine for the growing and school going babies, it is usually manufactured from the combination of hard and soft fats, followed by the addition of vitamins A, D and E. However, little is known regarding the supplementation of margarines with omega fatty acids of chia oil. This paper summarizes the physical and chemical characteristics of few ingredients that may be used in the formulation of *trans* free margarine with higher magnitude of omega fatty acids.

Keywords: margarine, omega fatty acids, *trans* free, chia oil

Introduction

Margarine is usually prepared from vegetable fats, the feedstock may originate from partially hydrogenated, interesterified and transesterified. Different categories of margarine are produced at industrial level e.g. table margarine, bakery margarine etc. According to the Codex Standards, margarine should contain at least 80% fat and 16% moisture at maximum (Codex, 2001). Margarine is used as an alternate source of butter. It can be manufactured from a wide range of materials including a combination of hydrogenated and non-hydrogenated stuffs (Richmond, 2007). Ever increasing population has led to a great deal of increase in the production of margarine during the last few decades. Now a days, most of the margarines are made from hydrogenated oils, partial hydrogenation of oil can induce *trans* fatty acids which have many health hazards (Shahidi, 2005). The partial hydrogenation of vegetable oils chemically transforms some of the unsaturated fats into the novel forms of *trans*-fatty acids leading to an increase in the proportion of saturated fats in these oils. *Trans* fatty acids are not required for any biochemical function in human body (Richmond, 2007). Health benefits associated with the intake of omega-3 margarine fortified foods is well known. Increased knowledge of food connected health ailments has considerably increased the demand of functional foods. Healthful properties of margarine can be improved through the fortification of omega-3 fatty acids with *trans* free options. Food is the basic requirement of life. Therefore, it should be safe. Scientific studies have proved that foods are directly related with many diseases such as cancer, cardiovascular diseases, obesity and hypertension etc. (Astrup *et al.,* 2011). Functional foods are getting popularity all over the world and researchers are trying to utilize the non-traditional sources of foods to develop functional foods with added health advantages (Kris-Etherton *et al*., 2002). This paper summarizes the physical and chemical characteristics of ingredients that can be used in the formulation of *trans* free margarine with numerous health benefits of omega-3 fatty acids.

*Author for correspondence; E-mail: muhammad.nadeem@uvas.edu.pk

Chemical composition of margarine. Fat content in margarine ranges from 80 to 90%. Margarine is a water-in-oil emulsion and fat phase is a network of fat crystals and agglomerates of fat with liquid oil entrapped in between. Ingredients like fat soluble flavours, vitamins, colourants and emulsifiers are the contents of fatty phase of margarine. Aqueous phase contains maximum 16% of water and remaining 4% are water-soluble ingredients. To produce the desired quality margarine, it is favourable to have optimum processing and desirable fat blending. Types of margarine are dependent on the solid fat content, melting point of fat, structure and characteristics of margarine (Vereecken, 2010).

***Trans* fatty acids.** Most of *trans* fatty acids enter in body through the intake of partially hydrogenated fats. There is scientific evidence that *trans* fatty acids are not necessary for any physiological function of the human body (Lokuruka, 2007). The concentration of *trans* fatty acids in partially hydrogenated fats depends upon temperature and hydrogen gas pressure. Higher the concentration of nickel catalyst, temperature, gas pressure and catalyst dose, greater would be the degree of geometric isomerization (Shahidi, 2005). As per recommendation of American Heart Association, intake of *trans* fatty acids should not be more than 2g/ day (AHA, 2000). In USA, coronary heart disease is the most common killer, many people are suffering from high blood pressure, stroke and rheumatic fever, with about 41.2% deaths due to cardiovascular diseases (AHA, 2000). Nadeem *et al.* (2014) reported that the concentration of *trans* fatty acids in partially hydrogenated fat was more than 21%. The existence of a strong correlation between harmful HDL cholesterol and *trans* fatty acids has led to the development of large number of *trans* free options. Nadeem *et al*. (2017) showed that margarine prepared from palm oil, palm kernel oil and chia oil blends *trans* fatty acids in margarine. Use of fractionation, inter esterification, trans esterification and blending of fats and oils in appropriate ratios can lead to the successful manufacturing of margarine, partially hydrogenated fat (used commonly in subcontinent) and shortening with similar plasticity, melting point and solid fat index without partial hydrogenation (O'Brien, 2008). Karabulut and Tauran (2006) analyzed 15 types of margarine available in Turkey and showed that concentration of *trans* fatty acids ranged from 8.5% to as high as 39%. Oil processing industries have started focusing on the development of blends which have higher concentration of beneficially unsaturated fatty acids with lower/zero *trans* to minimize the risk of cardiovascular diseases (Miskandar *et al*., 2005).

Table 1. Fatty acid composition of chia oil and some vegetable oils

Fatty acid	Chia oil	Sunflower oil	Soybean oil	Canola oil
C16:0	9.6	6.5	10.2	3.8
C18:0	4.3	1.8	3.8	4.2
C18:1	6.8	3.9	22.8	63.7
C18:2	17.6	68.1	52.8	16.4
C18:3	64.1	1.2	7.6	9.5
Reference	Jin *et al.* (2012)	O'Brien (2008)	Shahidi (2005)	Anwar *et al.* (2007)

Chemical characteristics of chia oil. Chia (*Salvia hispanica* L.) is native to Mexico; it was the staple food in Mexico in pre-historic times. It is an emerging oil seed crop that contains about 35-40% praise worthy oil. Chia oil contains α-linolenic acid up to 68% (Ayerza, 1995). Chia oil also contains significant concentrations of natural antioxidant such as chlorogenic acid, caffeic acid, myricetin, quercetin and kaempferol (Reyes-Caudillo *et al.,* 2008).

Chia oil a powerhouse of beneficial omega fatty acids. Omega-3 poly unsaturated fatty acid are essential fatty acids, which must be consumed through the diet (Nadeem *et al*., 2017). Chia oil is a great source of Omega-3 and Omega-6 fatty acids, which are essential for proper development and functioning of brain and it is also cellular component within the body (Jin *et al.,* 2012). Chia oil is the most efficient source of omega-3 fatty acids. This omega-3 fatty acids obtained from fish have fishy flavour, which limits the application of omega-3 fatty acids in large number of foods. Whereas, omega-3 fatty acids obtained from chia oil is not associated with any flavour defect and unfavourable physiological function (Ayerza *et al.,* 2002). Chia seeds have high content of omega-3 fatty acids, which have been implicated in the reduction of cholesterol level. They prevents blood clotting, improve tissue regeneration, control sugar level in blood, diabetes, cardiovascular diseases, regulate the immune system and development of retina and brain (Vuksan *et al.,* 2007). Eicosapentaenoic acid and decosapentaenoic acid prevent the cardiovascular disease and their daily recommendation is 500 mg for cardiovascular disease risk reduction (Gebauer *et al.,* 2006). A number of medical and epidemiological studies demonstrated that consumption of lipids that contain omega-3 fatty acids reduce the risk of cardiovascular disease (Zhao *et al.,* 2004). Chia oil has good oxidative stability, it was the part of Mexican diet in pre-historic times (Beltran-Orozco and Romero, 2003). Increased intakes of omega fatty acids have been related with low cholesterol level in blood, reduces the blood pressure, prevent the growth of tumors and also decreases the risk of heart attack (Ruiz-Rodriguez *et al.,* 2010). Omega-3 fatty acids are anti-carcinogenic, anti-atherogenic, anti-lipogenic, prevent the hypertension, control immune disorders and

possess immuno-suppressive properties (Williams, 2000). Omega-3 fatty acids have antiarrhythmic, antithrombotic, anti-inflammatory and vasodilator properties. It may also prevent type-2 diabetes and insulin resistance (Lombardo and Chicco, 2006). Docosahexaenoic acid (DHA) which contains 22 carbons and 6 double bonds is the largest unsaturated fatty acid in human body. DHA has been related to alleviation of a number of human illnesses, including heart diseases, cancers and neurological disorders (Stillwell *et al*., 2006). DHA is the major structural fatty acid in nervous system and retina. Pregnant and nursing women should take about 2.6g of omega-3 fatty acids and 100-300 mg of DHA on daily basis. The intake DHA during preschool years may also have a beneficial role in prevention of attention deficit hyperactivity disorder and increase learning ability and academic concern (Gebauer *et al.,* 2006). Concentration of omega-3 fatty acids in margarine can be increased through chia oil, which is a rich source of beneficial omega-3 fatty acids. Nadeem *et al*. (2016a) blended milk fat with high oleic acid fraction of *Moringa oleifera* oil, blends had higher magnitude of unsaturated fatty acids with no *trans* fatty acids. Nadeem *et al*. (2016b) added interesterified *Moringa oleifera* oil in ice cream, physical and oxidative stability characteristics of ice cream were improved without generation of harmful *trans* fatty acids.

Milk fat. Milk fat is usually regarded as precious fat for having appreciable amounts of short-chain fatty acids. The rich natural aroma of milk fat is mainly the contribution of short-chain fatty acids (McSweeney and Fox, 2003). It also contains about 22-26% oleic acid, with scientifically proven health benefits. Further, fats having an appreciable amount of oleic acid are getting a high degree of fame because of large number of health benefits and superior oxidative stability. Fatty acids and triglycerides composition of milk fat restricts its application in large number of bakery and other food products. Milk is mainly used in the manufacturing of butter and butter oil, whereas, partially hydrogenated fats have a wide range of applications in large number of food products (Deffense, 2002). Milk fat is good source of essential fatty acids and fat-soluble vitamins (A, D, E, and K). Short-chain fatty acids in milk fat are mainly responsible for superior sensory characteristics of dairy products, use of milk fat in margarine may improve the sensorial. Milk fat also contains about 1.5-2% omega-6 fatty acids and 0.5-0.7% omega-3 fatty acid (Nadeem *et al*., 2017; 2016a)

Palm oil. Palm oil has melting point 37-39 °C and iodine value is 50-55 (Nadeem *et al*., 2017). Palm oil is solid at room temperature which allows its application in large number of food preparations, such as Vanaspati, margarine, bakery and cake shortenings etc. without partial hydrogenation and generation of *trans* fatty acids. Palm oil is used worldwide in margarine manufacturing and also food products as an ingredient. Palm oil is most important for food industry in manufacturing of *trans* free margarine (Gillespie, 2012). Fatty acid (about 50% saturated and 50% unsaturated) and triglyceride composition of palm oil suggest wide range of *trans* free options (Mukherjee and Mitra, 2009**).** It is rich source of palmitic acid (44%), oleic acid about (40%) and stearic acid 5%. It has high oxidative stability and possesses reasonable amounts of antioxidants like beta-carotene (DeGraef, 2009). It has beneficial health perspectives as it contains no *trans* free fat, high beta-carotene and vitamin E (Henderson and Osborne, 2000). Palm oil has superior thermal stability, therefore, it can be successfully added into large number of bakery products (Foster *et al.,* 2009).

Conclusion

Margarine may be produced from wide range of *trans* free options, such as blending, inter esterification, trans esterification etc. Palm oil, palm olein, palm kernel oil and milk fat may be used for the preparation of *trans* free margarine. Concentration of omega-3 fatty acids in margarine may be improved with chia oil.

Acknowledgement

Financial assistance for this project was provided by Higher Education Commission of Pakistan (HEC/4947/NRPU).

References

AHA,2000. *Cholesterol.Heart and Stroke Guide*. American Heart Association. <http://www. americanheart. org.com > (17 April, 2001).

Anwar, F., Hussain, A.I., Iqbal, S., Bhanger, M.I. 2007. Enhancement of the oxidative stability of some vegetable oils by blending with *Moringa oleifera* oil. *Food Chemistry*, **103:** 1181-1191.

Astrup, A., Dyerberg, J., Elwood, P., Hermansen, K., Hu, F.B., Jakobsen, M.U., Kok, F.J., Krauss, R.M., Lecerf, J.M., LeGrand, P., Nestel, P., Riserus, U., Sanders, T., Sinclair, A., Stender, S., Tholstrup, T., Willett, W.C. 2011. The role of reducing intakes

of saturated fat in the prevention of cardiovascular disease: where does the evidence stand in 2010? *American Journal of Clinical Nutrition*, **93:** 684-688.

Ayerza, R., Coates, W., Lauria, M. 2002. Chia as an omega-3 fatty acid source for broilers influence on fatty acid composition, cholesterol and fat content of white and dark meat, on growth performance and on meat flavour. *Poultry Science*, **81:** 826-837.

Ayerza, R. 1995. Oil content and fatty acid composition of chia (*Salvia hispanica* L.) from five northwestern in Argentina. *Journal of American Oil Chemists Society*, **72**:1079- 1081.

Beltran-Orozco, M.C., Romero, M.R. 2003. La chia, alimento milenario. Mexico, Departamento de Graduados e Investigacion en Alimentos, E. N. C. B., I. P. N, Mexico.

Codex Alimentarius. 2001. Codex general standard for margarine. vol **8**, 2nd edition, FAO & WHO, Remo, Italy.

DeGraef, V. 2009. Microstructural Properties of Isothermal Palm Oil Crystallization. *PhD Thesis*. Ghent University, Belgium. 181pp.

Deffense, E. 2002. Fractionation of butterfat, In: *Oils and Fats Series*, Ch.5. *Dairy Fats*, Edn. B. Rossell.

Foster, R., Williamson, C.S., Lunn, J. 2009. Culinary oils and their health effects. *Nutrition Bulletin*, **34:** 4-47.

Gebauer, S.K., Psota, T.L., Harris, W.S., Kris-Etherton, P.M. 2006. Omega-3 fatty acid dietary recommendations and food sources to achieve essentiality and cardiovascular benefits. *American Journal of Clinical Nutrition*, **83:** S1526.

Gillespie, P. 2012. The challenges of corporate governance in Indonesian oil palm opportunities to move beyond legalism? *Asian Studies Review*, **36:** 247-269.

Henderson, J., Osborne, D.J. 2000. The oil palm in all our lives: how this came about. *Endeavour*, **24:** 63-68.

Jin, F., Nieman, D.C., Sha, W., Xie. G., Qiu, Y., Jia, W. 2012. Supplementation of milled chia seeds increases plasma ALA and EPA in postmenopausal women. *Plant Foods for Human Nutrition*, **67:** 105-110.

Kamla-Raj. 2009. Health effects of palm oil. *Journal of Human Ecology*, **26:** 197-203.

Karabulut, I., Tauran, S. 2006. Some properties of margarines and shortenings marketed in Turkey. *Journal of Food Composition and Analysis*, **19:** 55-58.

Kris-Etherton, P.M., Hecker, K.D., Bonanome, A., Coval, S.M., Binkoski, A.E., Hilpert, K.F., Griel, A.E., Etherton, T.D. 2002. Bioactive compounds in foods: their role in prevention of cardiovascular disease and cancer. *American Journal of Medicine*, **113:** 71-88.

Lokuruka, M.N.I. 2007. Role of fatty acids of milk and dairy products in cardiovascular diseases: A review. *African Journal of Food, Agriculture, Nutrition and Development*. **7:** 1-16.

Lombardo, Y., Chicco, A.G. 2006. Effects of dietary polyunsaturated omega-3 fatty acids on dyslipidemia and insulin resistance in rodents and humans. A review. *Journal of Nutrition and Biochemistry*, **17:** 1-13.

McSweeney, Paul, L.H., Fox, P.F. 2003. *Advanced Dairy Chemistry, Proteins*. Parts A&B, vol. **1,** 3rd edition, Kluwer Academic Plenum Pub, NY, USA.

Miskandar, M.S., Che Man, Y.B., Yusoff, M.S.A., Rahmann, R.A. 2005. Quality of margarine: fats selection and processing parameters. *Asia Pacific Journal of Clinical Nutrition*, **14:** 387-395.

Mukherjee, S., Mitra, A. 2009. Health effects of palm oil. *Journal of Human Ecology*, **26:** 197-203.

Nadeem, M., Imran, I., Taj, I., Ajmal, M., Junaid, M., 2017. Omega-3 fatty acids, phenolic compounds and antioxidant characteristics of chia oil supplemented margarine. *Lipids in Health and Disease*, **16:** 102.

Nadeem, M., Rahman Ullah. 2016a. Enhancement of oleic acid in butter oil by high oleic fraction of *Moringa oleifera* oil. *Pakistan Journal of Scientific and Industrial Research. Ser. B: biol. sci*. **59:** 105-110.

Nadeem, M., Rahman Ulla and Ansar Ullah. 2016b. Improvement of the physical and oxidative stability characteristics of ice cream through Interesterified *Moringa oleifera* oil. *Pakistan Journal of Scientific and Industrial Research*. *Ser. B: biol. sci.* **59:** 38-43.

Nadeem, M., Situ, C., Mahmud, A., Khalique, A., Imran, M., Rahman, F., Khan, S., 2014. Antioxidant activity of sesame (*Sesamum indicum* L.) cake extract for the stabilization of olein based butter. *Journal of the American Oil Chemists Society*, **91:** 967- 977.

O' Brien, R.D. 2008. *Fats and Oils*: *Formulating and Processing for Application*. 3rd edition, 680 pp., CRC Press; Taylor & Francis Group, FL, USA.

Reyes-Caudillo, E., Tecante, A., Valdivia-López, M.A.

2008. Dietary fibre content and antioxidant activity of phenolic compounds present in Mexican chia (*Salvia hispanica* L.) seeds. *Food Chemistry*, **107:** 656-663.

Richmond, H.D. 2007. *Dairy Chemistry, A Practical Handbook for Dairy Chemists and Others having Control of Dairies*. 3rd edition, C Griffin & Co., London, UK.

Ruiz-Rodriguez, A., Reglero, G., Ibanez, E. 2010. Review-recent trends in the advanced analysis of bioactive fatty acids. *Journal of Pharmacy and Biomedicine Analysis*, **51**:305-326.

Shahidi, S. 2005. *Baileys' Industrial Edible Oil and Fat Products*. 6th edition, John Wiley and Sons, Pub. Co., NY, USA.

Singh, M. 2003. Nutrition, brain and environment. How to have smarter Babies? *Indian Pediatrics*, **40**: 213-220.

Stillwell, W., Shaikh, S.R., LoCascio, D., Siddiqui, R.A., Seo, J., Chapkin, R.S., Wassall, S.R., Hauppage. 2006. The role of polyunsaturated lipids in membrane raft function. *Scandinavian Journal of Food and Nutrition*, **50:** 107-113

Vereecken, J. 2010. Effect of Acylglycerol Composition on the Microstructural and Functional Properties of Bakery Fats and Margarines. *Ph.D Thesis*, Ghent University, Belgium. 251pp.

Vuksan, V., Whitman, D., Sievenpiper, J., Jenkins, A., Rogovik, A., Bazinet, R., Vidgen, E., Hanna, A. 2007. Supplementation of conventional therapy with the novel grain Salba (*Salvia hispanica* L.) Improves major and emerging cardiovascular risk factors in type 2 diabetes. *Diabetes Care*, **30:** 2804-2810.

Williams, C.M. 2000. Dietary fatty acids and human health. *Annales de Zootechnie,* **49:** 165-180.

Zhao, G., Etherton, T.D., Martin, K.R., West, S.G., Gillies, P.J., Kris-Etheron, P.M. 2004. Dietary linolenic acid reduces inflammatory and lipid cardiovascular risk factors in hypercholesterolemic men and women. *Journal of Nutrition*, **134:** 2991-2997.

Pakistan Journal of Scientific and Industrial Research
Series B: Biological Sciences
Volume 61

Contents

Series B: Biological Sciences
Vol. 61, No.1, January - April, 2018

Short Communication

Vol. 61, No.2, May - August, 2018

Short Communication

Review

Vol. 61, No.3, September - December, 2018

Special Paper

Reviews

Pakistan Journal of Scientific and Industrial Research
Series B: Biological Sciences
Volume 61

Author Index

Pakistan Journal of Scientific and Industrial Research
Series B: Biological Sciences
Volume 61

Subject Index

INSTRUCTIONS TO AUTHOR

Pakistan Journal of Scientific and Industrial Research, Series A: Physical Sciences and ***Series B: Biological Sciences publish original researches as full research papers, short communications and reviews papers. The manuscript written in clear, concise English will be accepted for review. Authors who are not native English speakers should note that the manuscript must be edited for linguistic errors by some professional experts.***

The manuscripts will be entertained covering the following subjects of Physical and Biological Sciences: Agriculture, Agronomy, Biochemistry, Biotechnology, Botany, Chemistry, Ecology, Environmental Sciences, Epidemiology, Food Sciences, Genetics, Geography, Geology, Microbiology, Nanotechnology, Natural Products, Pharmaceutical Sciences, Physics, Proteomics, Radiation, Soil Sciences, Spectroscopy and Applied Sciences, Toxicology, Veterinary Sciences and Zoology. However, we do not entertain articles of pure Mathematics, Computer Sciences, pure Engineering and Medical Sciences.

All manuscripts are evaluated by subject experts of advanced countries. However, authenticity of the contents remains the sole responsibility of the author/s. The Journal reserves the rights to edit, wherever considered necessary to make contribution harmonized with the format of the journal.

Manuscripts should be submitted online *via* PJSIR website:

- Simply log on to our website and follow the onscreen instructions for all submissions (you will need to register before your first submission to PJSIR).
- If you have any technical problems or questions related to the electronic submission process or uploading your files, please contact to our Editorial Office.

Types of Manuscript:

The journal publishes **(1) Regular manuscripts, (2) Short communication and (3) Reviews.**

(1) Regular Manuscripts: The manuscript being submitted must consist of original research performed by the authors and the research must include new information that is significant.
(2) Short Communications: Short communication should contain description of findings in a concised form that would be of interest to readers of the journal. The material should be formatted as per PJSIR style and should not exceed 1500 words/3 printed pages.
(3) Reviews: *Regular Reviews* : Reviewing scientific discoveries including the recent results of author(s) and articles must having total length of the paper along with references approximately 10 printed pages. ***Invited Reviews*:** Submitted by invitation from the Editorial Board and encompass recent important scientific discoveries of approximately 10 printed pages (including tables, figures and references).

Prerequisites for Publication:

- **Certificate:** A certificate must be submitted that the paper has neither been submitted nor will be sent to any other journal after submitting to PJSIR.
- **Co-authors verification:** Author should certify that the co-author(s) has / have been informed about the submission of the paper to the journal.

- **Reviewers' names:** The author should submit at least four experts and their e-mail for review of the paper out of their country.
- **Illustrations/photographs:** It should be prepared for reproduction as halftones or line drawings. All illustrations are to be submitted in complete and finished form, with adequate labeling. Photographs in JPEG/TIFF format preferably direct from original with minimum reduction and clear resolution. Symbols and lines should be chosen to remain legible after the degree of reduction that will be used (single column 7.5:double column 16 cm) for better results.
- **Copyright:** PJSIR owns all copyright to any work published in the Journal. Any material submitted, whether appearing in the journal or not, may not be used, reproduced, or transmitted without the written consent of PJSIR.
- **Plagiarism Report:** PJSIR observes/follows HEC guidelines/Criteria for plagiarism. It is the author's responsibility to apprise themselves of plagiarism in any form. All the papers submitted to PJSIR should be accompanied by Turnitin Plagiarism Report up to 19% similarity index. If plagiarism report has been taken earlier, submit a certificate for the same.
- **Conflict of interest/Financial disclosure statement:** At the time of submission declaration must be submitted that the authors have no conflict of interest. Authors should disclose in the online submission system any commercial affiliation or consultancy that could be construed as a conflict of interest with respect to the submitted data.
- **Authors' contribution:** All papers submitted to PJSIR should be accompanied with statement describing contributions of each author of the paper.

Manuscript preparation:

- Manuscripts should be submitted with double spaced with proper margin on left and right side preferably in MS-Word (version 2003 and upwards) in 12-point size font on A4 size sheet with pages numbers from 1st to last in the bottom of the paper .Author/s may submit electronic copies in Ms Word via e-mail or through our website.
- **Cover letter:** Manuscript should be submitted with a covering letter at every time of submission.
- **Title of the paper:** The manuscript should contain title of the paper and unabbreviated names of all authors with their complete postal addresses e-mail and phone numbers. An asterisk (*) should be added to the right of the corresponding author's name.
- **Abstract:** The manuscript should contain abstract (not more than 300 words for research paper and 150 words for short communication).
- **Key words:** Up to six keywords identifying the nature of the subject matter may be used to alert readers. Key words should be listed below the summary of the paper.
- **Introduction:** The section should contain a clear statement of the purpose of work, the reasons for undertaking the research and pertinent background of the study that allows readers outside the field to understand the purpose and significance of the work.
- **Materials and Methods:** Description of methods should be brief, but with sufficient details to enable the experiments to be repeated by the readers. The design of the study or experiments, any specific procedures used, and statistical analyses must be described clearly and carefully. References to other papers describing the techniques may be given. The name and location (city and state/country) of commercial suppliers of uncommon chemicals, reagents, or instruments should be mentioned.
- **Results and Discussion:** The results and discussion should be presented concisely. Tables and figures should be used only if they are essential for the comprehension of the data. The purpose of the discussion is to interpret the results and to relate them to existing knowledge in the field.

- **Conclusion:** Conclusion should be provided under separate heading and highlight new aspects arising from the study. It should be in accordance with the objectives.
- **Tables:** Tables should be numbered (Arabic numerals) in the order in which they are referred to in the text. Each Table should have brief title, be on a separate page, and be double-spaced throughout. Non-standard abbreviations should be used sparingly and must be defined in a legend at the bottom of the Table. For Table title, use lower case letters with only the first letter capitalized.
- **Figures:** All illustrations (line drawings and photographs) are classified as figures. Digital photograph files should have a resolution of at least 300 dpi. Figures should be cited in consecutive order in the text. The abbreviation "Fig." should be used (e.g., (Fig. 1), If a figure consists of multiple parts, letters (a, b, c, etc.) should be used to label them (e.g**,** Fig. 1a). Legends for the figures should be double-spaced, in numerical order, and on a separate page. Non-standard abbreviations should be defined in legends. For figure title, use lower case letters with only the first letter capitalized.
- **Units:** IUPAC rules for units and their abbreviations should be used, i-e length (m, cm, mm, µm, nm, Å), mass (kg, g, mg, µg, ng pg, mol, mmol, µmol), volume (L, mL, µL), time (s, min, h, d), temperature (°C, °K), radiation (Bq, dpm, Gy, Sv), and concentration (M, mM) mol/L, mmol/L, mg/mL, (v/v), (w/v), ppm, ppb).
- **Spectral and elemental analysis data:** Please report spectral and elemental analysis data in the following format. ^{1}H-NMR ($CDC1_3$) δ : 1.25 (3H, D, J=7.0Hz) 3.55 (1H, q, J=7.0Hz), 6.70 (1H, m) ^{13}C-NMR ($CDC1_3$) δ :20.9 (q) 71.5 (d), 169.9 (s). IR (KBr) cm^{-1}: 1720, 1050, 910 UV λmax (EtOH) nm (ε) : 241 (1086), 288 (9380). UV λ_{max} (H_2O)nm(log ε): 280 (3.25). FAB-MS *m/z*: 332.1258 (Calcd for $C_{18}H_{20}O_6$: 332.1259). MS *m/z*:332 (M^+) 180, 168, $[\alpha]^{23}_D$ – 74.5 (c=1.0 MeOH). Anal. Calcd for $C_{19}H_{21}NO_3$:C, 73.29; H, 6.80; N, 4.50 Found: C, 73.30; H, 6.88: N, 4.65.
- **References formation:** All references should be as per PJSIR format, and cited in the text by the last name of the author and year into a chronological descending order. All references in the bibliography should be listed in alphabetical order of the authors' last names followed by date of publication and other complete details with full names of Journals as given below:

In "Text" References :

Park (2005), Aksu and Kabasakal (2004) and Evans *et al.* (2000)
(Park, 2005; Aksu and Kabasakal, 2004; Evans *et al.,* 2000)

In "List" References :

Evans, W.J., Johnson, M.A., Fujimoto, Cy. H., Greaves, J. 2000. Utility of electrospray mass spectrometry for the characterization of air-sensitive organolan-thanides and related species. *Organometallics,* **19:** 4258-4265.

For Standards:

ASTM, 2007. Standard Test Method for the Determination of Iron Ion Ores and Related Materials (E247-OD) P.O. Box C700, West Conshohocken, USA, *Annual Book of ASTM Standards*, vol, 30.5 pp.163-165.

PCRWR, 2007. *Water Quality Monitoring, Fifth Monitoring Report* 2005-6 . ISBM 978-969-8469-184, Pakistan Council of Research in Water Resources. Pakistan

Books:

Cinar, A., Parulekar, S.J., Undey, C., Birol, G. 2003. *Batch Fermentation: Modeling, Monitoring, and Control,* 250 pp.Marcel Dekker Inc., New York, USA.

Chapters in Edited Books:

Newby, P.J., Johnson, B. 2003. Overview of alternative rapid microbiological techniques. In: *Rapid Microbiological Methods in the Pharmaceutical Industry,* M.C. Easter (ed.), vol. **1**, pp. 41-59, 1st edition, Interpharm / CRC, Boca Raton, Florida, USA.

Papers in Proceedings:

Marceau, J. 2000. Innovation systems in building and construction and the housing industry in Australia. In: *Proceedings of Asia-Pacific Science and Technology Management Seminar on National Innovation Systems,* pp. 129-156,Japan Int. Sci. Technol. Exchange Centre, Saitama, Japan.

Reports:

*SIC, 2016. Annual Report, 2014-15,*Scientific Information Centre, Pakistan Council of Scientific and Industrial Research, PCSIR Laboratories Campus, Shahrah-e-Dr. Salimuzzaman Siddiqui, Karachi, Pakistan.

Thesis:

Saeed, A. 2005. Comparative Studies on the Biosorption of Heavy Metals by Immobilized Microalgal Cultures, Suspended Biomass and Agrowastes. *Ph.D. Thesis*, 248 pp., University of the Punjab, Lahore, Pakistan.

Patents:

Young, D.M. 2000. Thermostable Proteolysis Enzymes and Uses Thereof in Peptide and Protein Synthesis, US Patent No. 6,143,517, 7th November, 2000.

Data Set from a Database: (information should be retrievable through the input)

Deming, D; Dynarski, S. 2008. The lengthening of childhood (NBER Paper 14124) Cambridge, MA: National Bureau of Economic Research, Retrieved July 21, 2008 from http://www.nber.org/papers/w14124) DOI No.....

Other Important Points:

1. In general, after submission of a paper, no authors may be added deleted or replaced with other names from the paper, and the order of the names of the authors cannot be changed at any stage i.e. modification, revision and galley proof.
2. The authors are given an opportunity to proofread the galley of an accepted manuscript. No additions and revisions are allowed other than the correction of typographical errors.
3. No reprints will be sent. Single hard copy will be sent to the corresponding author and co-authors may subscribe it. Full-text will be available on our website.
4. Any change in postal address or e-mail of the corresponding author should be communicated immediately to the Editorial Office.

- **Publication fees:** At the time of submission of an article, the author has to submit Publication fees of the paper PKR. 2000/= (through Cross Cheque in favour of **PCSIR NIDA 16-3, NBP** and send at the address given below).

The manuscripts should be addressed to :

The Executive Editor
Pakistan Journal of Scientific and Industrial Research
Scientific Information Centre, PCSIR Laboratories Campus,
Sharah-e-Dr. Salimuzzaman Siddiqui, Karachi-75280, Pakistan
Phone: (92-21)-99261914,16,17,99261949 **Fax:** (92-21) 99261913
Web: http://www.pjsir.org; **E-mail:** info@pjsir.org

Pakistan Journal of Scientific & Industrial Research (PJSIR)

Call For Papers

Pakistan Journal of Scientific and Industrial Research (PJSIR) is the premier research journal of Pakistan and has been publishing for the last 60 years, comprising of original research papers in the fields of Physical and Biological Sciences. Since January 2011, the Journal has been bifurcated and now published bimonthly i.e. 6 issues in a year in two series as follows:

Series A: Physical Sciences (appearing as issues of January-February, May-June and September-October) including articles of Physics, Physical and Analytical Chemistry, Geology, Geography, Environmental Sciences, Technology) and

Series B: Biological Sciences (appearing as issues of March-April, July-August and November-December) including articles of Agriculture, Botany, Zoology, Microbiology, Biochemistry, Biological Chemistry, Genetics, Food Sciences, Pharmaceutical Sciences, Environmental Sciences).

PJSIR is an interdisciplinary open access peer-reviewed refereed journal and complies with the international standards and ethics of scientific community.

We publish original primary research articles/Review papers related to Biological Sciences, Physical Sciences and Technology. However, we do not entertain articles of Pure Mathematics, Computer Sciences, Social/Management Sciences and Medical Sciences. You may send it via e-mail info@pjsir.org as well as through our website www.pjsir.org.

CALL FOR PAPERS

Please submit your research papers for upcoming volume 62 of PJSIR for the year 2019.

Pakistan Journal of Scientific and Industrial Research

PCSIR - Scientific Information Centre

PCSIR Laboratories Campus, Shahrah-e-Dr. Salimuzzman Siddiqui, Karachi - 75280, Pakistan

Ph: 92-21-99261949-99261917, Fax: 92-21-99261913, E-mail: info@pjsir.org Website: www.pjsir.org

EXCHANGE FORM

We wish to receive Pakistan Journal of Scientific and Industrial Research Ser. A: Phys. Sci: and/or Ser. B: Biol. Sci. in exchange of :

Name of Journal: _____
Frequency: _____
Subjects Covered: _____
Institution: _____
Address: _____
_____
_____

Signature: _____
Name: _____
Designation: _____
Date: _____
E-mail: _____
Fax: _____
Phone: _____

Pakistan Journal of Scientific and Industrial Research

PCSIR - Scientific Information Centre

PCSIR Laboratories Campus, Shahrah-e-Dr. Salimuzzman Siddiqui, Karachi - 75280, Pakistan

Ph: 92-21-99261949-99261917, Fax: 92-21-99261913, E-mail: info@pjsir.org Website: www.pjsir.org

SUBSCRIPTION FORM

I / we wish to subscribe to 'Pakistan Journal of Scientific and Industrial Research' Ser. A: Phys. Sci: and/or Ser. B: Biol. Sci. The filled in proforma is being returned for compliance.

Subscriber's data:

Name: _____
Address: _____
_____
_____
E-mail: _____
Fax: _____
Phone: _____

Signature: _____
Order Membership No. (if any): _____

Tick the relevant box: ☐ Send invoice ☐ Bill later on ☐ Cheque for .....enclosed

RevisedSubscription Rates:
Local: Rs. 2500/ = per volume; Rs. 425/ = per copy
Foreign: US$ 450/ = per volume; US$ 75/ = per copy

Payment should be made through cross cheque in favour of PCSIR NIDA 16-3 NBP and mailed to the Director PCSIR - Scientific Information Centre, PCSIR Laboratories Campus, Shahrah-e-Dr. Salimuzzaman Siddiqui, Karachi-75280, Pakistan.