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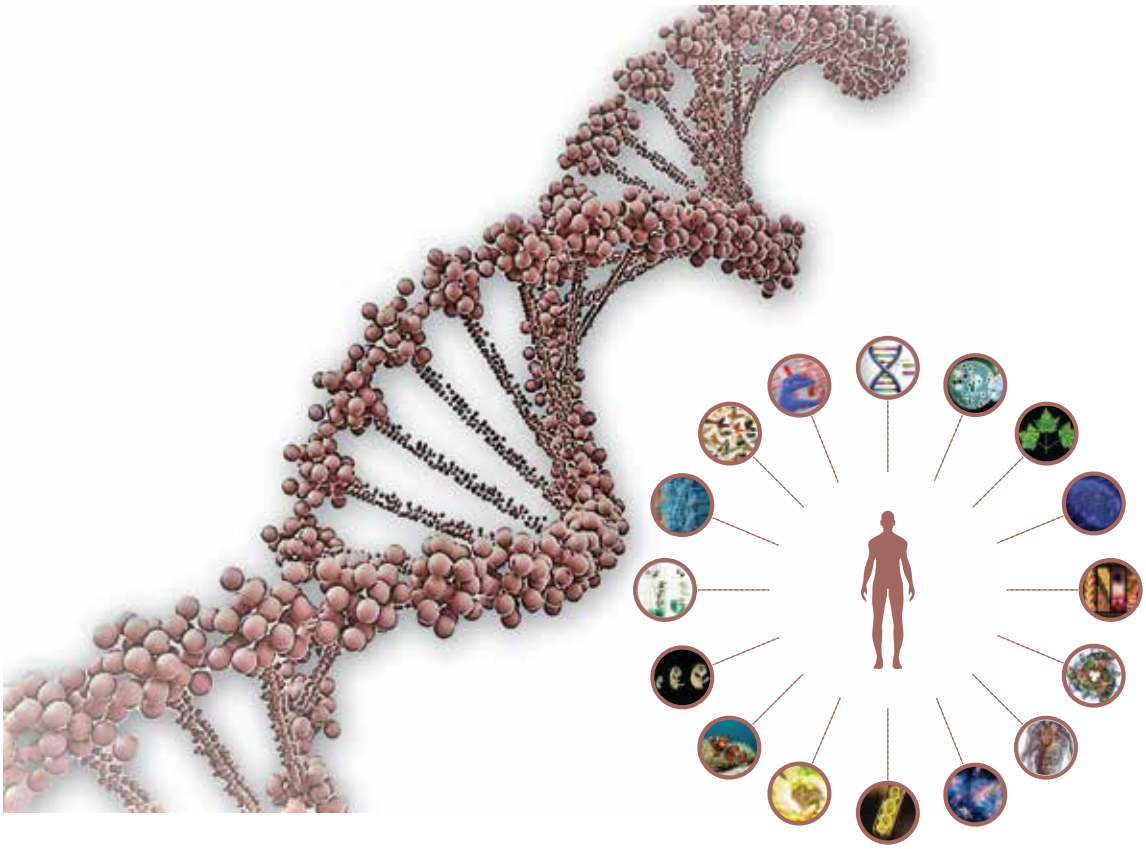

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Evaluation of Water Quality of Nearby Village During Process of Composting at Industrial Scale

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ABSTRACT: *The Lahore Compost Private Limited operates a composting plant. This company utilizes tons of organic waste to make a conditioner and organic fertilizer. The organic waste is transported to the Mehmood booti , landfill site ring road Lahore. This study was conducted to evaluate the parameters that effect the water quality of nearby village due to process of composting takes place in composting plant. The results from this study concluded that composting plant do not affect the water quality and all physical, chemical parameters are according to the WHO standards.*

Keywords: *LCL, Composting, Organic waste, Muncipal waste*

INTRODUCTION

The Lahore Compost (Private) Limited (LCL), part of the Saif Group of Companies, is operating a composting plant utilizing organic component of the municipal solid waste collected and transported to the Mehmood Booti landfill site, Ring Road, Lahore. The composting facility has been developed under the exclusive concession awarded by the City District Government Lahore (CDGL). The awarded concession required LCL to build, own, operate and transfer the compost fertilizer after twenty years from the date of award of such mandate. In March 2006, company inaugurated the production facility installed by M/s Menart of Belgium. About 8,000 ton of municipal waste is generated daily in Lahore; with a moderate estimate of over 50 percent organic content the need for final disposal could be reduced substantially by large scale composting. LCL operates the first large scale composting plant in the country with ability for further expanding composting of municipal, garden, and farm waste. The LCL facility is located within the premises of the Mehmood Booti dumping site the largest of the three managed landfills in Lahore. LCL has adopted (Windrow Technology) aerobic composting process. Windrows are formed on rectangular platform. Regular turning of the windrow are done to ensure availability of oxygen (Stombaugh and Nokes, 1996). Hence turning of windrows at fixed intervals are strictly followed (Yamada and Kawase, 2006). The effluent basin situated beside the windrow pad where effluent from the composting pad is collected to be sprinkled back on the windrows

to moisten the compost in dry periods (Bruns-Nagel et al., 1998). LCL is an environmentally alive organization having all basic facilities to fulfill the health and safety requirements of employees and community. Similarly LCL is conducting all those tests which are necessary for ambient air monitoring and water quality monitoring. LCL is following all its SOPs starting from receiving of waste to transport of compost in the market.

Solid, liquid and air emission are the products of every community. The liquid water can be used to supply the community after using different methods to clean it. Untreated waste water contains various pathogens that are extremely harmful. These pathogens can be incorporate toxicity in human intestinal tract. The waste water also contains many mutagens or different compounds that causes mutagenesis and also cause various types of cancer. This type of water is also very harmful for the growth of healthy plant and form it to toxic plant (Tchobanoglous and Buron 1991).

Now a days, long term health effects caused by waste water are emphasized by the scientists.

The process of composting takes place in the presence of 50% moisture content. The treatment of organic waste at industrial level may add pollutants in the form of heavy metals or pathogens released from composting pile and effect the nearby community in different forms (Sharma et al., 1997). The water can be polluted by composting plant.

MATERIALS AND METHODS

The composting of municipal solid organic waste was done in Lahore compost Pvt Ltd plant near Mahmood Booti Lahore. 8000 tons of municipal solid waste was sorted and divided in to eight windrows. Each windrow contains 1000 tons of municipal organic waste. The C: N was optimized for each windrow by adding cow dung and saw dust (Table 1).

Table 1: Optimized C: N for each windrow

Windrows	Ingredients	Optimized C:N
1	Municipal solid waste +Cow Dung+ Saw Dust	33:1
2	Municipal solid waste +Cow Dung+ Saw Dust	31:1
3	Municipal solid waste +Cow Dung+ Saw Dust	33:1
4	Municipal solid waste +Cow Dung+ Saw Dust	32:1
5	Municipal solid waste +Cow Dung+ Saw Dust	32:1
6	Municipal solid waste +Cow Dung+ Saw Dust	33:1
7	Municipal solid waste +Cow Dung+ Saw Dust	31:1
8	Municipal solid waste +Cow Dung+ Saw Dust	30:1

The process of composting initiated with moisture % ranging from 48.34 and 50.34, it was provided to each windrow at the start of process (Table 2).

Table 2: The adjusted moisture content of each windrow

Windrow No.	Ingredients	Moisture %
1	Municipal solid waste + Cow Dung+ Saw Dust	50.08
2	Municipal solid waste + Cow Dung+ Saw Dust	49.14
3	Municipal solid waste + Cow Dung+ Saw Dust	48.34
4	Municipal solid waste + Cow Dung+ Saw Dust	49.08
5	Municipal solid waste + Cow Dung+ Saw Dust	50.14
6	Municipal solid waste + Cow Dung+ Saw Dust	49.94
7	Municipal solid waste + Cow Dung+ Saw Dust	49.08
8	Municipal solid waste + Cow Dung+ Saw Dust	50.14
8	Screening Material + Cow Dung + Green Waste +paper	50.34

The aeration was provided by proper turning of windrows with the help of wheel loaders. The temperature of windrow rise and the thermophilic stage dominated, when the process started (Pietro and Paola, 2004). The process of composting took three months to completely degrade waste. The samples of water were taken from the nearest village to check the phytotoxicity and pathogenicity present in water due to composting plant. Physical and chemical analysis of water was performed. Physical analysis were based on the color, odor, TDS, turbidity and taste of the water. Chemical analysis was done by using waste water analysis kit. Conductivity, hardness, chloride concentration, magnesium, calcium and alkalinity was checked in chemical analysis.

PHYSICAL ANALYSIS

Taste: 10 ml of water sample was taken in sterile beaker. One drop was tasted with the help of dropper.

Odor: 15 ml of water was taken in a glass flask and left for almost 2 hours and checked for odor.

Turbidity: 5 ml water sample was taken in test tube and compared with sterilized water for any turbidity

Total Dissolved Solids: 100 ml of water sample was taken in 200 ml beaker and checked for TDS.

CHEMICAL ANALYSIS

The water testing kit was used for chemical analysis.

RESULTS AND DISCUSSION

The results showed that there is no pathogenicity and phytotoxicity was found in

water sample taken from nearby village and residential area according to WHO standards. The pH value according to WHO standards must ranging from 6.5-9.2. The results showed the pH range 6.70 in October, 7.20 in November and 7.2 in December. The pH value was not increased up to 9.2. The water was colorless. The turbidity was ranging from 0.7 N.T.U to 1 N.T.U and must follow the WHO standards. The amount of TDS as low in experimental water ranging from 250 to 365 mg/l. the amount of calcium was ranging from 40-52 mg/i. The amount of magnesium was observed 25 mg/l in October, 22mg/l in November and 192 mg/l in December and fall under the WHO standards. Total hardness was ranged from 140 mg/l to 200 mg/l. The estimated amount of chloride was recorded 40-35 mg/l.

Table 3: water quality test results

Sr. #	Parameter	WHO Standard	October 2014	November 2014	December 2014
1	pH	6.5 – 9.2	6.70	7.20	7.2
2	Color	50 units	Colour Less	Color Less	Color Less
3	Turbidity	25	0.7 N.T.U.	1 N.T.U.	0.7 N.T.U.
4	Odor	Odorless	Odorless	Odorless	Odorless
5	Total Dissolved Solids	1500	365 (mg/l)	350(mg/l)	250 (mg/l)
6	Calcium	200	40 (mg/l)	52 (mg/l)	52 (mg/l)
7	Magnesium	150	25 (mg/l)	22 (mg/l)	192 (mg/l)
8	Total Hardness	500	200 (mg/l)	220 (mg/l)	140 (mg/l)
10	Chloride	600	30 (mg/l)	40 (mg/l)	35 (mg/l)
12	Taste Less	Taste Less	Taste Less	Taste Less	Taste Less

Composts produced from municipal solid waste (MSW) contain trace amounts of metals and metalloids and it may affect the water quality by incorporating these heavy metals and metalloids. When this compost mixed with plant and water causes phytotoxic effects. These metals effect the quality of water and make it unhygienic for drinking purposes, this toxic water can be able to cause many diseases that could be lethal for the individual (Smet et al., 1999). Most plant species incorporate small amount of cadmium, but uptake of cadmium from MSW causes compost amended soils by species that most readily accumulate cadmium has not been examined under field conditions. Some mushroom species can accumulate cadmium and mercury from MSW compost (Liu et al., 2007) and it could have some lethal affects. The average values of lead, copper, and zinc in MSW composts may exceed limits recommended to protect invertebrates in soil when Compost is provided to plant, but these limits may be conservative. There is some evidence that metals in MSW composts can harm some soil microbiota, but such effects have not always been found (Parkinson et al., 2004). Metal concentrations in MSW compost leachates can exceed U.S.A. and E.E.C. drinking water standards, but under field conditions subsoil will presumably serve as a sink for metals, at least for many decades.

CONCLUSION

The LCL is utilizing tons of organic waste to make end product called compost. This compost having a wide value in agriculture sector, it can be used to condition the soil and can also be combine with other fertilizers to get better crop yield. The tons of

waste are transported to landfill site of Lahore compost (Mehmood Booti). However, this Composting plant could affect the environment in near future if proper measures are not taken to make it better. This study revealed that the LCL plant do not affect the water quality of nearby villages and LCL site so far. There is need of some precautionary measures to secure our environment in future.

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Antimicrobial Efficacy of Cinnamon Oil for the Production of Muffins

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ABSTRACT: *There is a renewed interest in the antimicrobial properties of spice oil. Spice plants have been shown to possess medicinal significance. Cinnamon oil has high antimicrobial activity. Oil could act as preservatives by eliminating food-borne pathogens. Antimicrobial activity of the oil extracted from Cinnamomum verum was tested in vitro against four microbial species by following well diffusion assay. Two gram positive (Bacillus cereus, Staphylococcus aureus) and two gram negative (Escherichia coli, Salmonella sp.) were selected in the first phase of the research project. Minimum Inhibitory Concentration (MIC) ranges were calculated by micro-broth dilution method. During second phase, effect of oil was checked by adding it in different ratio in muffins. Muffins were incorporated with 0.2 %, 0.4%, 0.6%, 0.8% and 1% of the oil. Sensory analysis of the muffins was assessed. Storage stability was checked by performing sensory evaluation with the interval of five days upto fifteen days. Data obtained from microbial and sensory analysis was evaluated through two way and one way analysis of variance, respectively at a confidence level of $P \leq 0.05$. The separation of means was done by applying Duncan Multiple Range test (DMRt). Stability test indicated superb preservative properties of the incorporated oil on muffins at ambient temperature.*

Keywords: *Cinnamon oil; Antimicrobial activity; Food spoilage; Natural preservatives*

INTRODUCTION

Currently due to the increased susceptibility of fresh food towards the microbial stresses, it has become more important to develop certain techniques that can be effectively used against the fresh food spoilage microorganisms. Traditionally, spices have proved to be potential antimicrobial and antifungal agents. Thus we tried to evaluate the essential oil of different natural agents for its antimicrobial activity. The essential oil of aromatic plants and their components have a wide range of applications in ethnic medicine, preservation, food flavoring, fragrances and perfume industries (Ahmad et al., 2013). Therefore, considerable attention has been focused on the various biological effects of these naturally occurring agents. Among them is the cinnamon essential oil and its constituents which are known to possess various antifungal and antimicrobial activities.

Cinnamon, a spice, is commonly used in sweet and savory and baking. Its oil is commonly used in the food industry because of its aroma (Babu et al*.,* 2011). Of all the available species of cinnamon *Cinnamomum verum* had been focused in this research. Cinnamon spice has extracts, oils (EOs), resins, cinnamic acid, cinnamate and cinnamaldehyde. It has been revealed to impart antioxidant and strong antimicrobial effects in food products, in which active substances are phenolic constituents Raventos (2014). General composition of cinnamon bark includes 20.3% fiber, 59.5% carbohydrates, 9.9% moisture, 4.6% protein, 2.1% fat and 3.4% total ash (Ariamuthu et al*.,*2013). Spice oils have been shown to contain inhibitory activity against *L. monocytogenes*, *C.botulinum*, *Staphylococcus spp*., *Micrococcus*, *Bacillus spp*., *Enterobacteriaceae*, *Salmonella* and *E. coli*. They are generally more inhibitory against Gram-positive organisms than Gram-negative. While this is true of some spice oils that they are useful against both Gram positive and Gram negative groups (clove, cinnamon and citral). There are also some non-phenolic components of spice oil which are more efficient (allyl isothiocyanate) or quite effective against Gram-negative bacteria (Daud et al., 2013). Spice oil contains antimicrobial effect that has been screened as possible source of new antibacterial components. Some of these oils show activity against pathogenic organisms like *S. aureus.* Oils could act as preservatives, eliminating or decreasing the pathogenic bacteria and increasing overall quality of foodstuff (Mishra and Bihal, 2010). Important quality of spice oil and its different components is hydrophobicity, which allows them to make partition with lipids of cell membrane and mitochondria, damaging the cell structure and render them permeable. Escape of important cell constituents and ions due to more permeability leads to the death of the bacteria (Aruna and Baskaran, 2010). An Emerging body of data depicts that there is significant effect for use of spice in dietary stuff, especially application to bakery items, for improving the quality and nutritional value of foodstuff, in addition to their strong antimicrobial properties (Hoque et al., 2008). Oil of spices apply direct or indirect effects to increase the stability and quality of food items (Jauharah et al*.,* 2014, Saranraj and Geetha, 2015). Pure oils are mixtures of more than two hundred components with nominal differences between compounds, contain volatile fraction (alcohols, aldehydes, monoterpene) and non-volatile portion (hydrocarbons, carotenoids, flavonoids etc.) as well (Asghari et al.,2010; Elumalai et al*.*, 2011).

The aim of this study is to assess the efficacy and antimicrobial activity of essential oil of *cinamomum verum,* in different concentrations against the food spoilage microorganism, two gram positive (*Bacillus cereus, Staphylococcus aureus*) and two gram negative *(Escherichia coli, Salmonella sp*.). Conclusively selection of best concentration of essential oil of *cinnamomum verum* for the production of best quality muffins so that the results can be further used to make an effective food spoilage resistant spray/preservative.

MATERIALS AND METHODS

The present research project was carried out in two phases. During first phase antimicrobial efficacy of cinnamon oil was checked after extracting oil through Soxhlet

apparatus. During second phase effect of oil was checked by adding it in different ratio in bakery product (muffins). These trials were conducted in the Department of Food Science & Human Nutrition, University of Veterinary and Animal Sciences (UVAS), Lahore.

10ml sterilized nutrient broth was taken in each test tube.Fresh bacterial cultures (*E.Coli, Salmonella, B.cereus, S.aureus*) were inoculated in nutrient broth and cultures were incubated at 37^{O}C for 24 hrs. Inoculum was standardized by using 0.5 McFarland solutions. The O.D. value of 0.5 MacFarland was found to be 0.1. Bacterial growth was determined in terms of optical density by taking absorbance at 600 nm (OD 600nm).The OD values of all 24 hours old bacterial cultures were taken and adjusted to 0.1 with the help of sterilized nutrient broth. (Elumalai et al*.,* 2011)

Agar Well Diffusion Method

Oil of the spice was screened for its antimicrobial activity against four microorganisms. In order to determine the antimicrobial spectrum, antimicrobial activity was performed by using agar well diffusion assay (CLSI standards).

Sterile cotton swab was dipped in to the prepared inoculums and spread all over the nutrient agar plate by rotating through an angle of 60°. After each swabbing finally, the swab was passed round the edges of the agar surface and left to dry for few minutes at room temperature with lid closed. Then with the help of sterilized cork borer, wells were made in the inoculated plate and labeled as 10µl, 15µl, 20µ l, 25µl. Prepared suspension of the spice oil was distributed in the respective wells with the help of the micropipette under sterilized conditions. Then the plates were incubated at 37°C for 24 hr and zone of Inhibition was determined for different oil concentrations. Diameter of zone of inhibition (DIZ) was observed and shown as clear area in millimeters (Nazia and Parveen, 2006).

Three replications were maintained in each treatment.

Micro-broth dilution Method

Minimum Inhibitory Concentration (MIC) was determined for the organisms that were sensitive to the oil using a micro-broth dilution method. Micro-broth dilution method was used to determine the MIC of tested organism with little modifications. It was carried out in 96-well micro titer plates. The tested organisms were inoculated in nutrient broth and 18 hour old culture was selected for further dilutions after standardization with 0.5 McFarland. Stock solution of oil was prepared by adding 10% DMSO (Dimethyl sulfoxide) and 100µl oil. 100µl of nutrient broth was added in micro titer plates from well 1 to 12. 100µl from stock solution was transferred to first well and mixed properly. Then 100µl from first well was shifted to second and so on up to 11th well in order to make two fold dilutions, and 12th well was used as the negative control. Then 100µl standardized inoculums was added to each well from 1 to 12th. DMSO concentration never surpassed 10% (v/v). Micro titer plates were incubated at optimum growth temperature for each bacterial strain. Growth was monitored by calculating absorbance through ELISA reader at 630 nm. (El-Baroty et al*.,* 2010).Three replicates were used for each oil concentration.

Five treatments (T1-T5) were prepared in which different ratio of Cinnamon oil (0.2, 0.4, 0.6, 0.8 and 1%) were added to the recipe of muffins. Muffins were prepared from different treatments of flour and oil along with control according to the method (Yaseen et al*.,* 2012). A batter was prepared in a bowl manually by mixing dry ingredients with the wet ingredients. Batter was poured in muffins tray grease with oil. Oven was pre-heated at 210 ºC. Muffins were baked at 180 ºC for 15-20 min. Samples were transported and stored at room temperature.

Organoleptic Evaluation: On the basis of concentration of oil, organoleptic evaluation and sensory acceptability one best treatment was selected along with the control and its storage stability was checked for a period of fifteen days with the interval of 5 days. Effect of oil on storage stability was examined by doing sensory evaluation after each five days. Data thus obtained from microbial analysis was evaluated through two way analysis of variance at a confidence level of $P \leq 0.05$. Data obtained from sensory analysis was evaluated through one way analysis of variance at a confidence level of $P \leq 0.05$. The separation of means or significant difference comparison was done by using DMR test (Steel et al., 1997).

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Results and Discussion

The antimicrobial activity of *Cinnamomum verum*, was assessed on four food spoilage and water borne bacteria, *Staphylococcus aureus, Salmonella, Escherichia coli* and *Bacillus cereus.* Analysis of the effect of cinnamon oil at different concentrations was done after an incubation period of 24 hour . The zone of inhibition was measured for *Cinnamon verum* oil for the gram negative- *Salmonella, Escherichia coli* and gram positive *Bacillus cereus* and *Staphylococcus aureus* bacteria was calculated at different concentrations (Table1)

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Results showed maximum values for zone of inhibition by *Bacillus cereus* followed by *Staphylococcus aureus*. Comparing all the four results, *C.verum* was found to have a better antimicrobial activity at effective concentration of 0.6% and 0.8% (Table 1) clearly shows that among the four bacteria *Bacillus* was found to be most susceptible against the action of cinnamon oil while *Salmonella* to be least.

The results of this work were found to be consistent with the work done by (Ates and Erdogrul, 2003; Shan et al*.*, 2007) who showed different effective concentration of essential oil of cinnamon against *Staphylococcus aureus* in another study (Nazia and Parveen, 2006) it was found that cinnamon oil was effective against *E. coli* (Dobre et al*.*, 2011), Gupta and Garg (2008), who showed that the essential oil of cinnamon inhibit the growth of *Staphylococcus aureus* (Magetsari 2012). These findings are also quite similar with the results of Witkowska et al (2013) who reported that cinnamon bark oil completely inhibited the growth of some gram positive and gram negative bacteria, fungi and yeasts (Gende et al*.,* 2008) and (Saraf et al*.,* 2011). As the main component, cinnamaldehyde has proven to be particularly effective against some species of gram positive and gram negative bacteria (Friedman et al., 2000). It has been proposed that cinnamaldehyde and eugenol inhibit production of an essential enzyme by the bacteria and/or cause damage to the cell wall

of the bacteria (Tajkarimi et al., 2010; Revati et al., 2013). Therefore, the high antimicrobial activity of cinnamon oil is due to the presence of the high amount of cinnamaldehyde and due to high antibacterial activity of *C. verum* ascertained by this study. Mean values for sensory evaluation of muffins by the addition of different concentration of cinnamon oil is presented in Table 2. Mean value of color was seen highest in 0.22% i.e. 13.22±1.49 and lowest in 0.8% and 0% 12.07±1.56 and 12.07±1.49 respectively. 0.2% and 0.4% were significant for texture parameter with each other. Similarly for texture 0.2% illustrated the highest value i.e. 13.22±1.04. However for taste, highest value was shown in 0.4% and 0.6%. Mean values for flavor and overall acceptability were seen highest in 0.2% *i.e*., 13.42±0.76 and 13.17±0.84. Similarly data for storage stability of muffins depicted highest score for 0.2% and 0.4% respectively for storage interval of 10 days (Table 3).

CONCLUSION

By the present study it can thus be concluded that *Cinnamomum verum* can be very successfully be used against the food spoilage bacteria *E.coli*. *Staphylococcus* bacteria are easily encountered in during food handling and treatment hence *C.verum* can also be employed for limiting the spread of these bacteria through handling or reducing their concentration at minimal damaging limit. The minimum concentration required for *C. verum* to act upon these spoilage bacteria was found to be 0.06 % v/v. Such a small concentration can be easily imparted in food products like apple juice (spoiled by *E.coli*), flavored milk (spoiled by *Pseudomonas aeruginosa*) and bakery products (cakes and muffins) to inhibit the spoilage.

Table.1: Zone of inhibition of different microflora at different concentrations

Sr.No	Concentration of oil (%) v/v	*Salmonella Sp.*	*Staphylococcus aureus*	*E.coli*	*Bacillus cereus*
		Zone of Inhibition (mm)	Zone of Inhibition (mm)	Zone of Inhibition (mm)	Zone of Inhibition (mm)
1.	0.0	0.0	0.0	0.0	0.0
2.	0.2	23	28	24.33	29.67
3.	0.4	24.33	28.67	31.67	25.33
4.	0.6	27.33	28.33	35.67	25.67
5.	0.8	28.33	28.33	26.33	36.67
6.	1	27.38	28.69	24.37	29.65

Table.2: Sensory evaluation scores of cinnamon oil added muffins

Treatment	Color	Texture	Taste	Aroma	Flavor	Overall acceptability
0%	12.07±1.49	12.68±1.02	11.83±1.16	11.58±1.03	12.13±1.13	12.42±1.29
0.2%	13.22±1.04	13.48±0.60	12.9±1.05	12.8±1.02	13.42±0.76	13.17±0.84
0.4%	13.05±0.90	12.18±0.88	12.18±1.16	11.77±1.05	12.07±1.25	12.66±1.36
0.6%	12.87±1.33	12.5±0.98	12.18±1.07	11.56±1.09	12.05±1.17	12.47±1.49
0.8%	12.07±1.56	12.39±1.22	11.37±0.95	11.33±1.37	11.68±1.28	11.82±1.29
1%	12.05±1.56	12.49±1.22	11.45±0.95	11.4±1.37	11.38±1.28	11.42±1.29

Table 3: Effect of different time intervals and concentrations of Cinnamon oil on sensory attributes of muffins

Sensory Attributes	TIME INTERVAL																							
	0 Day						5 Days						10 Days						15 Days					
	T1	T2	T3	T4	T5	T6	T1	T2	T3	T4	T5	T6	T1	T2	T3	T4	T5	T6	T1	T2	T3	T4	T5	T6
Color	14	14	12	12	13	13	14	14	13	13	13	13	13	13	12	11	12	12	13	14	12	11	11	11
Texture	14	14	14	13	13	13	14	14	14	13	13	13	13	13	13	11	11	11	11	13	11	11	11	11
Taste	14	14	14	14	13	14	14	13	14	13	12	13	13	14	13	12	12	11	12	13	11	11	11	11
Aroma	14	15	14	14	14	13	14	13	13	14	13	12	12	13	14	13	12	11	11	13	12	14	11	12
Flavor	14	14	13	14	13	14	14	14	13	12	13	11	13	14	13	12	12	12	11	13	12	12	11	11
Overall Acceptability	14	15	13	13	13	13	14	13	13	12	13	12	14	13	13	13	12	12	12	12	13	12	12	12

* T_1:0.0%, T_2:0.2% , T_3:0.4% , T_4:0.6%, T_5:0.8%, T_6:1%

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Quality of Life in Patients of Melasma

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ABSTRACT: *To determine the impact of Melasma on patient's quality of life using life quality index "Melasma quality of life scale." A cross sectional study was carried out at dermatology departments of Mayo, Services, and Shalimar hospitals of Lahore city. 160 patients, aged 16-55 suffering from Melasma were enrolled in the study. Using the Melasma quality of life scale questionnaire, consisting of 10 questions. Patients were asked to score on a scale from 1-7 for each of 10 items. The data were analyzed after compiling the results. The higher the Melasma quality of life scale) score, the poorer is the quality of life. Mean age of the patients was 30.5± 7.694 years. The mean Melasma quality of life scale score was 55.62 ± 12.3444. The finding indicates several areas in which Melasma had an impact on individual's quality of life, particularly in relation to symptoms, feelings, and personal relationships. Melasma has an adverse effect on patient's quality of life. It has observed that embarrassment and family relationship are badly affected domains of patient's quality of life. It was concluded that Melasma has an adverse effect on patient's quality of life. It is needed to encourage patient or enhance the educational training and suitable psychological therapies to attain a complete and effective management.*

Keywords: *Melasma, Quality of life, MELASQOLS*

INTRODUCTION

The word melasma comes from Greek word "Melas" (black). It is referred to as 'Chloasma' when it occurs during pregnancy. Melasma is a skin disorder, a psychological distressing of the skin, which causes patchy brown discoloration that mostly appear on face and sun exposed areas (Moin et al., 2006). Melasma is a very common disorder, mostly in young women who take oral contraceptives or hormone replacement therapy medications, but it can affect anyone (Jadotte and Schwartz, 2010). The brown discoloration is due to over production of melanin, (a natural element that gives color to our hair, skin and eyes)(Moin et al., 2006). In most countries, the reason for the widespread of Melasma is unknown; however, it is a known factor that Melasma is found 10% in males and 90% in females, because of hormone-related reasons like pregnancy and use of birth control pills (Jadotte and Schwartz, 2010). So far, the exact cause of melasma is unknown. However, researchers have determined multiple factors, which increase the risk of melasma. While these factors are not direct causes, they do increase the chance of developing melasma, are known as risk factors, and are defined as the factors responsible to enhance the development of disease in person. Some of these expected risk factors are Pregnancy, Ultraviolet light, oral

contraceptives, genetic predisposition, cosmetic ingredients, hormone replacement therapy, phototoxic drugs and thyroid autoimmunity (Kang et al., 2002).

Quality of life (QOL) is the aptitude of a person's daily activity applicable to his/her age and role in the society[Adalatkhan and Amani, 2007]. All human especially females are very conscious of their facial appearance. Any unwanted mark, deformity or blemish like Melasma can severely hurt their ego and self-esteem, and has negative effect on their quality of life (Balkrishnan et al., 2003). The present study was conducted to assert on the effect of melasma on the QOL of a person with the help of a QOL index. There are many indices, Dermatology Life Quality Index (DLQI), Skindex 16, Melasma Quality of life scale (MELASQOL), etc., are available in questionnaires form to measure the degree of incapacity due to skin diseases. However, Melasma has a greater influence on psychosocial instead of physical aspects of a patient's life (Ali et al., 2013).Objective of the present study is to define the influence of Melasma on patient's quality of life using Melasma Quality of Life Scale (MELASQOL).

MATERIALS AND METHODS

The target population of the study was the dermatology OPD patients coming to public hospitals of Lahore and sampled population was the patients who come to be diagnosed melasma on expose areas especially on face. The study was conducted during September 2013 to March 2014 and the sample of 160 patients was selected from the following hospitals, Mayo Hospital, Services Hospital, and Shalimar Hospital.

A questionnaire was developed with the help of the dermatology consultants of Mayo Hospital Lahore to collect the information from both, the cases and the controls. Questionnaires were filled in by the researcher in a face to face interviewing process. Only female patients with age ranged between 16 to 45 were included in this study. The data was collected in different visits from different outdoor patients of dermatology skin departments from the hospitals under study. MELASQOL is a validated and an effective questionnaire to assess the psychosocial aspect of Melasma, by Balkrishnan et al (2003). All patients were asked to fill a MELASQOL questionnaire that comprised of 10 questions covering different areas of QOL e.g. symptoms and feeling, family relationship, emotional well-being, sexual relationships, recreation leisure. The patients were asked to score on a Likert scale from 1-7, 1: strongly not bothered, 2: not bothered, 3: somewhat not bothered, 4: some time bothered and sometime not bothered, 5: somewhat bothered, 6: bothered, 7: bothered all time.

The score ranged between 7 and 70. The higher score indicated poorer quality of life.The data were analyzed through SPSS version 16.0. The factors were included in the study were age, and MELASQOL score. MELASQOL scale description (Balkrishnan et al. 2003). On a Likert scale of 1 (not bothered at all) to 7 (bothered all the time), the subject rates how she feels about the appearance of your skin condition.

1. Frustration about your skin condition
2. Embarrassment about your skin condition
3. Feeling depressed about your skin condition
4. The effects of your skin condition on

your interactions with other people (e.g. interactions with family, friends, close, relationship, etc.)

5. The effects of your skin condition on your desire to be with people

6. Your skin condition making it hard to show affection

7. Skin discolorations making you feel unattractive to others

8. Skin discolorations making you feel less vital or productive

9. Skin discolorations affecting your sense of freedom

RESULTS

In this research, we studied the case history of 160 Melasma patients. Their mean age was (30.58 ± 7.694) years. 121 cases were married and 39 unmarried. Most of females were not working. They were mostly housewives and belonged to poor class family. They faced excessive sun exposure during their household chores like gardening and washing. According to the pattern of Melasma 95 (59.375%) were Centrofacial, 42 (26.25%) Malar, 23 (14.375%) Mandibular. Patients having 3 months disease history were 17 (10.625%), 3-6 months were 17 (10.625%), 7-12 were 23 (14.375%), 13-24 months were 17 (10.625%) and > 24 months were 86 (53.75%).Mean score of MELASQOL scale was 55.62 ± 12.344.

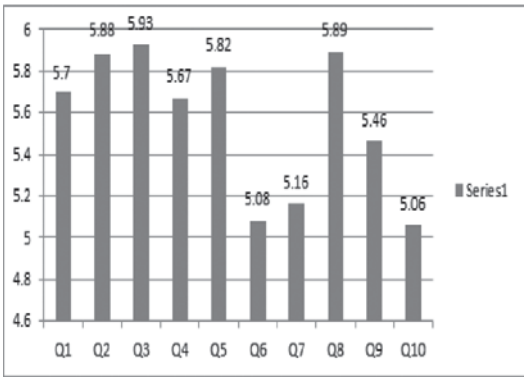

Fig 1: Mean MELASQOL scores for each of the 10 questions.

Fig 1 revealed that the highest score was determined for question number Q3 (5.93 ± 1.559) related to feeling of patients followed by Q8 (5.89 ± 1.703) related to feel unattractive, Q2 (5.88 ± 1.560) related to Frustration, and Q5 related to personal relationship. The comparison between MELASQOL means scores and different age groups were done (Table 1) and significant difference was seen (Table 2). According to Kendall's tau-b (Table 3), as age increases mean score decreases.

Table 1: Age-group & MELASQOL mean score Cross tabulation

Age-gp	Score-gp					
	10-20	21-30	31-40	41-50	51-60	61-70
16-25	1	0	3	6	10	24
26-35	0	2	6	14	24	31
36-45	0	1	8	7	9	12
46-55	1	0	0	0	0	1
Total	2	3	17	27	43	68

Table 2: Chi-Square Test

	Value	Df	Significance
Linear-by-Linear Association	6.416	1	.011
No. of Valid Cases	160		

Table 3: Symmetric Measures

	Value	Approx. significance
Ordinal by Kendall's tau-b Ordinal	-0.167	.020
No. of Valid Cases	160	

DISCUSSION

Melasma is a skin disease that displays as irregular macules and patches of hyperpigmentation predominantly on the face. This long-lasting and recurring situation causes an adverse effect on many areas of patient's QOL.

In this study, patient's mean age was 30.58 ± 7.694 years, which is accordance to the results of Ali et al. (2013). Previous studies showed the mean age between 36 to 40 years .This difference is because of social, racial and cultural, difference around the world. In our culture, most marriages are held at the age of 20-30 years. This makes people conscious to consult and get advice earlier about their disease

It was observed in this study that QOL was less disturbed by Melasma in effected age group of 46-55 years, in agreement with the results of Balkrishanan et al, (2003). Where QOL was also observed less affected in the same. It was observed that the MELASQOL mean score was higher in the age group 26-35 in comparison to other age-groups

In this study, the patients suffered from Melasma for a longer period of time had adverse effect on QOL. Same results were carried out in the studies of Dominguez et al. (2006); Ali et al. (2013) which determined that QOL was more worsened patients with long duration of disease.

In this study mean MELASQOL score is 55.62 while in previous study, by Balkrishanan et al, (2003). was found to be 36.The difference can be described by the fact that in this study mostly patients belonged to poor class family, where psychosocial effect of Melasma was higher.

In this present study, the most badly affected life area feeling of patients linked to embarrassment represented by the largest MELASQOL mean score for question No .3 . The second largest affected life area was self-consciousness and then personal relationship of patients insisting them to avoid social interactions with close friends , relatives or partner. Comparable to our study, emotional well-being was reported to be one of the most adversely affected life domains due to Melasma, by Balkrishnan et al. (2003).

The present study suggested that a blemishing facial mask like Melasma that postures an adverse effect on patient's QOL. It

is needed to encourage patient or enhancement of educational training and suitable psychological involvement to attain a complete and effective management.

CONCLUSION

It is concluded that Melasma has an adverse effect on patient's QOL. It has been observed that embarrassment and family relationship are badly affected domains of patient's QOL. From the findings of the study, the following suggestions are recommended:

- There could be reasonable precautionary measures to stop Melasma from developing. These measures include protection from the harmful sunray (UVR). This protection can be ensured by avoiding unnecessary sun-exposure especially between 7 Am to 7 Pm and further strengthening this protection by covering the face, wearing a wide hat, use of umbrella and application of a sunscreen product with SPF 30-60, at least half an hour before going out door. A well balanced diet, proper hydration and avoiding stress may also help.

- The present study showed that a blemishing facial mask like melasma that reflects an adverse effect on patient's QOL, It is needed to encourage patient or enhance the educational training and suitable psychological therapies to attain a complete and effective management.

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Statistical Aspects of Thalassemia Major: A Case Study in Punjab Pakistan

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ABSTRACT: *Thalassemia major is becoming one of the leading health problems in Pakistan. A case study on 204 patients was carried out at Fatimid Foundation Lahore. The objective of the current study was to investigate the prevalence of the disease in patients related to physical and socio-demographic aspects. The questionnaire was designed to collect the information on factors regarding the gender, age, weight, height, blood group, Hb level, ferritin level, age at the diagnosis, age at the first transfusion, complications associated with the disease and some information was collected from parents relating their education, awareness about disease and their relation. Descriptive and analytical analysis were performed by using numbers & percentages, Chi- Square, Phi and V values, Kendall's Tau-B techniques. Descriptive analysis showed that disease is more common in males (61.3%) and risk factor blood group observed where (91.6%) patients had positive blood groups. Consanguinity was found to be a great risk factor. In bivariate analysis factors age group, caste, Splenectomy, enlarged liver, patients' education, hepatitis C are found to be significantly associated with the gender and blood transfusion in a month, ferritin level, bones problem and hepatitis Care associated with age. It has observed from the results that thalassemia is an inherited blood disease and can be prevented by creating awareness in people of our country.*

Keywords: *thalassemia major, awareness, socio-demographic*

INTRODUCTION

Thalassemias are the form of inherited autosomal recessive blood disorders that affect the synthesis of globin chain in the patients. The disease is caused by the imperfect production of the hemoglobin and too much destruction of the red blood cells (RBC) (Rund and Rachmilewitz, 2005). Thomas Cooley described thalassemia first time in 1925 (Weatherall, 2001). He described it a syndrome amongst children of Italian decent regarded as anemia, splenomegaly and bony abnormalities. The word "thalassemia" was taken from the Greek word θαλασσα (the sea) and haimia (in the blood) to show the relation between the disease of blood and the Mediterranean by Whipple and Bradford in 1936. Thalassemia was originated in the Mediterranean, African and Asian region along the familiar belt that extends from Asia to Africa. Due to migration, thalassemia gene now appears in every part of

the world and affects the people of every ethnic group. β – Thalassemia cases have been found in the Middle East, Pakistan, Iran, China and India (Al-awami, 2000).

Approximately 4.5% of the world population carries the defective hemoglobin genes of thalassemia. There are approximately 250 million carriers worldwide and at least 2 million patients are born annually with thalassemia major (Ahmad et al., 2011). Annually 50,000 to 100,000 children die with thalassemia major in developing countries (Qurat-ul-Ain et al., 2011). Pakistan is a developing country where thalassemia is found in all the parts of the country and patients are multiplying each year at a very fast rate. According to the thalassemia experts, about 5 - 7% of the total population in Pakistan carry thalassemia gene, which accounts approximately nine million carriers in the total population. It is estimated that more than 40,000 people are suffering with thalassemia major in Pakistan and 5000 people are born each year (Arif and Fayaz, 2008, Iqbal, 2009).

The main objectives of this study were:

(i) to study and analyze the physical aspects and socio - demographic profile of the patients suffering from Thalassemia major by using statistical techniques
(ii) to find the prevalence of disease among the people of different casts to determine the risk factor associated with the disease
(iii) to evaluate the awareness level among the parents regarding the disease
(iv) to advise the government to pay attention on this serious issue by making a legislation for premarital screening to diagnose the carrier.

RESEARCH METHODOLOGY

The study was descriptive in nature. The principle of this study was to explore different aspects and risk factors contributing to thalassemia major from Lahore, Pakistan. Data was collected for thalassemia major patients registered at Fatimid Foundation Blood Bank & Hematological Services Centre, Lahore, from 1986 to 31st June, 2014. The data were obtained on the variables gender, age, blood group, caste, residential area, age at diagnosis, 1st blood transfusion, number of transfusion in a month, weight, height, average Hb level, red blood cells, serum ferritin level and associated complications for 204 patients from available files. The data consists of the patients who visited to the foundation for regular blood transfusion from Lahore and nearby districts (Kasur, Sheikhupura and Okara) of the province of Punjab, Pakistan. A questionnaire was designed with the guidance of the concerned doctor to collect the data from lived patients and their parents on different socio-demographic aspects of the disease. The direct personal interviewing method was used. The questionnaire contained almost all the questions related to the risk factors and the number of aspects like education of the patient, employment status, taking medicine regularly and associated complications. Some information was collected from the parents relating to their education, socio- economic status, relation of the parents and to check their basic knowledge about the deadly disease and the tests and support for premarital screening to be mandatory by making legislation by the

government. All the factors were coded for the computer analysis and the data were entered into the personal computer system. The software SPSS Statistics 17.0 was used for data processing and analysis. Descriptive and the analytical results were obtained using the different statistical techniques including averages, percentages and $\chi 2$ (Chi-square), Phi and V Statistics, Kendall's Tau-b. SPSS v. 17 was used for descriptive analysis and test of association.

RESULTS AND DISCUSSION

A comprehensive analysis is presented into two major sections, descriptive study and analytical study.

Descriptive Analysis

The descriptive analysis has been performed to discuss the frequency of different factors of the thalassemia major. An overall analysis was done on 204 patients for the factors age, residential area, blood group, gender, age at diagnosis, first blood transfusion and blood transfusion in a month, average Hb level, Splenectomy, ferritin level and on regular medication. The results are presented in Table-1.

Table-1 Classification of Variables

Variable	Categories	Frequency	Ratio (%)
Gender	Male	125	61.3
	Female	79	38.7
Age Group	0-4	10	4.9
	5-9	37	18.1
	10-14	36	17.7
	15-19	70	34.3
	20-24	37	18.1
	25-29	10	4.9
	>=30	4	2.0
Residential Area	Rural	77	37.7
	Urban	127	62.3
Blood Group	A+	34	16.7
	A-	09	4.4
	B+	89	43.6
	B-	03	1.5
	O+	49	24.0
	O-	05	2.5
	AB+	15	7.4
Age at Diagnosis(in months)	00-04	39	19.1
	05-09	86	42.2
	10-14	27	13.2
	>= 15	52	25.5
Blood Transfusion in Month	Once	23	11.3
	Twice	90	44.1
	>= Thrice	91	44.6
Average Hemoglobin level (mg/dl)	< 5	9	4.4
	5-9	141	69.1
	>9	54	26.5
Red blood cells (10^6/ml)	<=3	98	48.0
	>3	106	52.0
Serum Ferritin Level (ng/L)	< 3000	55	27
	3001-6000	78	38.2
	>6000	71	34.8
Splenectomy	Yes	16	7.8
	No	189	92.2
On Regular Medicine	Yes	92	76.0
	No	29	24.0
Hepatitis B	Yes	07	5.8
	No	114	94.2
Hepatitis C	Yes	31	25.6
	No	90	74.4
Enlarged Spleen	Yes	15	12.4
	No	106	87.6
Enlarged Liver	Yes	05	4.1
	No	116	95.9
Orofacial Complication	Yes	33	27.3
	No	88	72.7

From the table, it was observed that out of 204 patients 125(61.3%) are male and 79(38.7%) are females. Male to female ratio is 1.583: 1. The disease was found to be more frequent in males than in females. Zaman and Salahuddin explained that the thalassemia major occurred more frequently in males (60%) as compared to females (40%) (Zaman and Salahuddin, 2006). The results are similar to the outcomes of the study of Ishaq et al. (2012) in which the frequency of the male (64.3%) is higher relative to females (35.7%) (Ishaq et al., 2011).

The highest frequency of patients was observed between the age group of 15 and 19 years with 70 (34.3%) patients. Only 4 (2%) patients had the age of 30 years or more. It is observed that number of patients decreases as the age increases. Minimum age of the patient was 1 year and maximum age was observed as 48 years. The counts (percentages) of the individuals who are living in rural and urban areas are 77(37.7%) and 127 (62.3%), respectively. The frequency of disease is more in urban areas in comparison of rural areas. The highest percentage of disease is found in Arain (26%) followed by Rajputs (21.1%).

In blood group aspects, the results showed that majority of the patients (91.6%) had positive blood group with 16.7% (A +ve), 43.6(B +ve), 24.0% (O +ve), 7.4% (AB +ve) and only 8.4% had negative blood groups. 39 (19.1%) of the total patients were diagnosed to be thalassemia major between the age of 0 to 4 months, 86 (42%) of the patients were diagnosed between the age of 5-9 months, 27(13.2%) were diagnosed between the age of 10-14 months and 52(25.5%) were diagnosed more than 15 months of their age then they started their treatment. 90(44.1%) of the patients received the blood transfusion twice a month and 87 (42.6%) thrice a month.

69.1% of the patients had their Hb level between 5 - 9g/dl and 54(26.5%) had more than 9g/dl. 98(48%) of the total patients had less than or equal to 3× 106 /µL and 106(52.2%) patients had more than 3× 106 /µL red blood cells. 16 (7.8%) of the patients were splenectomized and 84.3% patients were on oral medication. Serum ferritin level in 55(27%) patients was recorded less than 3000 ng/ml, between 3001 and 6000 in 78(38.2%) patients and more than 6000 ng/ml in 71(34.8%) patients that indicated an excessive iron overload in their bodies. 7(5.8%) had hepatitis B, 31(25.6%) hepatitis C, 15(12.4%) had their spleen enlarged and 4(4.1%) were suffering with enlarged liver. 80(66.1%) of the 121 patients were studying, 18(14.9%) had left their education and 23(19.0%) never went to the school. An analysis is conducted for 121 lived patients and parents who come to receive regular blood transfusion. Results are given in Table-2. As concerned to the information obtained from the parents of alive patients (121), forty one (33.81%) fathers and 55(45.5%) mothers of the patients were below matric, among the literates only 14(11%) fathers and 2(1.7%) mothers were highly educated up to masters. 79(65.3%) couples had consanguineous marriage and 26 (21.5%) had no relation. 80 (71.1%) parents were aware of the disease and 41(33.9%) had heard about the test for detecting the carrier. 100 % parents did not have premarital screening but 92(76%) supported for a law for premarital screening of all the individuals in the country in order to prevent the disease. 71% families had one child, 23% had two and 5.8% had three or

more children with thalassemia major. 60(46.6%) parents wanted to start the family of their children and 58(47.9%) parents replied that they do not know about the future and only 3(2.5%) said no. 92(76%) patients had irritating behavior due to the disease.

Table-2 Classification of Variables

Variable	Categories	Frequency	Ratio (%)
Socio-Economic Status	Low(<=20000)	64	52.9
	Middle(21000-40000)	38	31.4
	Upper(>40000)	19	15.7
Parents' Relation	1st Cousin	79	65.3
	2nd Cousin	03	2.5
	Distant relative	13	10.7
	Unrelated	26	21.5
Support for legislation	Yes	92	76.0
	No	29	24.0
Number of Affected children in the family	1	86	71.1
	2	28	23.1
	3	07	5.8
Want to start the family of their child	Yes	60	46.6
	No	03	2.5
	Do not know	58	47.9
Behavioral Problem	Yes	92	76.0
	No	29	24.0

The socio-economic status is taken from the income of the parents. The numbers (percentages) of the total subjects who are falling in low, middle and upper status are 64 (52.9%) earning below Pakistani Rupees (PKR) 20000, 38 (31.4%) earning between Rs. 21000 and 40000 and 19(15.7%) earning more than Rs.40000 per month, respectively.

Bivariate Analysis

Bivariate analysis has performed to observe the association between the different socio demographic and clinical factors with gender, blood Group and age group. The Chi square, Phi/ Cramer's V statistics, Kendall's Tau-b were calculated to find the significance of association. If p-value will be less than 0.05, factor considered as significant. The factors are associated if the result is found to be significant.

Table-3 Association of Socio-demographical and Clinical Factors with Gender

Factor	Chi-square Value	P-Value	Phi/ V	Contingency Coefficient
Age Group	$\chi^2(6) = 17.384$	0.008	0.292	0.280
Status	$\chi^2(1) = 4.637$	0.031	0.151	0.149
Caste	$\chi^2(8) = 19.069$	0.014	0.306	0.292
Splenectomy	$\chi^2(1) = 4.303$	0.038	0.145	0.144
Hb Level	$\chi^2(2) = 5.396$	0.067	0.163	0.161
Enlarge Liver	$\chi^2(1) = 4.204$	0.040	0.186	0.183
Education	$\chi^2(1) = 7.127$	0.028	0.243	0.243
Hepatitis C	$\chi^2(1) = 4.102$	0.043	0.184	0.184

The factors age group, status, caste, splenectomy, enlarged liver, patients' education, hepatitis C were found to be significantly associated with the gender whereas there was no evidence of association found between blood group, residential area, age at diagnosis, age at first blood transfusion, blood transfusion in a month, average Hb level, ferritin level, enlarged spleen and complication at 5% level of significance (Table 3).

Association of Various Factors with Age Group

Statistical analysis using chi-square revealed that the factors blood transfusion in a month ($\chi 2 = 6.355$ with p= 0.000), ferritin levels ($\chi 2 = 82.151$, p= 0.000), bones problem ($\chi 2 = 15.180$, p = 0.019) and hepatitis C ($\chi 2 = 33.464$, p= 0.000) are associated with age at 5% level of significance, it means that as age increases requirement for the blood transfusion increases, ferritin level in the body become

higher, bones problem may arise and patients have more chances of having hepatitis C virus. Many of the complications are age dependent. The other factors are not affected by age.

Table-4: Association of Factors with Age Group

Factor	Chi-Square	P-Value	Phi/ V	Kendall's Tau –B
Blood Transfusion in a month	$\chi^2(18) = 105.001$	0.000	-	0.386
Ferritin Level	$\chi^2(12) = 82.151$	0.000	-	0.401
Bones Problem	$\chi^2(6) = 15.180$	0.019	0.354	-
Hepatitis C	$\chi^2(6) = 33.464$		0.526	-

Awareness Regarding Disease, Test and Parents' Education

Table-5 was used to determine the association between parents' education and their awareness about thalassemia disease. The chi-square test gave the large value 14.899, which showed that there is association between the education and awareness of thalassemia. Similarly, table also shows the association between parents' education and their knowledge regarding the test. Again from the large value of $\chi 2 = 24.701$, it was found that the educated parents have more knowledge about the test.

Parents' Education and Awareness about Disease:

$[\chi 2(\mathbf{5}) = 14.899,\ p = 0.011]$

Parents' Education and Awareness about Test:

$[\chi 2\ (\mathbf{5}) = 24.701,\ p = 0.000]$

Table-5: Association between Parents' Education and Awareness about Disease and test

		< matric	Matric	Inter	Graduates	Masters
Awareness about Disease	No	19	11	3	1	1
	Yes	22	27	12	12	13
Total		41	38	15	13	14
Awareness about Test	No	34	30	6	5	5
	Yes	7	8	9	8	9
Total		41	38	15	13	14

Correlations between Age, Weight and Height

The Spearman correlation coefficients of different factors age (years), weights (kg) and heights (inches) are calculated for thalassemia patients. It is a general concept that the height and the weights of thalassemia patient are affected by the disease. The results in the Table -6 show that the age and height, age and weight and weight and height all have the same tendency to increase in the same direction and found to be significant with p= 0.000.

Table-6: Correlations between Variable

Variables	Results of Spearman Correlation Coefficient			
	Overall	Male	Female	p-value
Age and Weight	0.852	0.840	0.872	0.000
Age and Height	0.828	0.823	0.812	0.000
Height and Weight	0.895	0.901	0.873	0.000

Thalassemia major is common in both sex. In the present study, it was observed that males' percentage (61.3%) is higher than females (38.7%). In our country the most common risk factor for the prevalence of the disease is due to the cultural and religious traditions of the consanguineous marriages. People prefer to marry in their own caste and family without knowing its consequences

especially in Arain and Rajputs where frequency of thalassemia patients is relatively high.

In our country poverty, less education and insufficient facilities of health care are the common hurdles in effective treatment of thalassemia patients of iron over load, higher ferritin levels and the complications which are the main causes of death in thalassemia major. The main reason for prevalence of disease is found to be low education. No permanent treatment for thalassemia major exists in Pakistan. The only strategy adopted is to prolong the lifespan of the patients by using different treatments. There is no concept of the premarital screening and genetic counseling of the people having a family history of the disease. In our community there is a big religious and ethical issue of termination of pregnancy of the affected fetus.76% parents supported that there should be legislation in the country for premarital screening of all the individuals in order to prevent the disease.

CONCLUSIONS

Finally, it is concluded that thalassemia is an inherited blood disease that can be prevented by creating education in the population of Pakistan. Thalassemia is a preventable disease. Preventive measures are required to reduce the problem of the disease in the country. The following general guidelines based on this study that can be helpful to tackle and control the spread of thalassemia.

- General awareness among the people about the features and complications of disease is the most effective tool for its prevention, which can be carried out via media like newspaper and television, seminars, debates, training of local general practitioners, obstetricians, nurses, social workers and generalists etc.

- Prenatal diagnosis is feasible and accepted by the affected families if pregnancies are at risk, identified in the lst trimester. Most religious scholars allow for termination of pregnancy for a severe inherited disorder if it is done before 17 weeks of pregnancy.

- Premarital screening should be mandatory in the country and there should be legislation by the government for premarital screening as in Muslim countries Saudi Arabia and UAE, the state has made pre-marital screening for thalassemia mandatory.

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Estimators of Population Mean under Two-Phase Sampling Technique

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ABSTRACT: *The present research work is the collection of estimators for mean of population of concerned variable under two-phase sampling technique. It is a comprehensive study whose purpose is to present an overview of estimators developed by different researchers and to provide a convenient and comprehensive access to these estimators to researchers and scholars for their follow-up studies.*

Keywords: *Estimators of population mean, Two-Phase sampling*

INTRODUCTION

Sampling is a well-defined statistical technique which is concerned with the small part of a given statistical population to determine the unknown qualities of interest in population. It is being widely applied in life sciences. The various designs of this technique have been introduced in surveys. The history of survey sampling started about century ago. Then with the passage of time contributions regarding different sampling techniques including single-phase and two-phase were undertaken by different researchers. Two-phase sampling technique was first introduced by a statistician Neyman (1938). In this technique some information is gathered from elements selected in sample at initial phase and some detailed information is collected from the sub-sample selected from the initial phase sample. It has been widely used in survey sampling when collection of data is very expensive on variable of interest. Some references in this area are Snedecor and King (1942); Rao (1973); Cochran (1977); Bredit and Fuller (1993) and Rao and Sitter (1995).

MATERIAL AND METHODS

In this study, various estimators used for estimating unknown mean of population for two phase sampling have been presented. One, two or multi-auxiliary variables have been used to describe these estimators.

RESULTS AND DISCUSSION

The notations related to these estimators have been described as follows:

1.1 Notations:

Let = (1,2,3… N') represents the population with finite size N' and the concerned variable is represented by Y. X and Z be auxiliary variables correlated with Y. To reproduce estimators used for unknown mean of population of study variable Y following notations have been used:

N' = Population size.

n° = Sample size under first phase.

$n^{\circ\circ}$ = Sample size under second phase.

$\overline{Y}' = \frac{\sum_{i}^{N'} Y}{N'}$ population mean

$\lambda_1^{\circ} = \frac{1}{n^{\circ}} - \frac{1}{N'}$, $\lambda_2^{\circ\circ} = \frac{1}{n^{\circ\circ}} - \frac{1}{N'}$, $\lambda_3^{\otimes} = \frac{1}{n^{\circ\circ}} - \frac{1}{n^{\circ}}$

Population variance for study variable is:

$$S_y'^2 = \frac{\sum_{i}^{N'} \left(y_i - \overline{Y}'\right)^2}{\left(N' - 1\right)}$$

Population variance for auxiliary variable is:

$$S_x'^2 = \frac{\sum_{i}^{N'} \left(x_i - \overline{X}'\right)^2}{\left(N' - 1\right)}$$

Population covariance of x and y:

$$S'_{yx} = \frac{\sum_{i}^{N'} \left(x_i - \overline{X}'\right)\left(y_i - \overline{Y}'\right)}{\left(N' - 1\right)}$$

Regression coefficient of y on x:

$$\beta'_{yx} = \frac{S'_{yx}}{S'^2_x},$$

Coefficient of Variation of x:

$$C'^2_x = \frac{S'^2_x}{\overline{X}'^2},$$

Correlation Coefficient of y on x:

$$\rho' = \frac{S'_{yx}}{S'_x S'_y}, \quad C' = \frac{\beta'_{yx}}{R'}, \quad R' = \frac{\overline{Y}'}{\overline{X}'}, \quad R'_2 = \frac{\overline{Y}'}{\overline{Z}'}$$

1.2 Estimators for Two-Phase Sampling

In this section estimators of mean for population for concerned variable under two phase sampling are given.

1.2.1 Hansen and Hurwitz (1953) Estimator :

$$T_1' = \overline{y}^{\circ\circ} + b_{yx}(\overline{x}^{\circ} - \overline{x}^{\circ\circ}) \qquad (1.2.1)$$

1.2.2 Robson (1957) Estimator :

Robson (1957) gave the idea of product estimator when there is highly negative correlation.

$$T_2' = \overline{y}^{\circ\circ} \frac{\overline{x}^{\circ\circ}}{\overline{x}^{\circ}} \qquad (1.2.2)$$

1.2.3 Sukhatme (1962) Estimator :

Sukhatme (1962) developed ratio estimator when information about auxiliary variable was available at second phase.

$$T_3' = \frac{\overline{y}^{\circ\circ}}{\overline{x}^{\circ\circ}} \overline{x}^{\circ} \qquad (1.2.3)$$

3.2.4 Cochran's (1963) Estimators :

a) Cochran (1963) suggested ratio and

regression estimator with population information on auxiliary variable. This case is known as complete information case.

$$T_4' = \frac{\bar{y}^{\circ\circ}}{\bar{x}^{\circ\circ}} \bar{X}' \quad (1.2.4)$$

b) $T_5' = \bar{y}^{\circ\circ} + b_{yx}\left(\bar{X}' - \bar{x}^{\circ\circ}\right)$ (1.2.5)

3.2.5 Raj (1965) Estimator:

$$T_6' = wU + (1-w)V \quad (1.2.6)$$

Where $U = \bar{y}^{\circ\circ} + b_{yx}\left(\bar{x}^{\circ} - \bar{x}^{\circ\circ}\right)$ and

$V = \bar{y}^{\circ\circ} + b_{yz}\left(\bar{z}^{\circ} - \bar{z}^{\circ\circ}\right)$ and w is a suitable constant.

1.2.6 Mohanty (1967) Estimators:

a) Mohanty (1967) proposed regression estimator when information on supporting variables was accessible at second phase.

$$T_7' = \left[\bar{y}^{\circ\circ} + b_{yx}\left(\bar{x}^{\circ} - \bar{x}^{\circ\circ}\right)\right]\frac{\bar{z}^{\circ}}{\bar{z}^{\circ\circ}} \quad (1.2.7)$$

b) Mohanty (1967) gave the regression-cum-ratio estimator when complete information on X is not accessible on Z is available. This situation is known as partial information case.

$$T_8' = \left[\bar{y}^{\circ\circ} + b_{yx}\left(\bar{x}^{\circ} - \bar{x}^{\circ\circ}\right)\right]\frac{\bar{Z}'}{\bar{z}^{\circ\circ}} \quad (1.2.8)$$

where b_{yx} is calculated from second phase sample.

1.2.7 Srivastava (1971) Estimator:

A general ratio estimator was proposed by Srivastava (1971) when information of auxiliary variable was available at second phase.

$$T_9' = \bar{y}^{\circ\circ}\left(\frac{\bar{x}^{\circ}}{\bar{x}^{\circ\circ}}\right)^{\alpha} \quad (1.2.9)$$

Where α is unknown constant and the value of α for which the MSE of this estimator is minimum is $\alpha = \frac{C_y'}{C_x'}\rho_{xy}'$

1.2.8 Chand (1975) Chain Ratio Estimator:

Under partial information case the estimator is:

$$T_{10}' = \bar{y}^{\circ\circ}\frac{\bar{x}^{\circ}}{\bar{x}^{\circ\circ}}\frac{\bar{Z}'}{\bar{z}^{\circ}} \quad (1.2.10)$$

3.2.9 Cochran's (1977) Estimators:

a) The usual ratio estimator for unknown population mean $\bar{Y}'$ recommended by Cochran (1977) is:

$$T_{11}' = \frac{\bar{y}^{\circ\circ}}{\bar{x}^{\circ\circ}}\bar{x}^{\circ} \quad (1.2.11)$$

b) Cochran (1977) suggested following simple regression estimator as:

$$T_{12}' = \bar{y}^{\circ\circ} + b_{yx}\left(\bar{x}^{\circ} - \bar{x}^{\circ\circ}\right) \quad (1.2.12)$$

c) Another simple regression estimator suggested) is:

$$T_{13}' = \bar{y}^{\circ\circ} + b_{yx}\left(\bar{X}' - \bar{x}^{\circ\circ}\right) \quad (1.2.13)$$

where b_{yx} is based on second phase sample.

1.2.10 Kiregyera (1980) Estimator:

Kiregyera (1980) proposed ratio-to-regression estimator under partial information case.

$$T'_{14} = \frac{\overline{y}^{\circ\circ}}{\overline{x}^{\circ\circ}}\left[\overline{x}^{\circ} + b_{xz}\left(\overline{Z}' - \overline{z}^{\circ}\right)\right] \qquad (1.2.14)$$

where is calculated from the first-phase sample.

1.2.11 Khaire and Srivastava(1981) Estimator:

Khaire and Srivastava (1981) suggested regression-cum-ratio estimator in case of complete information.

$$\mathrm{T}'_{15} = \left[\overline{\mathrm{y}}^{\circ\circ} + \mathrm{b}_{\mathrm{yx}}\left(\overline{\mathrm{X}}' - \overline{\mathrm{x}}^{\circ}\right)\right]\frac{\overline{Z}'}{\overline{\mathrm{z}}^{\circ\circ}} \qquad (1.2.15)$$

1.2.12 Kiregyera (1984) Estimator:

Kiregyera (1984) proposed regression-in-regression estimator by applying two supporting variables under partial information case.

$$T'_{16} = \overline{y}^{\circ\circ} + b_{yx}\left[\left(\overline{x}^{\circ} - \overline{x}^{\circ\circ}\right) - b_{xz}\left(\overline{z}^{\circ} - \overline{Z}'\right)\right] \qquad (1.2.16)$$

Where b_{yx} depends on second-phase b_{xz} sample while on first-phase sample.

1.2.13 Mukerjee et al. (1987) Estimator:

a) Mukerjee et al. (1987) developed three regression type estimators. The first estimator was developed after the development of regression-in-regression estimator by Kiregyera (1984) when information about both auxiliary variables is unavailable for population. This situation is known as no information case.

$$T'_{17} = \overline{y}^{\circ\circ} + b_{yx}\left(\overline{x}^{\circ} - \overline{x}^{\circ\circ}\right) + b_{yz}\left(\overline{z}^{\circ} - \overline{z}^{\circ\circ}\right) \qquad (1.2.17)$$

Where b_{yx} and b_{yz} are based on second-phase sample.

b) under partial information the estimator is:

$$T'_{18} = \overline{\mathrm{y}}^{\circ\circ} + \left\{\mathrm{b}_{\mathrm{yx}}\left(\overline{\mathrm{x}}^{\circ} - \overline{\mathrm{x}}^{\circ\circ}\right) + \mathrm{b}_{\mathrm{yz}}\left(\overline{\mathrm{Z}}' - \overline{\mathrm{z}}^{\circ\circ}\right)\right\} \qquad (1.2.18)$$

Where b_{yx} and b_{yz} are based on second-phase sample.

1.2.14 Singh and Shukla (1987) F-T (Factor Type) Estimator:

$$T'_{19} = \overline{y}^{\circ\circ}\left[\frac{(A+C)\overline{x}^{\circ} + fB\overline{x}^{\circ\circ}}{(A+fB)\overline{x}^{\circ} + C\overline{x}^{\circ\circ}}\right] \qquad (1.2.19)$$

Where A=(d-1)(d-2), B=(d-1)(d-4), C=(d-2)(d-3)(d-4) are the known quantities based on the chosen values of d and $d \geq 0$ is a constant.

1.2.15 Srivastava et al. (1990) Estimator:

Under partial information case the general ratio estimator is:

$$T'_{20} = \overline{y}^{\circ\circ}\left(\frac{\overline{x}^{\circ}}{\overline{x}^{\circ\circ}}\right)^{\alpha_1}\left(\frac{\overline{Z}'}{\overline{z}^{\circ}}\right)^{\alpha_2} \qquad (1.2.20)$$

The values of α_1 and α_2 for which the MSE for this estimator is minimum are:

$\alpha_1 = \frac{C'_y}{C'_x}\rho'_{xy}$ and $\alpha_2 = \frac{C'_y}{C'_z}\rho'_{yz}$ respectively.

1.2.16 Sahoo et al. (1993) Estimator:

Under partial information case the regression type estimator is:

$$\mathrm{T}'_{21} = \overline{\mathrm{y}}^{\circ\circ} + \mathrm{b}_{\mathrm{yx}}\left(\overline{\mathrm{x}}^{\circ} - \overline{\mathrm{x}}^{\circ\circ}\right) + \mathrm{b}_{\mathrm{yz}}\left(\overline{\mathrm{Z}}' - \overline{\mathrm{z}}^{\circ}\right) \qquad (1.2.21)$$

where b_{yx} and b_{yz} are based on second-phase sample.

1.2.17 Singh et al. (1994) Estimator:

Using an unknown constant t > 0

$$T'_{22} = \bar{y}^{\circ\circ} h_3(1,0)\left[\frac{\psi\{\phi_1(t)\}}{\psi\{\phi_2(t)\}}\right] \quad (1.2.22)$$

$$\psi\{\phi_i(t)\} = \phi_i(t) + \{1-\phi_i(t)\} h_2(0,1); i = 1,2$$

$$h_2(\alpha,\beta) = \left(\frac{\bar{X}_1}{\bar{x}'_1}\right)^{\alpha}\left(\frac{\bar{X}_2}{\bar{x}'_2}\right)^{\beta} \text{ for } \alpha = 0 \text{ and } \beta = 1$$

$$h_3(\alpha,\beta) = \left(\frac{\bar{x}'_1}{\bar{x}_1}\right)^{\alpha}\left(\frac{\bar{x}'_2}{\bar{x}'_2}\right)^{\beta}, \text{ for } \alpha = 1 \text{ and } \beta = 0$$

$$\phi_1(t) = \theta B \Big/ (A + \theta B + C) \quad \phi_2(t) = C \Big/ (A + \theta B + C)$$

$$A = (t-1)(t-2),\ B = (t-1)(t-4),$$

$$C = (t-2)(t-3)(t-4),\ \theta = \frac{n^{\circ\circ}}{N'}$$

1.2.18 Singh and Upadhyaya (1995) Estimator:

Under partial information case the estimator suggested by Singh and Upadhyaya (1995) is:

$$T'_{23} = \bar{y}^{\circ\circ}\left(\frac{\bar{x}^{\circ\circ}}{\bar{x}^{\circ}}\right)\left(\frac{\bar{Z}' + C'_z}{\bar{z}^{\circ\circ} + C'_z}\right)^{\alpha_2} \quad (1.2.23)$$

3.2.19 Kott and Stukel (1997) Estimator:

$$T'_{24} = \frac{1}{n}\sum_{h=1}^{H}\sum_{i\in A_{h2}}\frac{n_h}{r_h}y_i \quad (1.2.24)$$

Where A_{h2} is the set of indices for Second-Phase Sample elements that belong to stratum *h.*

H: No. of strata for Second-Phase Sampling.

n_h : First-Phase Sample elements.

r_h : Second-Phase Sample elements.

1.2.20 Fuller's (1998) Regression Estimator:

$$T'_{25} = \hat{\mu}_{Y(F)} = \hat{\mu}_{Y(2)} + \left(\hat{\mu}_{X(1)} - \hat{\mu}_{X(2)}\right)\hat{\beta}_{(2)} \quad (1.2.25)$$

Where $\hat{\mu}_{X(1)}$ shows the estimator for population mean of variable X from first sample.

$\hat{\mu}_{X(2)}$: estimated mean of population for X from second sample.

$\hat{\mu}_{Y(2)}$: estimated mean of population for Y from second sample.

$\mu_{Y(F)}$: vector of finite population means.

$\hat{\beta}_{(2)}$: coefficient vector computed from second sample.

$\hat{\beta}_{(2)} = \left(\sum_{t=1}^{n_2}\pi_t^{-1}X'_tX_t\right)^{-1}\sum_{t=1}^{n_2}\pi_t^{-1}X'_tY_t$, π_t^{-1} where is the sampling weight.

1.2.21 Upadhyaya and Singh (2001) Estimator:

a) $T'_{26} = \bar{y}^{\circ\circ}\left(\frac{\bar{x}^{\circ}}{\bar{x}^{\circ\circ}}\right)\left(\frac{Z' + C_z}{\bar{z}^{\circ} + C_z}\right)$ (1.2.26)

b) $T'_{27} = \bar{y}^{\circ\circ}\left(\frac{\bar{x}^{\circ}}{\bar{x}^{\circ\circ}}\right)\left(\frac{\beta_2(z)\bar{Z}' + C'_z}{\beta_2(z)\bar{z}^{\circ} + C'_z}\right)$ (1.2.27)

c) $T'_{28} = \bar{y}^{\circ\circ}\left(\frac{\bar{x}^{\circ}}{\bar{x}^{\circ\circ}}\right)\left(\frac{C'_z\bar{Z}' + \beta_2(z)}{C'_z\bar{z}^{\circ} + \beta_2(z)}\right)$ (1.2.28)

$\beta_2(z)$ is coefficient of kurtosis

C'_z is coefficient of variation

1.2.22 Singh (2001) Estimator:

a) $T'_{29} = \bar{y}^{\circ\circ}\left(\frac{\bar{x}^{\circ}}{\bar{x}^{\circ\circ}}\right)\left(\frac{\bar{Z}' + \sigma_z}{\bar{z}^{\circ} + \sigma_z}\right)$ (1.2.29)

σ_z is standard deviation.

b) $T'_{30} = \bar{y}^{\circ\circ}\left(\frac{\bar{x}^{\circ}}{\bar{x}^{\circ\circ}}\right)\left(\frac{\beta_1(z)\bar{Z}' + \sigma_z}{\beta_1(z)\bar{z}^{\circ} + \sigma_z}\right)$ (1.2.30)

$\beta_1(z)$ is coefficient of skewness.

1.2.23 Roy (2003) Estimator:

Roy (2003) proposed a general regression estimator of mean for population in case of information on supporting variable Z is available as:

$$T'_{31} = \bar{y}^{\circ\circ} + k_1\left[\left\{\bar{x}^{\circ} + k_2\left(\bar{Z}' - \bar{z}^{\circ}\right)\right\} - \left\{\bar{x}^{\circ\circ} + k_3\left(\bar{Z}' - \bar{z}^{\circ\circ}\right)\right\}\right] \quad (3.2.31)$$

The optimum values of unknown constants are:

$$k_1 = \frac{\bar{Y}'C'_y\left(\rho'_{yx} - \rho'_{xz}\rho'_{yz}\right)}{\bar{X}'C'_x\left(1 - \rho'^2_{xz}\right)}, \quad k_2 = \frac{\bar{X}'C'_x}{\bar{Z}'C'_z}\rho'_{xz}$$

and $k_3 = \frac{\bar{X}'C'_x\left(\rho'_{yz} - \rho'_{xz}\rho'_{yz}\right)}{\bar{Z}'C'_z\left(\rho'_{xy} - \rho'_{yz}\rho'_{xz}\right)}$

1.2.24 Singh et al. (2004) Estimator:

a) Singh et al. (2004) proposed a generalized estimator with partial information case.

$$T'_{32} = \bar{y}^{\circ\circ}\left(\frac{\bar{x}^{\circ}}{\bar{x}^{\circ\circ}}\right)^{\alpha_1}\left(\frac{a\bar{Z}' + b}{a\bar{z}^{\circ} + b}\right)^{\alpha_2}\left(\frac{a\bar{Z}' + b}{a\bar{z}^{\circ\circ} + b}\right)^{\alpha_3} \quad (1.2.32)$$

where

$$\alpha_1 = \frac{C'_y}{C'_x}\left(\frac{\rho'_{yx} - \rho'_{yz}\rho'_{xz}}{1 - \rho'^2_{xz}}\right),$$

$$\alpha_2 = \frac{1}{\phi}\frac{C'_y}{C'_z}\left(\frac{\rho'_{xz}\left(\rho'_{yx} - \rho'_{yz}\rho'_{xz}\right)}{1 - \rho'^2_{xz}}\right)$$

$$\alpha_3 = \frac{1}{\phi}\frac{C'_y}{C'_z}\left(\frac{\left(\rho'_{yz} - \rho'_{yx}\rho'_{xz}\right)}{1 - \rho'^2_{xz}}\right)$$

1.2.25 Singh and Espejo (2007) Estimator: Singh and Espejo (2007) proposed following regression ratio estimators.

a) $T'_{33} = \bar{y}^{\circ\circ}\left(\frac{\bar{x}^{\circ}}{\bar{x}^{\circ\circ}}\right)^{\alpha_1}\left(\frac{\bar{Z}' + \rho'_{xz}}{\bar{z}^{\circ\circ} + \rho'_{xz}}\right)^{\alpha_2}$ (1.2.33)

b) $T'_{34} = \bar{y}^{\circ\circ}\left(\frac{\bar{x}^{\circ}}{\bar{x}^{\circ}}\right)\left(\frac{\bar{Z}' + \rho'_{xz}}{\bar{z}^{\circ} + \rho'_{xz}}\right)$ (1.2.34)

1.2.26 Samiuddin and Hanif (2007) Estimator:

Samiuddin and Hanif (2007) developed regression-in-ratio estimator by applying two auxiliary variables.

a) $T'_{35} = [\bar{y}^{\circ\circ} + \beta_{yz}(\bar{z}^{\circ} - \bar{z}^{\circ\circ})]\frac{\bar{X}'}{\bar{x}^{\circ\circ}}$ (1.2.35)

b) The regression estimator under Full information Case is:

$$T'_{36}=\bar{y}^{\circ\circ}+\alpha_1\left(\bar{X}'-\bar{x}^{\circ\circ}\right)+\alpha_2\left(\bar{Z}'-\bar{z}^{\circ\circ}\right) \quad (1.2.36)$$

Where

$$\alpha_1=\frac{\bar{Y}'C'_y}{\bar{X}'C'_x}\left(\frac{\rho'_{yx}-\rho'_{yz}\rho'_{xz}}{1-\rho'^2_{xz}}\right)$$

$$\alpha_2=\frac{\bar{Y}'C'_y}{\bar{Z}'C'_z}\left(\frac{\rho'_{yz}-\rho'_{xy}\rho'_{xz}}{1-\rho'^2_{xz}}\right)$$

Where $\rho'^2_{y.xz}$ is the partial coefficient of correlation for y on x and z.

c) A regression estimator under Partial Information Case.

$$T'_{37}=\bar{y}^{\circ\circ}+\alpha_1\left(\bar{x}^{\circ}-\bar{x}^{\circ\circ}\right)+\alpha_2\left(\bar{z}^{\circ}-\bar{z}^{\circ\circ}\right)+\alpha_3\left(\bar{Z}'-\bar{z}^{\circ\circ}\right) \quad (1.2.37)$$

The optimum values for unknown constants are:

$$\alpha_1=\frac{\bar{Y}'C'_y\left(\rho'_{xy}-\rho'_{yz}\rho'_{xz}\right)}{\bar{X}'C'_x\left(1-\rho'^2_{xz}\right)},$$

$$\alpha_2=\frac{\bar{Y}'C'_y\rho'_{xz}\left(\rho'_{xy}-\rho'_{yz}\rho'_{xz}\right)}{\bar{Z}'C'_z\left(1-\rho'^2_{xz}\right)} \text{ and } \alpha_3=\frac{\bar{Y}'C'_y\rho'_{yz}}{\bar{Z}'C'_z}$$

d) A regression estimator under no information case is:

$$T'_{38}=\bar{y}^{\circ\circ}+\alpha_1\left(\bar{x}^{\circ}-\bar{x}^{\circ\circ}\right)+\alpha_2\left(z^{\circ}-\bar{z}^{\circ\circ}\right) \quad (1.2.38)$$

e) The ratio estimator in case of complete information is:

$$T'_{39}=\bar{y}^{\circ\circ}\left(\frac{\bar{X}'}{\bar{x}^{\circ\circ}}\right)^{\alpha_1}\left(\frac{\bar{Z}'}{\bar{z}^{\circ\circ}}\right)^{\alpha_2} \quad (1.2.39)$$

$$\alpha_1=\frac{C'_y\left(\rho'_{xy}-\rho'_{xz}\rho'_{yz}\right)}{C'_x\left(1-\rho'^2_{xz}\right)} \quad \alpha_2=\frac{C'_y\left(\rho'_{yz}-\rho'_{xy}\rho'_{xz}\right)}{C'_z\left(1-\rho'^2_{xz}\right)}$$

f) The ratio estimator with partial information is:

$$T'_{40}=\bar{y}^{\circ\circ}\left(\frac{x^{\circ}}{\bar{x}^{\circ\circ}}\right)^{\alpha_1}\left(\frac{\bar{z}^{\circ}}{\bar{z}^{\circ\circ}}\right)^{\alpha_2}\left(\frac{\bar{Z}'}{\bar{z}^{\circ\circ}}\right)^{\alpha_3} \quad (1.2.40)$$

Where $\alpha_1=\frac{C'_y\left(\rho'_{xy}-\rho'_{yz}\rho'_{xz}\right)}{C'_x\left(1-\rho'^2_{xz}\right)}$,

$$\alpha_2=\frac{C'_y\rho'_{xz}\left(\rho'_{xy}-\rho'_{yz}\rho'_{xz}\right)}{C'_z\left(1-\rho'^2_{xz}\right)} \text{ and } \alpha_3=\frac{C'_y}{C'_z}\rho'_{yz}$$

g) Another ratio estimator given by Samiuddin and Hanif (2007) under no information case.

$$T'_{41}=\bar{y}^{\circ\circ}\left(\frac{\bar{x}^{\circ}}{\bar{x}^{\circ\circ}}\right)^{\alpha_1}\left(\frac{\bar{z}^{\circ}}{\bar{z}^{\circ\circ}}\right)^{\alpha_2} \quad (1.2.41)$$

Where $\alpha_1=\frac{C'_y\left(\rho'_{xy}-\rho'_{xz}\rho'_{yz}\right)}{C'_x\left(1-\rho'^2_{xz}\right)}$,

$$\alpha_2=\frac{C'_y\left(\rho'_{yz}-\rho'_{xy}\rho'_{xz}\right)}{C'_z\left(1-\rho'^2_{xz}\right)}$$

1.2.27 Singh and Espeejo (2007) Estimator: Singh and Espejo (2007) proposed ratio-product estimator under no information case.

$$T'_{42}=\bar{y}^{\circ\circ}\left\{k\frac{\bar{x}^{\circ}}{\bar{x}^{\circ\circ}}+(1-k)\frac{\bar{x}^{\circ\circ}}{\bar{x}^{\circ}}\right\} \quad (1.2.42)$$

Where $k=\frac{1}{2}\left(1+\frac{C'_y}{C'_x}\rho'_{xy}\right)$ and $0\le k\le 1$

1.2.28 Ahmad (2007) Estimators:

a) Ahmad (2007) proposed the following generalized ratio estimator with q auxiliary variables x_1 , x_2 ,… x_q when information on all of them is known for population,

$$T'_{43}=\bar{y}^{\circ\circ}\prod_{i=1}^{q}\left(\frac{\bar{X}'_i}{\bar{x}_i^{\circ\circ}}\right)^{\alpha_i} \qquad (1.2.43)$$

b) The following generalized ratio estimator for population mean $\bar{Y}'$ has been developed in case of information on first r supporting variables is known (partial information) where random sample having n° size be chosen at the first phase to get information on $q = r + s$ auxiliary variables x_1, x_2 ,..... x_q and from the second-phase, random samples of size $n^{\circ\circ}$, to get information on $y^{\circ\circ}$ and r auxiliary variables x_1, x_2 ,....., x_r .

$$T'_{44}=\bar{y}^{\circ\circ}\prod_{i=1}^{r}\left(\frac{\bar{x}_i^{\circ}}{\bar{x}_i^{\circ\circ}}\right)^{\alpha_i}\prod_{i=1}^{r}\left(\frac{\bar{X}'_i}{\bar{x}_i^{\circ\circ}}\right)^{\beta_i}\prod_{i=r+s=q}\left(\frac{\bar{x}_i^{\circ}}{\bar{x}_i^{\circ\circ}}\right)^{\alpha_i} \qquad (1.2.44)$$

c) The following generalized ratio estimator $\bar{Y}'$ in case where information about all helping variables is not known for population i.e no information case where a random sample with size n° be chosen to have information on q helping variables x_1 , x_2 … x_q and random samples of size $n^{\circ\circ}$ is chosen to get information on $y^{\circ\circ}$ and q auxiliary variables on second phase.

$$T'_{45}=\bar{y}^{\circ\circ}\prod_{i=1}^{q}\left(\frac{\bar{x}_i^{\circ}}{\bar{x}_i^{\circ\circ}}\right)^{\alpha_i} \qquad (1.2.45)$$

d) Ahmad (2007) proposed the following generalized regression estimator $\bar{Y}'$ of population mean by applying q auxiliary variables $x_1, x_2,$ x_q in case of information about all helping variables is accessible i.e complete information, Where $y^{\circ\circ}$ be a random variable for which a sample having size $n^{\circ\circ}$ is selected at second-phase and $x_1, x_2, \ldots x_q$ are q auxiliary variables.

$$T'_{46}=\bar{y}^{\circ\circ}+\sum_{i=1}^{q}\alpha_i\left(\bar{X}'_i-\bar{x}_i^{\circ\circ}\right) \qquad (1.2.46)$$

e) The following generalized regression estimator for population mean $\bar{Y}'$ has been developed when information about first r helping variables is accessible (partial information) where random samples of size n° be selected at the first phase to get information on $q = r + s$ auxiliary variables $x_1, x_2,\ldots\ldots, x_q$ and from the second-phase, random samples of size to get information on and r auxiliary variables x_1 , x_2 ,.....,x_q .

$$T'_{47}=\bar{y}^{\circ\circ}+\sum_{i=1}^{r}\alpha_i\left(\bar{x}_i^{\circ}-\bar{x}_i^{\circ\circ}\right)+\sum_{i=1}^{r}\beta_i\left(\bar{X}'_i-\bar{x}_i^{\circ\circ}\right)+\sum_{i=r+1}^{r+s=q}\alpha_i\left(\bar{x}_i^{\circ}-\bar{x}_i^{\circ\circ}\right) \qquad (1.2.47)$$

f) The following generalized regression estimator where information about all helping variables is not accessible i.e no information case where a random samples having size n° be selected from first-phase to get information about q helping variables $x_1, x_2 \ldots x_q$ and from second phase, random samples of size $n^{\circ\circ}$ selected to get information on $y^{\circ\circ}$ and q auxiliary variables.

$$T'_{48}=\bar{y}^{\circ\circ}+\sum_{i=1}^{q}\alpha_i\left(\bar{x}_i^{\circ}-\bar{x}_i^{\circ\circ}\right) \qquad (1.2.48)$$

3.2.29 Hanif et al. (2010) Estimator:

Hanif et al. (2010) suggested some regression type estimators with help of two helping variables under various situations. These are as follows:

a) No Information Case:

$$T'_{49} = \left(\bar{y}^{\circ\circ} + b_{yx}\left(\bar{x}^{\circ} - \bar{x}^{\circ\circ}\right)\right)\left\{K\frac{\bar{z}^{\circ}}{\bar{z}^{\circ\circ}} + \left(1 - K\frac{\bar{z}^{\circ\circ}}{\bar{z}^{\circ}}\right)\right\} \quad (1.4.49)$$

The optimum value of K that minimizes MSE is as under:

$$K = \frac{1}{2} + \frac{1}{2}\frac{C'_y}{C'_z}\rho'_{yz} - \frac{1}{2}\frac{C'_y}{C'_z}\rho'_{xy}\rho'_{xz}$$

b) Partial Information Case:

$$T'_{50} = \left(\bar{y}^{\circ\circ} + b_{yx}\left(\bar{X}' - \bar{x}^{\circ\circ}\right)\right)\left\{K\frac{\bar{z}^{\circ}}{\bar{z}^{\circ\circ}} + \left((1-K)\frac{\bar{z}^{\circ\circ}}{\bar{z}^{\circ}}\right)\right\} \quad (1.2.50)$$

c) Full Information Case

$$T'_{51} = \left(\bar{y}^{\circ\circ} + b_{yx}\left(\bar{X}' - \bar{x}^{\circ\circ}\right)\right)\left\{K\frac{\bar{Z}'}{\bar{z}^{\circ\circ}} + \left((1-K)\frac{\bar{z}^{\circ\circ}}{\bar{Z}'}\right)\right\} \quad (1.2.51)$$

3.2.30 Butt et al. (2010) Estimator:

Butt et al. (2010) produced multivariate estimator as:

$$T'_{52} = \bar{y}^{\circ\circ} + \left(\bar{x}^{\circ} - \bar{x}^{\circ\circ}\right)k + \left(\bar{W}' - \bar{w}^{\circ}\right)AK + \left(\bar{W}' - \bar{w}^{\circ\circ}\right)BK \quad (1.2.52)$$

Where X and W are the auxiliary variables. $\bar{y}^{\circ\circ}$ is the mean vector of estimates based on second-phase sample, K has been defined as vector having constants with A and B diagonal matrices.

The i^{th} component of the estimator is:

$$t_i = \bar{y}_i^{\circ\circ} + k_i\left[\begin{array}{l}\left\{\bar{x}^{\circ} + \alpha_i\left(\bar{W}' - \bar{w}_i^{\circ\circ}\right)\right\} - \\ \left\{\bar{x}^{\circ\circ} + \beta_i\left(\bar{W}' - \bar{w}^{\circ\circ}\right)\right\}\end{array}\right]$$

$$\alpha_i = \frac{S'_x}{S'^2_w} = \beta_{xw}\,;\ \beta_i = \frac{S'_{wx}}{S'^2_w} - \frac{1}{k_i}\frac{S'_{wy_i}}{S'^2_w} = \beta_{xw} - \frac{1}{k_i}\beta_{y_i w}\,;$$

$$k_i = \left(\frac{\rho'_{xy_i} - \rho'_{wx}\rho'_{wy_i}}{1 - S'^2_{wx}}\right)\frac{S'_y}{S'_x}$$

3.2.31 Hamad et al. (2013) Estimator:

Following estimator has been obtained under no information case.

$$T'_{53} = \left(\bar{y}^{\circ} + k_1\left(\bar{x}^{\circ} - \bar{x}^{\circ\circ}\right)\right)\left\{k_2\frac{\bar{z}^{\circ}}{\bar{z}^{\circ\circ}} + \left(1 - k_2\right)\frac{\bar{z}^{\circ\circ}}{\bar{z}^{\circ}}\right\} \quad (1.2.53)$$

Where

$$-\infty \le k_1 \le \infty\ ,\ 0 \le k_2 \le 1$$

CONCLUSION

As the sampling techniques are very important in research throughout the centuries so this paper is an effort of collecting a number of estimators for mean of population for concerned variable under two-phase sampling technique. It is a comprehensive study on estimators which may be investigated by different scholars for future study. They may throw more light on these estimators by making comparisons or by suggesting some new estimators.

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Effectiveness Of Gamma Radiation Technology For Abating Postharvest Losses Of Banana (*Musa Sapientum*) By Targeting The Residential Microflora

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ABSTRACT: *Banana (Musa spp.), member of the family Musaceae, is the perennial herb mostly grown in the tropical areas situated at latitude 30° above and below the equator covering the warmer countries of the world. This regional belt has wide seasonal variability with respect to temperature and rainfall. In the international trade, Banana holds 22% share in world fresh fruit production and is ranked as second amongst the most important fruits after citrus. Gamma irradiation of pre climacteric bananas at optimal doses, which vary with the variety of banana, delays the ripening process. For Pakistan, radiation processing technology supports remarkable potential not only for local food securities but also for greater exports earnings in agriculture sector. Farmers could be stimulated for greater production of fragile fruits like banana when putrefaction could be prevented by appropriate preservation practices resulting in increased and balanced consumption.*

Keywords: *Banana, postharvest losses, gamma irradiation*

INTRODUCTION

Bananas are produced by numerous types of enormous herbaceous flowering plants that are monocots and technically defined as 'herbs'. It is a unique fruit due to its high calories and nutritive value. Being a good source of carbohydrates, minerals and vitamins it leads all other fruits in food value. The general term "Banana" encompasses cultivated varieties of the genus Musa (Family Musaceae) that is divided into two subgroups: the sweet or dessert banana which accounts for approximately 43% and the cooking bananas which accounts for approximately 57% of the world production (Pillay et al., 2004). The term plantain is used to describe a specific subgroup of cooking banana (Valmayor, 2000). Sweet bananas show enormous diversity with respect to plant stature, fruit size and fruit colour which ranges from yellow and green to red and orange (Ploetz et al., 2007).

The annual production of banana amounts to around 80 million tons (Smith, 2010). The climatic conditions of the developing countries of Asia, Caribbean and Latin America are best suited for the banana cultivation. In Pakistan, banana was introduced from Bombay (India) to Sindh province in

1937 but the commercial production was started in 1947 after independence. Banana is cultivated on 34.8 thousand hectares in Pakistan (GOP, 2011). More than 90% of this area falls in the Sindh province, contributing 80% of the total production in the country. Pakistan exports banana mainly to Afghanistan, United Arab Emirates and Iran (CABI, 2008). Important cultivars include Dwarf Cavendish, Robusta, Monthan, Poovan, Nendean, Red Banana, Nyali, Safed Velchi, Ardhapuri, Rasthali, Karpurvalli, Karthalli and Grand Naine etc.

Banana is highly fragile commodity as per its extraordinary water content and is disposed to sundry diseases, particularly fungal infection. Being a climacteric fruit, it supports considerable amount of ethylene gas production which is responsible for swift alterations in physical and chemical properties, such as aroma, texture, color, chemical composition, respiration rate and senescence. The climacteric phase is accompanied by enhanced ethylene production, higher oxygen consumption, chlorophyll degradation, starch to sugar conversion, and relocation of the micro and macro nutrients between the pulp and other plant parts (Mohapatra et al., 2010). Banana quality quickly drop after the fruit is completely ripened. One of the restricting factors in banana exports to far off locations is the short shelf-life primarily due to postharvest deterioration during transport and storage. The major postharvest infections include anthracnose and crown rot disease caused by a fungal complex, *Colletotrichum musae, Fusarium* spp. and *Lasiodiplodia theobromae* (Win et al., 2007). Global postharvest losses of fruits and vegetables are as high as 30 to 40% and even much higher in some developing nations (Zaman W et al., 2007). Reducing these postharvest losses is vital; guaranteeing that adequate food of superlative quality is accessible for every single dweller of our planet. Minimization of post-harvest losses would reduce the cost of production, trade and distribution, lowering the price for the consumer and increasing the farmer's income.

The improper postharvest management practices result in huge economic losses. Post production losses of banana can be lowered by employing numerous post-harvest management practices that are in current use all over the world to enhance its shelf life. Scientific studies and genetic research into banana production has assisted in maximizing the output and quality, apart from increasing the resistance to diseases. However, parallel to improvements in productivity and quality, losses of banana produced are still prevalent (Pommer and Barbosa, 2009). Postharvest management practices such as sorting, cleaning, and pre-storage treatments like pre-cooling, modified atmospheric packaging, chemical treatment for disinfection, for banana have been proposed but all these processes have their limitations.

Ionizing radiation has been considered a prospective technology for prolonging the shelf life of food supplies as it can reduce pathogenic bacteria, disinfest fresh fruits and vegetables as a postharvest cordon sanitaire practice and diminish or exclude microorganisms. Additionally, radiation can hold back the ripening process in some fruits and vegetables, modifying the physiological developments in plant tissues (Follett and Weinert, 2009). This technique gains advantage over others for the reason that

gamma radiation offers effective and uniform penetration power in tissues for short time duration with not raising the temperature at the same time (Dionísio et al., 2009). Irradiation can interrupt with ripening of a number of tropical fruits and therefore extends shelf life. The longer shelf lives will improve the trade prospects among nations by lengthening time checks under which fresh produce must be supplied to remote geographical markets or by consenting the usage of unhurried and less costly methods of transportation (Kader, 1986).

ORIGIN AND DISTRIBUTION

According to Fuller and Madella (2009) Alexander the great reported seeing the people of India eating a fruit resembling the banana. Most of the primitive species of banana are still found growing wild in Asia which led us to believe that bananas have their origin in this region. South East Asia is the centre of diversity for bananas (ranging from South Western India through to Papua New Guinea) Early explorers propagated the banana around the world. Spanish brought them over to Western Hemisphere in 16th century and spread them to South and Central America and the Caribbean.

THE BANANA PLANT

Nelson et al. (2006) reported that the length of the plant can be upto 25 ft in some cultivars but generally remains 6 – 15 ft. The pseudostem of the plant is formed by the clustered cylindrical aggregation of the leaf stalk base. The banana plants have the largest leaves among all other plants, becoming upto 9 ft long and 2 ft wide. The number of leaves on each plant ranges from 25 – 50 with the minimum of 10 for proper maturation of a bunch of fruit. The mature banana plants possess spike like in flourescences originating from the rhizome. Turner et al. (2007) described that the male flowers are present at the top, neuter or hermaphrodite flowers in the center and female flowers with inferior ovaries occupying the lower five to fifteen rows on the stalk. The negative geotropic flowers turn upright during the first ten weeks of growth. Fruit is borne in hands of upto 20 fruits, with five to twenty hands per spike. Fruit appears angled, slender, green “fingers” during the growth period reaching harvest maturity in 90 – 120 days after flower opening. Generally the fruit is harvested when the fingers of the second hand are three-fourth rounded.

BANANA PHYSIOLOGY

Liu et al. (1999) researched that the banana being a climacteric fruit have a high respiration rate. The endogenous ethylene induces ripening making it strenuous to market the product in more distant localities. Three main events occur after the banana fruit is harvested namely the preclimacteric phase, the ripening phase and the senescent phase. Ripening makes the fruit edible and comprises the most crucial phase in their life both physiologically and commercially. Banana acquires proper weight, shape and volume and matures during the developmental phase. In the next phase of ripening, the fruit gains appropriate colour, aroma, texture and flavor. Surendranathan (2005) outlined that with the onset of the third and the final phase, senescence, the fruit begins to undergo degenerative changes marking the start of the end of the life of the fruit. The preclimacteric period after harvesting is of extreme

importance to the ripeners and importers as banana is transported before it is ripened. This period is characterized by low basal respiration rate and almost undetectable levels of ethylene production by the mature green fruit. Ngoh Newilah et al. (2008) described the preclimacteric period as the "green life". The longest practical preclimacteric period is desired.

COMPOSITION AND CONSUMPTION OF BANANA

Zaman et al. (2007) reported that banana is a unique fruit due to its high calories and nutritional value. Being a good source of carbohydrates, minerals and vitamins it surpasses all other fruits in food value. It has moisture, protein, fat, minerals, fiber and carbohydrate content of 70.1%, 1.2%, 0.3%, 0.8%, 0.4% and 27.2% respectively. Phosphorous, calcium and iron are the main mineral elements found with the first two occurring in significant quantities. It also provides vitamin A and C with small quantities of vitamin B complex. As compared to the apple it provides five times more vitamin A and iron, four times proteins, three times phosphorous, twice the carbohydrate and vitamin content. Based on the FAO statistics, bananas average consumption in Asia as a whole is approximately 8kg/person/year with a much greater average for other countries e.g 34kg/person/year for Philippines. The average for Pakistan (0.9kg/person/year) is only about one tenth of the regional average.

MEDICINAL USES OF BANANA

Valmayor (2000) documented that although best known as food crop, virtually every single part of the banana plant can be consumed in one way or another. This is the reason that banana is traditionally well-known as 'Kalpatharu' meaning, 'herb with all imaginable uses in India'. The dessert bananas have enormous therapeutic qualities in many special diets. Kumar et al. (2012) reported that banana is regarded as the 'meal in a peel' as it has large quantities of energy (90 calories per 100g with no cholesterol). A single banana harbors more than an adult's daily requirements of potassium (380g). Being low in sodium content, having very little fat and no cholesterol, they are useful in managing patients with heart diseases and high blood pressure. Bananas do not have any substances that when metabolized can synthesize uric acid and thus are ideal for patients with arthritis or gout. Bananas are referred to as 'good mood food' in Australia, as high level of vitamin B_6 helps in relieving stress. The pounded peels of the ripe banana are used in making poultice for wounds in Africa. Due to antiseptic properties of the peel it can be covered directly around wounds. In USA, a natural extract from the psuedostem has been patented under the name of 'Cell Quest' to be sold as a dietry supplement and preventative and curative agent of cancer.

ECONOMIC SIGNIFICANCE OF BANANA

Bananas is considered as key food commodity for low-income populations in many parts of the world. For that reason, bananas production is of significant commercial and societal importance. Banana industry holds a lot of potential for earning foreign exchange and employment in Latin America, Africa and Asia which are the major

producers of bananas. Based on the FAO (2004) statistics, world banana exports reach over US $ 5 billion value per year. This results in the establishment of a robust link amid banana generated income and house hold food security. Alterations in the export volume or price bring about income vagaries for those directly linked to banana production both as small holder farmers and as bread winner on banana plantation.

WORLD BANANA PRODUCTION

According to Heslop-Harrison and Schwarzacher (2007) bananas are now grown pantropically in one hundred and thirty countries which is more than any fruit. Worldwide over 1000 banana cultivars have been recognized. As quoted by De Centroamerica and De Trabajo-Correspondencia (2003) "If all the bananas grown in the world every year were placed end to end, they would circle the earth two thousand times". As of FAO (2009) it is cultivated over an area of more than four million hectares with annual production of greater than 80 million tons. Based on FAO (2010) data world banana production in 2010 reached 102 million tones, showing an increase of almost 50% from 65 million tons in 2000 and more than double the production in1990. According to the estimates of FAO (2011) India, Uganda, China, Philippines, Ecuador, Brazil, Indonesia, Columbia, Cameron and Tanzania were the leading banana producing countries with the production of 29.7 million, 11.1 million, 10.7 million, 9.2 million, 8.0 million, 7.3 million, 6.1 million, 5.1 million, 4.8 million and 3.9 million tons respectively.

BANANA EXPORTS AND IMPORTS

Smith (2010) narrated that there has been a steady increase in the world exports of banana and 17 million of the 80 million tons production entered into world market in 2008. These statistics indicate that the bananas hold the highest export ratio of all fruits, much greater than that of apples and mangoes. Sant'anna et al. (2009) recounted that it is traded predominantly in developed countries, reaching 90% of the world net trade. FAO (2014) catalogued the International banana exports depicting that its exports are highly concentrated in the following six countries: Ecuador, Costa Rica, Columbia, Philippines, Guatemala and Panama. India and Brazil, two major banana producing countries, however do not export significant quantities. This can be supported by the data gathered by FAO (2007) that India exported only 0.1% and Brazil only 3% of its total annual production in 2006. According to FAO (2011) Costa Rica, Ecuador, Guatemala, Mexico, Nicaragua, Panama and Philippines had the highest exports of 1624800, 4158200, 1691700, 167100, 883000, 141600 and 1067000 tons respectively. Among the importers, the strongest demands are in USA, EU, Japan, China and Canada. In 2004, European Union, United States of America and Japan, together represented more than 65% of the world imports of banana.

BANANA SECTOR OF PAKISTAN

GOP (2011) proclaimed that banana is cultivated on 34.8 thousand hectares in Pakistan with the annual production of 157300 tonnes. More than 90% of this area falls in the

Sindh province, contributing 80% of the total production in the country. Banana occupies the second ranking among the fruits of Sindh and is mainly cultivated in the areas of Khairpur, Thatta, Hyderabad, Nawabshah, Nausharoferoz, Sanghar, Mirpurkhas and Badin districts. Area under the fruit in the province of Punjab, Sindh, KPK and Balochistan for the year 2010-2011 was 1.5 thousand, 32.1 thousand, 0.7 thousand, 0.7 thousand and 0.4 thousand hectares. The corresponding production for that year was 10.1 thousand, 128.9 thousand, 13.5 thousand and 4.3 thousand tones for Punjab, Sindh, KPK and Balochistan respectively.

CABI (2008) data showed that Khairpur, Thatta and Hyderabad districts are the leading banana producing districts with production of 35,324, 30,432 and 21,996 tons. According to the economic survey 2011-2012, banana production decreased from 1,39,000 tonnes in 2010-2011 to 99,000 tonnes in 2011-2012. Banana holds a great potential to earn foreign exchange. Pakistan exports banana mainly to Afghanistan, United Arab Emirates and Iran. Afghanistan imported 867136767 kgs of banana worth 8658750000 PKR in 2009. In Pakistan about 98% of the farmers are planting the Dwarf Cavendish variety commonly known as "Basri" while 2% are planting William Hybrid.

STORAGE TEMPERATURE AND RELATIVE HUMIDITY

Mohapatara et al. (2010) reported that banana can be held in reserve at the temperature a little above 13°C and a relative humidity of 85 to 95% for approximately three weeks in storage. Mostly after removal from storage for delivery purposes, fruits should be kept at 13.3°C to 15.6°C and 80 to 85% relative humidity to prevent spoilage. Hailu et al. (2013) detailed the shipping of green bananas at 13 to 14°C to retard the ripening. Temperatures lesser than that may cause chilling injury giving rise to dull, grey skin colour, improper ripening and poor flavor development and vulnerability to deterioration. The development of symptoms rest on exposed temperature and time duration and proneness depends upon the cultivar. Kerbel (2004) described that temperatures higher than 25°C abreviate the preclimacteric phase and change the quality of fruit by modification of the metabolism during ripening. Beyond 35°C the development in peel and pulp occurs at unrelated times with softening of pulp foregoing faster than the colouring of peel. The consequences are that the fruit would have soft pulp but green peel and is known as cooked/boiled green. Above 48°C climacteric does not induce effectively blocking ripening. Banana fruit should not be refrigerated as it blackens at lower temperature. Lower relative humidity results in greater loss of water leading to shorter preclimacteric phase. It also induces ethylene production and respiratory rise but the amount of ethylene gas produced rely on the variety.

POST HARVEST LOSSES OF BANANA

Hassan et al. (2004) narrated that a large amount of the world's fruitage gets wasted because of putrefaction and infestations on the way to the consumers. In growing nations, where tropical surroundings and below par infrastructure adds to the situation, losses are occasionally of astounding volume. Losses

take place at all stages from harvesting via handling, storage, processing and marketing. Essentially, these postharvest losses are the manifestation rather than the actual problem. It is therefore imperative to single out the root causes for concluding the measures to avoid them. Such steps may need to be processed at different levels ranging from small scale farmers, the private traders, the cooperatives, the marketing board or the other operators, handlers and transporters, whole sale dealers and retailers etc.

Irtwange (2006) attributed most of the losses in perishable crops to microbiological, mechanical and physiological factors. Fallik (2004) claimed the other causes as derisory harvesting, packing and handling skills, scarcity of proper packages for transport and handling of perishables, improper storage services for safety of the food and transportation incompetent to supply the food to the market afore its decomposition. Soluri (2009) stated that banana prone to abrasion and injury faces large scale marketing problems of export due to mechanical damage that is shown as black sunken area on the peel after ripening. To avoid this bunch should not be allowed to bow down on the ground and get spoiled. The use of plastic liner in the export containers reduces chafing wreckage to the fingers brushing the side of the carton in the course of handling and transportation. Brown and black stains appear as the skin oxidizes due to drying of the latex leading to the downgrading of the fruit. Diseases particularly crown rot which has the tendency to effect the whole carton and promote uneven fruit ripening are other major concerns. The fingers of some cultivars may detach easily from the hand exposing the pulp during ripening because of the weak pedicels.

POST HARVEST TREATMENTS

Chamara et al. (2000) reported that since the postharvest losses are the result of improper storage, mechanical injury and decay by microorganisms, systemic fungicides of benzimadazole group have been frequently used in the past for the effective control of post harvest diseases. However conforming to Niranjala and Karunaratne (2001) with the arising public demands of fresh bananas, nonfungicidal control of postharvest diseases has been proposed and studies on the use of modified atmosphere packing and cold atmosphere storage have been carried out with the purpose of extending shelf life of banana and reducing the use of fungicides. Ranasinghe et al. (2005) reported that the essential oils of clove and cinnamon have been found to possess antifungal properties The low polyethylene packaging used helps preventing the evaporation of volatile compounds of oils and creates a high carbondioxide and low oxygen atmosphere, further retarding an increase in the production of pathogens. An integrated strategy of using combinations of essential oils with modified atmosphere to lessen post harvest deterioration problems in banana has been suggested, extending the storage life of banana upto 21 days in a cold room and 14 days at 28±2°C without upsetting the organoleptic and physiochemical features. Ilyas et al. (2007) outlined that the storage life also improves in the presence of modified atmosphere having lower oxygen concentration and higher carbondioxide concentration.

MICROFLORA ASSOCIATED WITH BANANA FRUIT SURFACE

As in line with Postmaster et al. (1997) consumers demand to use those fresh fruits and vegetables which are bacteriologically safe. As the fresh fruit passes from farm to the table, it is predisposed to probable microbial contamination throughout the entire process involving cultivation, harvesting, transporting, packaging, storage and selling it to the ultimate destination i.e. the consumer. FAO (2000) reported that spoilage and contamination due to microbes and pathogens presents a grave risk in food shelter. The Centre for Disease Control and Prevention stated that 76 million circumstances of food borne diseases occur anually. Incidences with identified etiology were essentially of bacterial nature demonstrating that the analysis of microbial load on the surfaces of raw fruits and vegetables is requisite

Obetta et al. (2011) carried out an investigation to study the microflora of apple, carrot and banana. The sample were evaluated for bacterial (coliforms and mesophiles) and fungal (mold and yeast) loads. The results revealed that banana had the average absolute microbial load with mesophillic aerobic plate count of 113 $\log_{10}$ cfu/in^2, coliform 42.7 $\log_{10}$ cfu/in^2, yeast 23.7 $\log_{10}$ cfu/in^2 and mold 28 $\log_{10}$ cfu/in^2. Fajinmi et al. (2011) studied the influence of two storage procedures (open shelf and jute bag) on microbial load and ripening behavior of some Musa species (PITA 26, cooking banana and 'agbagba' plantains) at various phases of ripening. The microbial count (bacterial and fungal) of fruits increases continuously as ripening proceeded. The identified fungal isolates were *Aspegillus niger*, *Fusarium spp*, *Saccharomyces* and *Penecillium spp* and the bacterial isolates were *Bacillus spp*, *Staphylococcus aureus* and *Lactobacillus spp*. A remarkable variation in the ripening rate of three different Musa cultivars placed under two storage methods was observed. The fruits kept in jute bags ripened earlier (8 days for PITA 26 and 'agbagba' plantain and 11 days for cooking banana) than the ones placed on open shelves (10 days for PITA 26 and 'agbagba' plantains and 14 days for cooking bananas).

Al Hindi et al. (2011) isolated and identified some fruit spoilage fungi and screened them for possessing plant cell wall degrading enzymes. Ten types of different fresh post harvest fruit samples were collected. The fruit spoilage fungi were isolated and identified as follows: *Fusarium oxysporum* (banana and grape), *Aspergillus japonicas* (pokhara and apricot), *Aspergillus oryzae* (orange), *Aspergillus awamori* (lemon), *Aspergillus phoenicis* (tomato) *Aspergillus tubingensis* (peach), *Aspergillus niger* (apple), *Aspergillus flavus* (mango), *Aspergillus foetidillus* (kiwi) and *Rhizopus stolonifer* (date). Gawai (2011) carried out an investigation to study the biochemical changes in banana fruits due to post harvest fungal pathogens. Four fungi were focused which were responsible for bringing considerable biochemical changes to alter the quality of banana fruit. In contrast with the control group, the total soluble sugar, protein, ash, ascorbic acid and mineral elements of the effected banana were found to be decreased in quantity. *B. theobromae, R.oryzae, A. flavus* and *F. roseum* were responsible for causing rot during

storage and were associated with the ripening of bananas.

Alvindia and Natsauki (2008) examined the aerial and fruit surface population of fungi in nonchemical banana production in Phillipines. The aim of the study was to follow the source, find out the origin and determine the population dynamics of numerous fungi associated with the postharvest diseases of banana. There were a total of forty fungal genera found in the air on banana farm and 26 of them were present on the developing fruits. The dominant fungi associated with the air were *Penecillium* (13.7%), *Fusarium* (12.1%), *Aspergillus* (9%), *Lasiodiplodia* (8.3%), *Culvularia* (7.5%), *Trichoderma* (5.2%) and *Collotrichum* (5%). Other reported fungi in air included *Ullocladium, Neurospora, Drechslira, Diplodio, Bipolaris, Thysanophora, Stemphyliomma, Acladium, Besipetospora, Annellophorella, Tetraploa, Nigrospra, Gliocladium, Rhizopus, Thielaviopsis, Arthrinium, Mucor, Phoma, Cylindrocarpon, Nectria, Phomopsis, Glomerella, Plectosporium, Acremonium, Verticillium, Oidiodendron, Spiromyces, Oedocephalum and Monilia.* The maturing banana surface was characterized by two consistent fungal genera noticed from the week after flower emerged until the 11th week. They were *Aspergillus, Fusarium, Lasiodiplodia, Thielaviopsis, Collotrichum, Penecillium, Gliocladium, Nigrospora, Papularia* and *Trichoderma*. The study suggested that the fungi accountable for postharvest diseases of nontreated bananas were imported from the banana farm. This was based on the fact that all the fungi responsible for causing postharvest diseases of banana were present in the air at the farm as well as on the developing fruit surfaces. Furthermore, the most prevalent fungi in the air at the farm were the same as that of the ones prevaliing on the banana fruit from flowering until the harvest.

Postmaster et al. (1997) carried out a study to examine the epiphytic microflora on the developing banana fruit surface. The fruit surface was observed for microbial load in the months of April, August and December at three different developmental stages. The three stages of fruit development encompass: the first flower emergence (1-2 weeks old), immature stage (6-8 weeks old) and pre harvest or mature stage (16-20 weeks old). The bacterial, yeast and filamentous fungal count was enumerated and the fungal isolates were identified. In April, 59% of the isolates were bacteria, 34% were filamentous fungi and 7% were yeast. In August 35% of the bioburden were bacteria, 47% were filamentous fungi and 18% were yeast. Of the microbial isolations made in December 15% were found to be bacteria, 37% filamentous fungi and 48% were yeast. Greater number of epiphytic microorganisms were observed on the fruits at mature pre harvested stage than the ones at the other two earlier stages of development. The fruits sampled in August harbored a higher microbial load than those in April or December. Numerous yeasts were isolated from the fruits collected in December at the mature preharvest stage of fruit development. There was not a notable difference in the yeast, bacterial or filamentous fungal count when considered separately, but for the difference in seasons. It was therefore concluded that season influenced microbial population on banana fruit surfaces more than the stages of development. 12 species of yeasts, 83 species of bacteria (73 gram positive rods, 4 gram negative rods, 6 gram positive cocci) and 47

species of fungi (20 *Cladosporium species*, 20 *Aspergillus species*, 7 other fungi species) were identified in the month of April. In August, 32 species of yeast, 60 species of bacteria (55 gram positive rods, 2 gram negative rods, 3 gram negative cocci) and 80 species of fungi (60 *Cladosprium species*, 12 *Asppergillus species*, 8 other fungi species) were isolated. The microbial enumeration for the month of December included 65 yeast species, 20 bacterial species (17 gram positive rods,1 gram negative rod, 2 gram positive cocci) and 50 fungi species (40 *Cladosporium species*, 7 *Aspergillus species*, 3 other fungi species). Fungi that sporulated and were identified in the other fungi category were *Fusarium, Alternaria and Penecillium* for the month of April, *Nigrospora, Periconia, Alternaria, Trichoderma, Gliomastix and Fusarium* for the month of August and *Paecilomyces, Gliomastix* and *Penecillium* for the month of December. The yeast species present on the surface of bananas in the month of April included *Rhodotorula glutinis, Cryptoccocus albidus, Candida lusitaniae, Zygosaccharomyces species, Candida famata* and *Saccharomyces kluyveri.* In the month of August the identified species of yeast were *Cryptoccocus laurinie, Rhodotorula glutinis, Cryptococcus albidus, Candida membranaefaciens, Cryptococcus unigultulatus, Saccharomyces cerevisea, Candida famata.* In the month of December the banana peel harboured the richest diversity of yeast species than the other two months which included *Crytoccocus laurennii, Candida memebranaefaciens, Rhodotorula glutnis, Saccharomyces Kluyveri, Candida curvata, Candida guillirmondii, Cryptococcus neoformans, Trichosporum cutanium, Rhodotorula rubrae, Candida ciferii* and *Candida silvicola.*

GAMMA IRRADIATION AND FOOD SAFETY

According to Loaharanu (2007) since 2000, irradiation has been officially adopted as a quarantine treatment to check the spread of tropical fruit flies and the other insect pests present in fresh fruits and tubers. Food is mainly irradiated for accomplishing two main goals. Firstly to safeguard the hygienic quality of solid foods of both plant and animal origin that may get contaminated by certain spoilage and pathogenic microorganisms and secondly to satisfy the quarantine standards in trade in fresh produce particularly tropical fruits. Conventional food processing methods such as smoking, salting, heating, fermentation and drying have been practiced for ages to refine the quality, quantity and safety of food. Recent methods such as pulsed electrical fields, electrical conductivity, heating, ultra high hydrostatic pressure, fumigation, refrigeration, freezing, canning and heat pasteurization have been included to food processing technologies. Each of the above mentioned methods holds distinctive benefits in preserving the food supplies against spoilage, microbiological contamination and destruction. However none can be applied for all types of foods. Most of these are capable of causing notable changes in food quality and sensory attributes.

As published by Fielding (2007) serious food recalls and food borne disease incidences in the past decade have intensified the awareness of the risks associated with the food borne pathogens and the use of the irradiation to maintain the microbiological safety of foods. Approved by U.S FDA in Decemeber 2006 and some other countries, irradiation can be deployed to halt spoilage and pathogenic

bacterial growth in most solid foods in the raw, frozen or dried state without significantly raising the temperature or altering the sensory attributes. It is therefore also referred to as "cold pasteurization". The allowed maximum absorbed dose of 10 kGy can only increases the temperature of exposed food by less than 40 F. It is unable to reverse the food spoilage therefore safe food handling and good manufacturing practices must be observed for irradiated foods.

Farkas and Mohácsi-Farkas (2011) narrated that food irradiation has been permitted in about more than 50 countries currently with the volume of the food treated exceeding 500,000 metirc tons per annum worldwide. The safety of the irradiated food has been investigated more widely than that of any other food preservation process. As the cases with other food processes, irradiation may bring chemical alterations in food. The radiolytic products formed are similar in nature to the ones formed in heat treatment. However none of these products in irradiated foods have been found in quantities that may be toxic by any modern toxicological methods. According to the United States Department of Agriculture (USDA) estimates, the American consumer will gain approximately $2 in benefits in the form of reduced spoilage and less illness for every $1 spent on food irradiation.

EFFECT OF GAMMA IRRADIATION ON MICROORGANISMS

Da Silva Aquino (2012) explained the effects of radiation on live microorganisms are mainly related to the chemical alterations but are also associated with somatic and biological factors. Among the physical parameters, dose rate, dose distribution and radiation quality are included. The paramount biological factors are oxygen concentration, moisture content and temperature. The harmful effects of ionizing radiation on microorganisms as qualified by the inability of cells to form colonies on culture medium has been the focus of thorough investigaton.

Mohamed and Mahmoud (2010) carried out an investigation to examine the influence of gamma irradiation on the microbial load of banana fruit stored under different temperatures. Bananas were exposed to irradiation doses of 0.0, 0.25, 0.50, 1.0, 1.5, and 2.0 kGy. Both irradiated and nonirradiated fruits were kept under different temperatures of 5, 10, 15, and 20°C. Samples were analyzed after different storage periods at an interval of 7 days up to 42 days. For all treatments, decay percent of fruits, total bacterial count and fungal count were measured. The positive effect of gamma irradiation to minimize the decay percent and in turn to increase the shelf life was greatly correlated with the imposed storage temperature. The lower proportion of decay and injury was registered with the lowest temperature storage rate. Hence the storage temperature of 5°C was accountable for the most favourable results of reducing and retarding the deterioration of both irradiated and non irradiated banana fruits. For the fruits sample stored at 5°C, the experimental group and the control group bananas had no incidence of decay upto 14 days of storage, however, only the nonirradiated control ones showed decay after 21 days of storage. Irradiation dose of 1.0 kGy was proved to be the superior one to delay injury and decay percent at all different storage temperatures followed by the treatments of 0.5 and 1.5 kGy.

The exposed gamma irradiation showed perceptible effect on total bacterial count of banana fruits and this effect also depended greatly on the given temperature level. In realtion to this, the non irradiated bananas kept at 5°C had initial bacterial count of 2.4 X 10^5 cfu/g after the first week of storage. This count increased slowly until the 6^{th} week up to 16.5 X 10^5 cfu/g. However the applied gamma irradiation doses played a positive role in retarding the bacterial growth on the stored fruit surface. The total bacterial count reduced with the applied doses of 0.25 and 0.5 kGy. No count could be detected with applied doses of 1.0 and 1.5 kGy up to the storage period of 21 and 28 days respectively. On the other hand, complete inhibition was achieved by 2.0 kGy during all storage periods. With respect to the total fungal count, it seemed that fungi intended to be more sensitive towards the irradiation than the total bacterial count. Fungal count also showed remarkable reduction at lower storage temperature rather than the higher ones. Again with the increase in the applied doses, the total fungal count like that of the total bacterial count also got reduced with the completed inhibition attained at doses of 1.0, 1.5 and 2.0 kGy with respect for banana fruits stored at 5°C. It could, therefore, be concluded that the banana fruits receiving the irradiation treatment of 1.0 kGy and then stored at 5°C were the ones with lowest injury and decay incidence upto maximum days and hence with the most extended shelf life.

Jitareerat et al. (2005) studied the effect of gamma radiation on the fungal growth and their pathogenesis on banana. *Collotrichum musae*, *Lasiodiplodia theobromea* and *Fusarium* species were grown on potato dextrose agar and the effect of gamma radiation on their mycelium growth was analyzed. The exposed doses were 0.0, 0.5, 1.0, 2.0 and 4.0 kGy. The growth of *Collotrichum musae* was impeded above 2.0 kGy while increasing the doses upto 4.0 kGy did not produce the effect of inhibition on the growth of *L. theobromea* and *Fusarium* species. The mycelium survived after irradiation were compared to the non gamma treated mycelium for pathogenesity on banana fruit. The fruits were inoculated with the mycelium of *C. musae* irradiated at 1.0 kGy and mycelium of *L. theobromea* and *Fusarium spp*. at 4.0 kGy. The bananas were examined after being kept at about 30°C for 6 hours in ambient air. It was found that irradiation did not bring significant change in pathogenesity of *C. musae* and *Fusarium* species on banana but in case of *L. theobromea* pathogenesity was slightly decreased.

INHIBITION OF RIPENING BY GAMMA IRRADIATION

Gamma irradiation can be employed to improve the shelf life of tropical fruits by delaying the processes of ripening and senescence. Ngoh Newilah et al. (2008) explained that after harvesting, banana produces carbondioxide and consumes oxygen at a rate that slows down to a minimum called the “preclimacteric minimum” erstwhile the commencement of ripening process. As the ripening proceeds, the respiratory rate escalates escorted by the change in green to yellow pigmentation (chlorophyll degenaration and anthocyanin and carotenoid formation), softening of texture (insoluble protopectin converted to soluble pectins and pectic acids), taste and aroma (production of characteristic volatile aromatic compounds and conversion of sugar to acids), upsurge in

sugar content, astringency (polymerization of low molecular weight polyphenols into soluble forms), and ethylene production rate. The time requisite for reaching the maximum respiratory rate i.e. "the climacteric peak" depends upon the fruit maturity and storage temperature. Many of the climacteric fruits are good enough to eat at or after the climacteric maximum.

As per Code of Good Irradiation Practice of Department of Standards Malaysia for shelf life extension of banana, the optimal dose range for different varieties is 0.2 kGy to 0.4kGy with the maximum tolerable dose of 0.5 kGy. The dehanded bananas are separated into clusters of 5 to 6 fingers and given a hot water pretreatment for 5 mins at 55°C for fungal control. After irradiation, bananas are stored at 13°C to15°C in relative humidity of 90% to 95%. The Codex Alimentarius Commision has approved the maximum tolerable dose of 1.0 kGy to delay ripening of fresh fruits and vegetables. Strydom and Whitehead (1990) analyzed the effect of ionizing radiation on ethylene sensitivity and postharvest ripening of banana fruit. Green banana fruits at full three quarter grade were given a gamma radiation treatment from a cobalt source to average doses of 0.0, 0.2, 0.4, 0.6, 0.8 and 1.0 kGy at dose rate of 7.35 kGy per hour and 20 to 25°C. The fruits were incubated at 21 ± 2°C and 75 ± 2% RH. After 14 days the irradiated banana were flushed with gas mixture of 1000 µL C_2H_4 air for 24 hours at 21°C to induce ripening. 0.2 kGy was the optimum radiation dose for dwarf Cavendish bananas kept at 21°C and 75% RH for 25 days by decreasing the sensitivity of bananas to its own endogenous C_2H_4 as well as exogenous. Doses higher than 0.2 kGy were found to be lethal.

Aina et al. (1999) examined the effect of gamma radiation on postharvest ripening of plaintain fruit cultivars named Agbagba, Obina L'ewai and Carbada harvested at mature full three quarters and full grades. Prior to irradiation, banana were treated with 0.1% benomyl solutions. Irradiation of untarnished and equally sized fruits was carried out within 12 hours of harvesting. The gamma irradiation doses were 0.0, 0.15, 0.20, 0.25, 0.30, 0.50 and 1.0 kGy in air at 25°C at a dose rate of 1.98 kGy. After irradiation, fruits were placed in baskets at 25 ± 2°C and 68 to 75% relative humidity. The ripening rate was determined by examining each fruit every day for color development with respect to the numerical color score styled by Loesecke. The ideal dose range for every cultivar at different stages of maturity was scrutinized on the basis of maximum delay in ripening with minimum skin injury. For all the three varieties, the irradiation of fully mature fruits exhibited no optimistic results since they ripened within 7 days just like the nonirradiated control ones. However irradiation at 0.15 kGy to 0.3 kGy postponed ripening in fruits at three full quarter stage. The development of yellow coloration was inhibited for greater length of time (10 to 12 days) in case of Landracs Agbagba and Obino L'ewai than the Carbada (3 days). This finding specified that the response to irradiation was clearly cultivar dependent. During the process of ripening both treated and control group of bananas showed climacteric patterns in CO_2 production. The beginning of the respiratory climacteric phase though was different with fruit cultivar, being sooner in Carbada (3 days) then the other two cultivars. Irrespective of the varietal differences of fruit maturity, irradiation to 0.5 kGy proved to be lethal causing tissue injury,

softening and undesirable skin discolouration.

Chanloy et al. (2005) investigated the outcome of gamma irradiation on ripening process of kluai khai banana. The fruits were irradiated at 0.0, 0.3, 0.6 and 1.0 kGy. Later on they were stored at room temperature (approximately 30°C) in ambient air. Peel colour, fruit firmness and weight loss were measured. Upon analysis, it was found that irradiation induced ripening during storage at 30°C. This could be concluded in accordance with the values observed for peel colour, fruit firmness, and the weight loss at different time intervals.

Zaman et al. (2007) examined the effectiveness of gamma radiation on enhancing the shelf life of bananas. In the study the bananas were first irradiated at 0.3 kGy, 0.4 kGy and 0.5 kGy for 5 minutes and placed under ambient conditions (25 ± 5°C / 80 ± 5% RH) in dry place. The physical analysis of irradiated and nonirradiated banana for their organoleptic properties at an interval of two days till spoilage suggested that the ripening period of the controlled bananas was 6 days and for the irradiated ones was 26 days hence increase in shelf life by 20 days. The quantitative analysis of the nutrient content and some important physiochemical parameter at an interval of 24 days resulted in the conclusion that the only detrimental effect of gamma radiation was the decrease in the ascorbic acid content of irradiated banana. However no other noteworthy changes took place in the nutrient and organoleptic qualities of fruit. The statistical analysis of scores given by the taste testing panelists suggested that the treated bananas to be acceptable up to 26 days at room temperature. The optimal dose was found to be 0.3 kGy.

Silva et al. (2008) used the gamma radiation to enhance the shelf life of bananas. The bananas were pretreated before irradiation by dipping in sodium hypochlorite at 200ppm. The range of doses used was 0.15 kGy, 0.30 kGy, 0.45 kGy, 0.60 kGy and 0.75 kGy with the storage conditions set at 16 to 17°C temperatures and 60 to 98% relative humidity. The effect of gamma radiation on physical and chemical parameters was observed, assessing the changes in the ripening period and the possibility of commercial irradiation aimed at export. No effect of irradiation at pH and acidity was observed. Highest degree of maturation was observed by the banana in the control group and those exposed to the irradiation dose of 0.75 kGy. Bananas receiving the highest doses of 0.3 kGy showed higher firmness. With respect to the taste, more ripe banana especially those belonging to the control group had the higher acceptance level. The dose of 0.3 kGy was the optimum dose to delay the ripening process. The results also concluded that irradiation in appropriate dosage benefited the storage and export process.

Gloria and Adao (2013) irradiated the green prata banana at full three quarter stage using the gamma radiation doses of 0.0, 1.0, 1.5 and 2.0 kGy. Storing the bananas (treated and controls) at 16 ± 1°C and 85% relative humidity, samples were periodically examined for peel colour, pulp to peel ratio and levels of starch, soluble sugars and bioactive amines. First and zero order kinetics were observed in the case of degradation of starch and formation of fructose and glucose respectively. Degradation of the starch correlated negatively

with gamma radiation dose. Fruits irradiated at 1.5 and 2.0 kGy were found to be unacceptable due to browning of the peel. Irradiation at 1.0 kGy was the most appropriate dose as it showed no effect on peel colour, pulp to peel ratio or the levels of amines. Starch degradation, formation and accumulation of glucose and fructose were slowed down at this dose delaying the ripening of the fruit for 7 days.

CONCLUSION

Horticulture crops are famous for their high return per unit time and area. However a substantial amount of postharvest wastage of these produces take place around the world. These postharvest losses are responsible for the reduction of profits from these produces thus subsequently promoting food insecurity (Mashau et al., 2012). One of the primary factors that can be held accountable for the losses is the microbiological decay. In relation to that this review aimed at evaluating the microbial potential and ripening behavior of mature green bananas as affected by gamma irradiation. The ionizing radiations, also endorsed by WHO to increase food safety, play a positive role in preserving the quality and mean life of fruits and vegetables. Gamma radiations have been proven to be energetically more powerful than the x-rays (Ibarz, 2008). Fruits are routinely irradiated around the globe with a purpose of delaying ripening process. The maximum dose of ionizing radiation approved by the United States that can be applied to fresh fruits and vegetables for retarding growth, maturity and disinfestation is 1.0 kGy (Miller, 2006).

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Comparative assessment of antifungal potential of *Zingiber officinale* and *Allium sativum* against standard antifungal drugs Amphotericin B and Nystatin

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ABSTRACT: *This study was conducted to investigate theantifungal activity of garlic and ginger on a food derived fungus.Aqueous extracts of garlic (Allium sativum) and ginger (Zingiber officinale) were tested for their antifungal activity and their activities were compared with antifungal drugs nystatin and amphotericin B. The extracts were 40-50% serially diluted and different concentrations were obtained. Sterile strips were dipped in different concentrations of garlic and ginger. Strips were placed on petri plates containing fungus culture. After 24-48 hours incubation at 37°C, antifungal zones of different diameter were obtained and measured in different plates. The results showed that garlic is more effective in its antifungal activity than ginger. The study demonstrated the potent activity of garlic against tested fungi which encourages its use as a suitable alternative drug for controlling fungal food spoilage. Since garlic is an herbal product with no known side-effects on human healthiest active ingredients may serve as safe food preservatives. This can ultimately increase the shelf life and also maintain the quality of preserved food. Purification and formulation of the garlic would give a true antifungal activity comparable to standard antifungal drugs.*

Keywords: *Garlic, ginger, antifungal activity, fungus, antifungal drugs.*

INTRODUCTION

To treat infectious diseases by the use of plant parts as a source of medicine predates history as a result of which nearly all cultures and civilizations from ancient times to the present day have used herbal medicines to cure infections. Ethno-pharmacological use of plants is used in treating malaria, diarrhea, burns, gonorrhea, stomach disorders and other infectious diseases. These plants which are easily available and cheaper than the conventional drugs include garlic and ginger which in their natural state are widely used as herbal medicines in West Africa.The extracts of ginger and garlic are widely used for this purpose. Extraction refers to processes of the isolation of the active ingredients from drug material and this may be by physical means or by dissolving in a suitable liquid solvent e.g. water or alcohol.

The presence of bioactive compounds of such plant extracts have been linked to antimicrobial activities which protect the plants themselves against bacteria, fungi and viral

infections as well as exhibiting their antimicrobial properties on these organisms. At present it has been estimated that about 80% of the world population rely on botanical preparations as medicine to meet the requirements as they are considered safe and provided to be effective against certain ailments (Hora and Nair, 1994).

Ginger (*Zingiber officinale*), belongs to the Zingiberaceae family, which is characterized by their tuberous or non-tuberous rhizomes, which have strong aromatic and medicinal properties. It is a well-known spice used in the daily diet in many sub-continental areas. It is a rhizomatous plant grown throughout Southeastern Asia, China and in parts of Japan, Austria, Latin America, Jamaica and Africa.

Over three quarters of the world population still rely on plantsand plant extracts for health care. Moreover, it is consumed worldwide as flavoring agent which is used extensively in food, beverage, and confectionary industries in the products such as marmalade, pickles, chutney, ginger beer, ginger wine, liquors, and other bakery products (Shim et al., 2011). In South India, ginger is used in the production of a candy called Injimurappa meaning ginger candy in Tamil (Sasidharan and Menon, 2010).

In addition, it has been reported that the main ingredients of ginger like volatile oil, gingerol, shogaol and diarylheptanoids work as antioxidant, anti-inflammatory, anti-lipid, anti-diabetic, analgesic, antipyretic and anti-tumor (Wang et al., 2011). The British Herbal Compendium reported its action as carminative, anti- emetic, spasmolytic, peripheral circulatory stimulant and anti-inflammatory (Ficker et al., 2011). The oil of ginger is a mixture of different compounds, consisting of monoterpenes (phellandrene, camphene, cineole, citral, and borneol) and sesquiterpenes (zingiberene, zingiberol, zingiberenol, s-bisabolene, sesquiphellandrene, and others). Aldehydes and alcohols are also present (Tang and Eisenbrand,1992). Currently, there is a growing interest to detect natural compounds characteristics and activities, like plant extracts of herb and spices for the preservation of foods, flavor characteristic and sometimes show antioxidant activity as well as antimicrobial activity. This gives the motivation for our present study to focus on ginger. Plant derived products have been used for medicinal purposes for centuries. In traditional Indian medicine or Ayurveda, *Zingiber officinale* and many other herbs have been used as medicine.

Due to increase in the antibiotic-resistant strains of microorganisms, traditional plants are being investigated or found for their antibacterial and medicinal values. Traditional uses of plants have led to investigating their bioactive compounds, which have resulted in the detection of a significant number of therapeutic properties. Due to the development of resistance in known fungal pathogens and the emergence of fungal pathogensintrinsically resistant to the currently available antibiotics, it is important that novel antifungal agents be identified and developed.

Fresh ginger contains 80.9% moisture, 2.3% protein, 0.9% fat, 1.2% minerals, 2.4% fiber and 12.3% Carbohydrates. The minerals present in ginger are iron, calcium and phosphorous. It also contains vitamins such as

thiamine, riboflavin, niacin and vitamin C. The composition varies with the type, variety, agronomic conditions, curing methods, drying and storage conditions (Ding *et al.*,1991).

The following table 1 shows the physiochemical properties of ginger essential oils from different origins. It shows the percentage of each constituent present in it.

Table 1: Physicochemical properties of ginger essential oils from different Origins

Constituents Present	% of amount
Moisture	80.9
Protein	2.3
Fat	0.9
Minerals	1.2
Fibers	2.5
Carbohydrates	12.3

The composition of the bulbs is approximately 84.09% water, 13.38% organic matter, and 1.53% inorganic matter, while the leaves are 87.14% water, 11.27% organic matter, and 1.59% inorganic matter (Yamasaki et al*.,* 1991).

Garlic has been shown to inhibit the growth of a variety of microorganisms, not only bacteria but also fungi and viruses. The antimicrobial activity of garlic is believed to be due to the effect of allicin, the main ingredient in garlic, generated by the phosphopyridoxal enzyme allinase. It is necessary to investigate whether or not the other components of garlic have antimicrobial activity.

Amphotericin B:

Amphotericin B is a polyene antifungal drug, often used intravenously for systemic fungal infections. It was originally isolated from *Streptomyces nodosus*, a filamentous bacterium, In 1955 at the Squibb Institute for Medical Research from cultures of an undescribed streptomycete isolated from the soil collected in the Orinoco River region of Venezuela. Its name originates from the chemical's amphoteric properties. Two amphotericins, amphotericin A and amphotericin B are known, but only B is used clinically, because it is significantly more active *in vivo.*

Mechanism of action:

As with other polyene antifungals, amphotericin B binds with ergosterol, a component of fungal cell membranes, forming a transmembrane channel that leads to monovalent ion (K^+, Na^+, H^+ and Cl^-) leakage, which is the primary effect leading to fungal cell death. Recently, researchers found evidence that pore formation is not necessarily linked to cell death (Baginski and Czub., 2009).

Nystatin:

Nystatin (originally named Fungicidal) is a polyene antifungal that is derived from a bacterium, *Streptomyces noursei*. It was discovered by Rachel Fuller Brown and Elizabeth Lee Hazen in 1950. Nystatin is sensitive for many molds and yeast infections, most notably *Candida*. It is used primarily for infections of the skin, mouth, esophagus and vagina. (Dismukes et al*.,* 2010)

Mechanism of action:

Like amphotericin B and Natamycin, Nystatin binds to Ergosterol, a major component of the fungal cell membrane. When present in sufficient concentrations, it forms pores in the membrane that lead to K^+ leakage, acidification, and death of the fungus (Hammond, 1977).

MATERIALS AND METHODS

Antifungal Agents:

The antifungal drugs (Amphotericin B and Nystatin) were procured from commercial source. These agents were dissolved in distilled water. The antifungal activity of the agents was tested in the following dilution range of 10-50% drugs in 100ml of distilled water. The different drugs concentration was prepared freshly when it was tested against strain each time.

Test Microorganism:

The sample was taken from fungus spoiled sweet desert pudding (Gajrela). A common sweet desert in sub-continent.

Preparation of the Ginger and Garlic Extract:

Fresh rhizomes of ginger (*Zingiber officinal*) and bulbs of garlic (*Allium sativum*) were purchased from local market. The rhizome and cloves were separated and peeled to obtain the edible portion. Fifty grams of the edible portion of each was chopped separately and then filtered by passage through filter paper to give a crude extract.

Different concentrations of effective plant extracts were used to check efficiency of extract. For making different concentrations of ginger and garlic, crude extract was dissolved in 100ml of distilled water.

Following concentrations were used:

Agents	% Concentrations			
Ginger	70 %	100 %	-	-
Garlic	70 %	100 %	-	-
Amphotericin B	10 %	15 %	40 %	50 %
Nystatin	10 %	15 %	40 %	50 %

Isolation:

Fungus was derived from spoiled food sweet desert pudding (Gajrela). This was grown on Potato dextrose agar (PDA) overnight at 37°C for 24 hours and 48-72 hours. Some isolates take more time for their optimum growth. This plate is known as master/mother plate.The Potato dextrose agar (PDA) was used for the study.

Inoculum Preparation:

With the help of sterile cotton swab few mycelia from the master plate were picked and swabbedon dried plates of Potato dextrose agar (PDA) to get lawn culture. The swabbed plates were placed in incubator for few minutes.

Gel diffusion method:

Different equal sized porous strips were dipped in different concentrations of

agents.With the help of a forceps/applicator, the different strips were placed carefully in the middle of different media plates. Pressed the bandages gently and then placed in the incubator at 37 °C for 24 or 48 hours.

RESULTS

The present study was done to evaluate the antifungal efficacy of ginger and garlic in comparison to various antifungal drugs like nystatin and amphotericin B.

The antifungal characteristics of garlic, ginger, Nystatin and Amphotericin B are shown in Tables 2-5. All these plant derived herbs show different degrees of antifungal activities against the food derived fungus used.

Table 2: Different concentrations of Garlic extract used to observe the anti-fungal activity

Sr. #	% concentration of Garlic extract	Number of replica	Width of zone in cm
1.	70	1	0.4
	70	2	0.5
	70	3	0.3
	-	Control	No zone
2.	100	1	0.6
	100	2	0.5
	100	3	0.8
	-	Control	No zone

The observations show that different concentrations of garlic (70 and 100%) exhibited antifungal activities (Table 2). Since the antifungal zone is seen in all these plates, therefore, it can be concluded that garlic can be used for its anti-fungal properties against several fungi.

Table 3: Different concentrations of Ginger extract used to observe the antifungal activity

Sr. #	% concentration of Ginger extract	Number of replica	Width of zone in cm
1.	70	1	No zone
	70	2	No zone
	70	3	No zone
	-	Control	No zone
2.	100	1	No zone
	100	2	No zone
	100	3	No zone
	-	Control	No zone

It is observed in the experiment that different concentrations of ginger extracts used do not exhibit any significant antifungal property. The results obtained show that ginger is not efficient against fungi (Table 3).

Table 4: Different concentrations of Nvstatin used to observe the antifungal activity

Sr. #	% concentration of Nystatin	Number of replica	Width of zone in cm
1.	5	1	No zone
	5	2	No zone
	5	3	No zone
	-	Control	No zone
2.	10	1	No zone
	10	2	No zone
	10	3	No zone
	-	Control	No zone
3.	15	1	No zone
	15	2	No zone
	15	3	No zone
	-	Control	No zone
4.	40	1	0.3
	40	2	0.2
	40	3	0.4
	-	Control	No zone
5.	50	1	0.4
	50	2	0.1
	50	3	0.6
	-	Control	No zone

The results with antifungal drug Nystatin show that it is effective in higher concentrations against the food derived fungus used. It can be seen that no antifungal zone is formed when lower concentrations of the drug is used. However, a distinct zone is found in plates having higher concentrations of Nystatin (Table 4).

Table 5 : Different concentrations of Amphotericin B used to observe the antifungal activity.

Sr. #	% concentration of Amphotericin B	Number of replica	Width of zone in cm
1.	5	1	No zone
	5	2	No zone
	5	3	No zone
	-	Control	No zone
2.	10	1	No zone
	10	2	No zone
	10	3	No zone
	-	Control	No zone
3.	15	1	No zone
	15	2	No zone
	15	3	No zone
	-	Control	No zone
4.	40	1	0.5
	40	2	0.3
	40	3	0.6
	-	Control	No zone
5.	50	1	1.2
	50	2	0.8
	50	3	2
	-	Control	No zone

Similar results are obtained with Amphotericin B as those obtained with Nystatin. When higher concentrations of Amphotericin B are used, then, wide zones are measured. This showed the efficacy of the antifungal drug against the fungus.

The above results show that garlic has more antifungal activity as compared to ginger against food derived fungus. Therefore, garlic can be used easily for its antifungal properties since it is a natural herb and has no significant side effects. The antifungal drugs Nystatin and Amphotericin B are also effective in high concentrations against our food derived fungus but there are some side effects as these are chemicals. The low concentrations of these are less effective against the food derived fungus used.

The following diagrams show the different concentrations of antifungal agents used and the antifungal zones formed (Fig. 1-8):

Fig 1: 100% Concentration of Garlic extract used

Fig 2: 100 %Concentration of Ginger extract

Fig 3: 70% Concentration of Ginger extract

Fig 4: 40 % Concentration of Nystatin

Fig 5: 50% Concentration of Nystatin

Fig 6: 15% Concentration of Amphotericin B

Fig 7: 40% Concentration of Amphotericin B

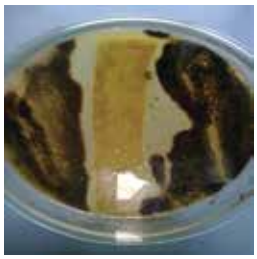

Fig 8:50% Concentration of Amphotericin B

The above results support the notion that herbs such as garlic, ginger have a role in antifungal activities and as preservatives in varying concentrations. According to our results garlic is effective in higher concentrations against the food derived fungus used but ginger is least effective. The drugs used Nystatin and Amphotericin B are also effective in high concentrations in their antifungal activities against the fungus.

In absolute and 70% garlic concentrations, distinct wide antifungal zones are observed. This shows that these concentrations of garlic extract are extremely useful in preventing the growth of fungus. On the other hand, different concentrations of ginger used showed no clear zone of inhibition. It means that these concentrations of ginger are not effective in preventing the growth of fungus against the food derived fungus used. Antifungal drugs used which are Nystatin and Amphotericin B effectively showed their antifungal activities against the fungus. These drugs showed a well-defined zone of inhibition when used in high concentrations.

Therefore, our results showed that garlic and these drugs are effective in preventing food items from the attack of fungus.Therefore, it is recommended to use garlic instead of nystatin to protect the food and health from the attacks of fungi.

Since garlic is a natural herb and has no side effects, therefore garlic is accepted worldwide as an effective antifungal agent.

DISCUSSION

Antifungal properties of medicinal plants are being increasingly reported from different parts of the world. The World Health Organization (WHO) estimates that plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population. Natural products of plant origin have played significant role in the search of new drugs such as quinine from cinchona (Hora and Nair, 1944). In the present work, the extracts obtained from garlic showed strong activity against the food derived fungus.

Different herbs such as garlic ginger and pharmaceutical agents such as nystatin and amphotericin B are used in our study to determine their antifungal characteristics against a food derived fungus. As described earlier, garlic is seen to be effective in inhibiting the growth of fungus as compared to ginger which showed no such results. Nystatin and amphotericin B are antifungal drugs and these are also effective in higher concentrations against the fungus used.

Garlic (*Allium sativum*) is a spice with global recognition. In the present study, it has beenshown to inhibit the growth of fungi when *in vitro* tested. The first definitive study was conducted on garlic chemistry and antimicrobial activity of garlic is believed to be due to the effect of allicin, the main ingredient

in garlic, generated by the phosphopyridoxal enzyme allinase and ajoene (Cavallito and Bailey, 1944).

Volatile oil of *Allium sativum* has antimicrobial activity against bacterial and fungal organisms (Petricic et al., 1977)

Moreover, all extracts of garlic in higher concentrations showed that the antifungal effects increased with increasing concentrations. When raw garlic is crushed, it starts a chemical process which creates allicin which exhibits potent anti-fungal properties.

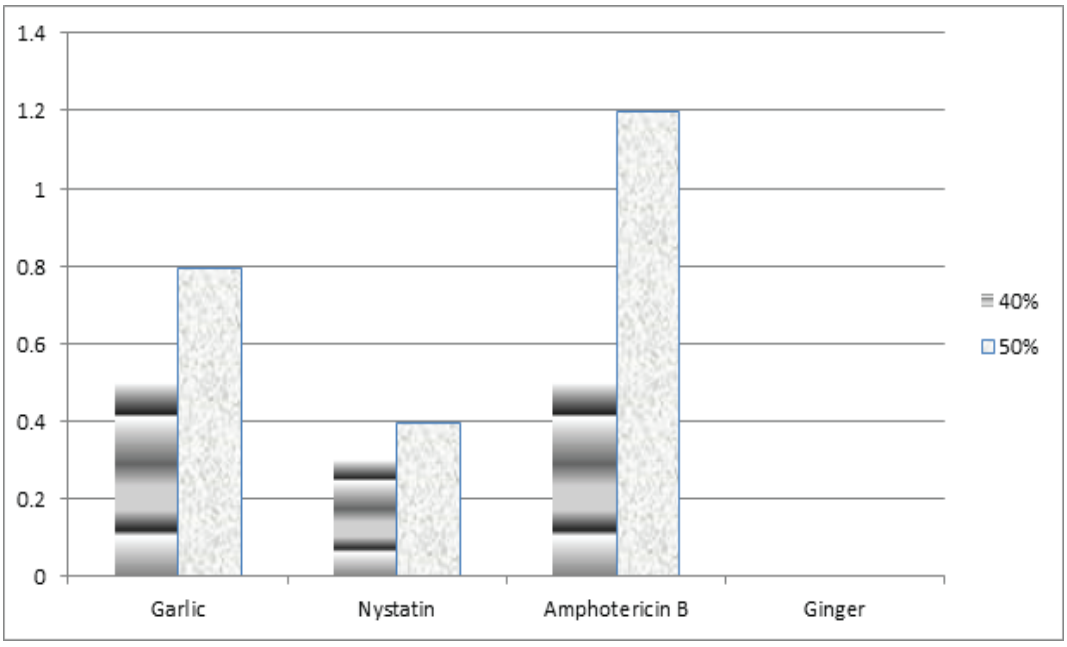

Figure 9: Antifungal activity against a food derived fungus

The above graph shows that garlic exhibits a significant zone of inhibition, which indicate its antifungal ability. No zone of inhibition is observed in plates having ginger concentrations. According to our result, amphotericin B and nystatin exhibit antifungal activities and therefore, are potent antifungal drugs.

Therefore, use of fresh garlic or its extract is highly recommended as a potent antifungal agent.Garlic extract may be useful as topical medicines or in the localized treatment against fungi. There is a need to explore the usefulness of garlic in various infectious diseases and to study the metabolism of action of various components present in such herbs.

CONCLUSION

The results obtained from this work showed that plant extracts of medicinal plants such as garlic exhibit antifungal effects against the food derived fungus. Even at moderate

concentrations, these herbs showed antifungal activity nearly equal to that of the commercial fungicide used as a positive control.Traditional healers have long used plants to prevent or cure infectious conditions.Further studies are needed to determine the chemical identity of the bioactive compounds responsible for the observed antifungal activity. Natural plant-derived fungicides may be a source of new alternative active compounds, in particular with antifungal activity.

This study confirms that many plant extracts such as garlic possess in vitro antifungal activity.

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