

**PESTICIDE RESIDUES AND NUTRITIONAL
QUALITY OF SELECTED VEGETABLES
GROWN IN SOUTHERN TELANGANA ZONE
OF ANDHRA PRADESH**

NEERVANI NIVEDITHA

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APPENDIX A:

Pesticide Residues and Nutritional Quality of Selected Vegetables Grown in Southern Telangana Zone of Andhra Pradesh

1. General information:

- 1.1. Name of the farmer : Address: H.No. :
1.1.1. Village :
1.1.2. Mandal :
1.1.3. District :
1.2. Age :
1.3. Educational Qualification:
1.4. Main Occupation :
1.4.1. Ancillary Occupation :
1.4.2. Agricultural allied activities: a) Horticulture, b) Poultry, c) Dairy, d) Floriculture
e) Sericulture f) If any
1.5. How much land is there for cultivation?
1.5.1. Tube wells/ Dug wells/ Canals, Tanks
1.5.2. What type of crop was harvested the previous year and what types of pesticide were used?

2. Cropping Pattern

2.1. Cultivation practices of the farmers:

Season	Main crop	Planting material used	Area (acres)	Other crops if any	Yield of main crop (tons/acre)

2.3. Source of procurement of the following agricultural inputs?

a) Seeds/ planting material b) Pesticides

3. Any specific pesticide sprayed before while harvesting the crop? If yes, give details

Crop	Name of the pesticide	Dosage/hectare	Reasons for applying pesticides
Tomato			
Brinjal			
Bitter gourd			

4. Mode of applying pesticides: a) manual sprayers b) power sprayers

5. Precautions taken while spraying pesticides:

- a) Masks
- b) Gloves
- c) None
- d) Both masks and gloves

6. From whom do you seek advice on pesticide usage?

- a) Labels on pesticide containers
- b) Neighbour farmer
- c) Books/ Vyavasaya panchangam
- d) Advice of head of the family
- e) Pesticide shop keeper
- f) Consult A.O. / A.E.O.
- g) Any other expert

7. Are you aware of any of the health problems faced by those involved in spray of pesticides on the crops?

Yes/ no. If yes_____

8. Adverse affects faced immediately after applying pesticides

a) Headache b) Nausea c) Vomiting d) Respiratory discomfort e) Skin rash f) Burning eyes
g) None

9. Any other alternative methods adopted to reduce the pesticide usage, if yes why?

a) b) c)

10. Are you aware of any health problems caused by the consumption of foods contaminated with pesticides? Yes/ no. if yes_____

11. How much amount of money were spend on pesticides over years?

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LIST OF ABBREVIATIONS

%	:	Per cent
<	:	Less than
>	:	Greater than
≤	:	less- than or equal to
l	:	Liter
ml	:	Milliliter
μg	:	Microgram
mg	:	Milligram
mm	:	Millimetre
d.f	:	Degree of freedom
kg	:	Kilogram
g	:	Gram
nm	:	nano meter
ng	:	Nanogram
v/v	:	Volume for Volume
a.i. / ha	:	Active ingredient per hectare
@	:	at the rate of
⁰ C	:	Degree Celsius
SD	:	Standard deviation
ANGRAU	:	Acharya N.G. Ranga Agricultural University
A.P	:	Andhra Pradesh
AChE	:	Acetylcholinesterse
ADI	:	Acceptable daily intake
AICRP	:	All India Coordinated Research Project

AOAC	:	Association of Official Analytical Chemists
AEE	:	Aerial exposure to endosulfan
BDL	:	Below detectable limits
BHC	:	Benzene Hexa Chloride
CG	:	Capillary gas chromatography
CML	:	Chronic myeloid leukemia
CNS	:	Central nervous system
DDT	:	Dichloro-Diphenyl-Trichloroethane
DDVP	:	Dimethyl 2, 2-Dichloro Vinyl Phosphate
DDE	:	Dichlorodiphenyldichloroethylene
DNA	:	Deoxyribonucleic acid
ECD / NPD	:	Electron capture detector / Nitrogen phosphorus detector
EFSA	:	European Food Safety Authority
FAO / WHO	:	Food and Agriculture Organization / World Health Organization
FTD	:	Full term delivery
GAP	:	Good Agricultural Practices
GC	:	Gas Chromatography
GPC	:	Gel permeation chromatography
GC / FID	:	Gas chromatography / Flame ionization detector
GSH	:	Reduced Glutathione
HPLC	:	High- performance liquid chromatography
HCH	:	Hexa Chlorocyclo Hexane
HCB	:	Hexachlorobenzene
Hb	:	Hemoglobin
ICMR	:	Indian Council of Medical Research
IPM	:	Integrated Pest Management

LC-MS/MS	:	Liquid Chromatography-Mass Spectrometry and Liquid Chromatography - Tandem Mass Spectrometry
LH	:	Luteinizing hormone
MRL	:	Maximum residue levels
MRM	:	Multiple reaction monitoring
MP	:	Methyl parathion
O.P	:	Organophosphate
O.C	:	Organochlorine
PDMS	:	Polydimethylsiloxane
ppb	:	Parts per billion
ppm	:	Parts per million
PTD	:	Pre- term delivery
PENTB	:	Pediatric environmental neuro- behavioural test battery
PPE	:	Personal protective material
RBC	:	Red blood cell
RARS	:	Regional Agricultural Research Station
SMR	:	Sensory motor rhythm
SPMG	:	Solid Phase Microextraction Method
SPs	:	Synthetic Pyrethroids
TSS	:	Total soluble solids
USD	:	US Department of Agriculture
USEPA	:	US Environment Protection Agency
UV	:	Ultra Violet
U.P	:	Uttar Pradesh
WBC	:	White blood cell

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ABSTRACT

The study entitled “**Pesticide Residues and Nutritional Quality of Selected Vegetables Grown in Southern Telangana Zone of Andhra Pradesh**” was conducted to estimate the pesticide residues in tomato, bitter gourd and brinjal in fresh form and after washing and the nutrient composition in fresh vegetables. The other objective was to determine the pesticide residue levels, of the selected vegetables before and after washing. Comparison was made between recommended package of practices versus farmers’ practices in the usage of pesticides and nutrient composition against reference values.

Ten farmers each of tomato, bitter gourd and brinjal were selected using purposive random sampling. The total thirty farmers were interviewed using the structured questionnaire that was prepared for the purpose. The questionnaire included information on the package of practices in relation to pesticide usage for different vegetable crops, dosage/hectare, mode of application, sources for the procurement of seeds and pesticides, processing techniques which included washing vegetables before consumption and the affect of pesticides on health.

Burning eyes, vomiting and headache were the most common immediate symptoms experienced and few of them used masks or gloves, very few used both. Nearly sixty seven percent did not use any precautionary measure while pesticide application. The reasons for application of pesticides were quoted as higher yield, better quality produce and prevention of pests. The farmers were aware of the use of natural pesticides like neem oil and crude

garlic oil instead of chemicals but are not practicing. Most of them did not feel the need for washing the vegetables before consumption as they were unaware of the serious health effects of pesticides.

The nutrient compositions of selected fresh vegetables (moisture, reducing sugars, β -carotene and ascorbic acid) were comparable with the reference values. The moisture content of bitter gourd, brinjal and tomato were 92.15%, 91.13%, 94.51% and ascorbic acid content were 55.21mg%, 10mg%, 23.44mg% and β -carotene content were 1340 μ g%, 1150 μ g%, 250 μ g% and reducing sugars content were 0.6%, 2.46% and 3.13% respectively.

Pesticide residue levels in all the selected vegetables before and after washing were below detectable limits (BDL). Pesticide residues in vegetables were influenced by the time lag between pesticide spray and fruit harvesting, storage, transport, handling and processing techniques like washing and peeling before consumption of vegetables. The lower dosages of pesticides applied by the farmers than recommended could have also contributed to the non-detectable levels of pesticide residues in the vegetable samples.

Thus, the results of the present study indicate that the pesticide residue levels in the selected vegetables tomato, bitter gourd and brinjal of Southern Telangana Zone are within the MRL levels and are therefore safe for human consumption. Pre-processing techniques like washing with water or 3% salt water and peeling for obtaining edible portion further reduced the residual burden as the residues fell below detectable levels.

Chapter I

INTRODUCTION

Vegetables are the important components of human diet since they provide protective nutrients that are required for most of the reactions occurring in the body. Certain vegetables perform special functions due to the presence of components such as polyphenolics, terpenoids, isoflavones, anthocyanins, amino acids, minerals, vitamins, and other antioxidants that are associated with protection from cancer, cardiovascular diseases, diabetes and hypertension (Islam, 2006). Vegetables, like, okra, egg plant, spinach, cauliflower, tomato, pumpkin, carrots, turnips etc. are produced in the country for local consumption as well as for export purposes.

India is known as the “*fruit and vegetable basket of the world*” as wide variety of fruits and vegetables are produced. It ranks second in fruits and vegetable production in the world, after China. India produces 71.516 million metric tons of fruits and 133.738 million metric tons of vegetables. The area under cultivation of fruits stood at 6.329 million hectares while vegetables were cultivated at 7.985 million hectares (National Horticulture Database, 2010).

Andhra Pradesh has a good potential for horticulture and about 1.53 million hectares of area covered under horticulture crops. The total production of horticulture crops is 11.30 million tons and is the second largest producer of fruits and vegetables in India. Fruit crops occupy major portion of area coverage with an estimated 7.60 lakh hectares followed by vegetables with a total area coverage of 2.36 lakh hectares. (Agriculture and industry survey, 2005).

India is the largest producer of pesticides in Asia and ranks 12th in the world for the use of pesticides with an annual production of 90,000 tons. A vast majority of population in India (56.7 per cent) is engaged in agriculture and is therefore exposed to the pesticides use in agriculture (Gupta, 2004). Although Indian average consumption of pesticide is far lower than many other developed economies, the problem of pesticide residue is very high and has also affected the export of agricultural commodities in the last few years (Abhilash and Nandita Singh 2009).

The presence of pesticide residue is a concern for consumers because pesticides are known to have potential harmful effects to other non-targeted organisms than pests and diseases. The major concerns are their toxic effects such as interfering with the reproductive systems and foetal development as well as their capacity to cause cancer and asthma (Gilden *et al.*, 2010).

Although the impact of pesticides on agricultural products vary according to the type of pesticide used, all pesticide residues cause nutrient imbalance and reduction in the quality of agricultural products (Bourn and Prescott, 2002).

Prolonged exposure to pesticides and their elevated concentration in the body causes adverse effects. Pesticides differ greatly in their mode of action, uptake by the body, metabolism and toxicity to humans. WHO (2001) has classified pesticides according to their toxic effects as class I (extremely hazardous) to class III (slightly hazardous). Depending upon the toxicity of the compound and exposure time, the symptoms of pesticide exposure vary from headache, vomiting, skin rash, respiratory problems, and convulsions (Dasgupta *et al.*, 2007).

Toxicologic and epidemiologic studies have demonstrated the association between acute and high levels of OP (organophosphorus) exposure and adverse health effects. The establishment of the relation between neurologic impairments and repeated low-level OP (organophosphorus) exposure that does not induce symptoms of acute poisoning in humans is far less concrete (Eskenazi *et al.*, 2007; Rauh *et al.*, 2006; Ruckart *et al.*, 2004).

Indiscriminate use and improper handling of pesticides in agriculture have caused serious health problems in many developing countries, which represent 30% of the global pesticide consumer market (Peres *et al.*, 2006). Some of the negative effects of pesticide misuse include low crop yield, destruction of soil micro-fauna and flora, and undesirable residue accumulation in food crops (Edwards 1986).

Several studies have been conducted on the effect of food processing techniques on the pesticidal residues. Reduction in pesticide residues has been observed in food due to the storage, handling and processing that occurs between harvesting of raw agricultural commodities and consumption of prepared food stuffs (Holland *et al.*, 1994).

Pesticide residues are reduced by processing or household preparation stages such as washing, peeling and cooking and so on (Dikshit *et al.*, 2003).

Fruits and vegetables are essential for a nutritious and healthy diet but the health benefits are compromised by consistent contamination with pesticide residues. Concern over pesticide toxicity has particularly increased over the past years owing to increasing evidence of carcinogenic, mutagenic and teratogenic effects in experimental animals and exposed humans. The general population is mainly exposed to pesticides through the ingestion of contaminated foods (such as cereals, vegetables and fruits), which are directly treated with these pesticides or are grown in contaminated fields.

Hence, the present study entitled “Pesticide Residues and Nutritional Quality of Selected Vegetables grown in Southern Telangana Zone of Andhra Pradesh” has been planned with the following objectives:

- To study the practices with respect to usage and application of pesticides in horticultural crops.
- To study primary processing techniques of the selected crops at house hold level.
- To estimate pesticide residues in fresh and processed forms of selected horticultural crops.
- To assess the nutritive value of selected horticultural crops.

CHAPTER-II

REVIEW OF LITERATURE

The study titled, “Pesticide Residues and Nutritional Quality of Selected vegetables Grown in Southern Telangana Zone of Andhra Pradesh” aimed to determine the levels of pesticide residue in the selected vegetables. Such an analysis is very essential as fruits and vegetables play a pivotal role in the diet for maintenance of health and prevention of disease. Like other crops, fruits and vegetables are attacked by pests and diseases during production and storage leading to damage that reduces the quality and the yield.

The term “pesticide” includes any substance used for the control of pests during production, storage, transport, marketing, or processing of food for man or animals and which may be administered to animals for the control of insects or arachnids in or on their bodies. Pesticides are toxic chemicals used in preventing, destroying, repelling, or mitigating pests (USEPA, 2005).

It is estimated that over 3 million tones of pesticide is used annually and there exists about 1600 varieties of pesticides. India is the second largest producer of pesticides after China in Asia and 76% of the pesticides used are insecticides (Mathur, 1999). There has been a steady growth in the production of technical grade pesticides in India, from 5000 metric tonnes in 1958 to 102,240 metric tonnes in 1998. In 1996-97 the demand for pesticides in terms of value was estimated to be around Rs.22 billion (USD 0.5 billion), which is about 2% of the total world market (Akhtar *et al.*, 2009).

In India, the first report of poisoning due to pesticides was from Kerala in 1958, where more than 100 people died after consuming wheat flour contaminated with parathion (Karunakaran, 1958).

Pesticides are most commonly used chemical substances in agricultural sector in order to increase the agricultural efficiency. Use of indiscriminative and improper pesticides in the agriculture sector poses serious environmental, human and animal health problems (Carson, 2007).

The literature was reviewed under the following headings.

2.1 Pesticide use and application in agriculture

2.2 Pesticide residues in foods

2.3 Pesticide recommendations for selected vegetable crops

2.4 Monitoring of pesticide residues in foods

2.5 Effect of storage and processing on pesticide residues

2.6 Impact of pesticide residues on human health

2.7 Future strategies-integrated pesticide management

2.1 Pesticide Use and Application in Agriculture

Pesticides are the last input in the agricultural process but are important for sustainable development of agriculture. Despite the fact that judicious use of pesticides can prevent crop losses and provide economic benefits to the farmers, pesticide consumption per hectare in India is far below the world average (Annual Review Report on Indian Pesticide Industry, 2012).

Agricultural development continues to remain the most important objective of Indian planning and policy. In the process of development of agriculture, pesticides have become an important tool as a plant protection agent for boosting food production. Majority of the population in India is engaged in agriculture and therefore the problem of pesticide residue is very high. Pesticide residue in several crops has also affected the export of agricultural commodities in the last few years. In this context, pesticide safety, regulation of pesticide use, proper application technologies and integrated pest management are some of the key strategies for minimizing human exposure to pesticides (Abhilash and Nandita Singh, 2009).

Farmers use pesticides without full understanding of the impact on human health and the environment (Mathews, 2008).

Practices of pesticides that were used by vegetable farmers in the tropical region of Cameroon revealed that weekly spray of pesticides was the most common practice:

40% of farmers sprayed insecticide and 28% sprayed fungicides. Farmers applied 0.5-9 liters of pesticide per year, 10-49 kg/annum and 10 to 49/annum packets of chemicals depending on farm size. Ninety percent of farmers used a knapsack sprayer and 20% of farmers noticed that their health was affected by pesticides. Seventy-five percent received information about agricultural production from other farmers, and have never received any training on pesticide use practices and health effects (Abang *et al.*, 2013).

The types of pesticides used by the small-scale farmers in Northern Tanzania were insecticides (59%), fungicides (29%) and herbicides (10%) with the remaining 2% being rodenticides. About a third of the farmers applied pesticides in mixtures. Up to 90% had a maximum of 3 pesticides in a mixture. Fifty three percent of the farmers reported that the trend of pesticide used was increasing, while 33% was constant and 14% decreased the usage. More than 50 percent of the respondents applied pesticides up to 5 times or more per cropping season depending on the crop. Insecticides and fungicides were routinely applied by 77% and 7%, respectively by these farmers (Ngowi *et al.*, 2007).

Out of the total pesticide use in the country, insecticides accounted for 80%, followed by herbicides (15%) and fungicides (1.46%). Organophosphorous compounds were the most widely used insecticides for controlling pests. According to Agricultural Statistics at a Glance (2009) the pesticide share is highest for cotton (45%), followed by rice (20%), vegetables/ fruits (13-24%) and cereals/millet/oilseeds (6-7%) in the recent decade.

Results of US Government pesticide test reported that 192 different pesticides were used on 46 popular fresh fruits and vegetables. Items such as apples, bell peppers, celery, cherries, grapes (imported), nectarines, peaches, pears, potatoes, red rasp berries, spinach and straw berries were reported to have highest pesticide residues. The ones with lowest pesticide residues levels reported were asparagus, avocado, bananas, broccoli, cauliflower, corn(sweet), kiwi, mangoes, onions, papaya, pineapple and peas. (Anonymous, 2003 www.foodnews.org).

Shrestha and Koirala (2010) conducted a study to evaluate the knowledge, practice and use of pesticides among thirty commercial vegetable growers of Dhading district of Nepal. The study revealed that more than four in five were using pesticides and nearly half of them spray pesticides five to six times/season/annum. More than one-

sixth of the pesticides used were extremely hazardous, which were banned for general agriculture use. Majority did not receive any official training on pesticides and nearly one-third did not read information available on the pesticide label. Nearly half were not using Personal Protective Equipment during pesticide application and experienced symptoms of health hazards.

A cross sectional survey was conducted on 631 farmers in Thanjavur District (South India) using a pre-tested interview questionnaire with an aim to determine the relationship between the extent of pesticide-use and signs and symptoms of illnesses due to exposure among farmers. About 68.6% farmers sprayed pesticides by themselves and did not use any form of protection while handling them. More than 75% of farmers used highly hazardous pesticides. Around 56% of farmers obtained information on pesticides from retail shop owners. Farmers reported the following acute signs and symptoms: excessive sweating (36.5%), burning/ stinging/ itching of eyes (35.7%), dry/sore throat (25.5%), and excessive salivation (14.1%) (Chitra and Muraleedharan, 2007).

2.2 Pesticide residues in foods

Pesticide residue contaminating food is the problem focused worldwide because of its direct implications on human health and international trade (Sanborn *et al.*, 2004).

Foods from Egyptian local markets have shown that pesticide residues for hexa chlorocyclo hexane (HCN) in potatoes exceeded in 8 samples and DDT in 2 samples. No organochlorine pesticide residues were found in citrus fruits (Salwa *et al.*, 1996).

Twelve most commonly used pesticides were selected to study residual effects on 24 samples of freshly collected vegetables by Sasi and Rashmi (2001). Most of the samples showed presence of high levels of malathion, DDE, a metabolite of DDT (dichlorodiphenyltrichloroethane), BHC, dimethoate, endosulfan and ethion were also detected in few samples. Leafy vegetables like spinach, fenugreek and mustard seem to be the most affected. Radish also showed levels of contamination.

In one study Beena *et al.* (2003) the tested samples were found to be 100% contaminated with low but measurable amounts of pesticide residues. Among the four major chemical groups, residue levels of organophosphorous insecticides were highest followed by carbamates, synthetic pyrethroids and organochlorines. About 32% of the

samples showed contamination with organophosphorous and carbamate insecticides above their respective MRL values

Aktar *et al.* (2009) reported that samples (186) of 20 commercial brands of infants formulae showed the presence of residues of DDT and HCH isomers in about 70 and 94% of the samples with their maximum level of 4.3 and 5.7 mg/kg (fat basis) respectively.

In 1998, four commodities (oranges, peaches, carrots, spinach) were analysed for 20 pesticides (acephate, benomyl group, chlorpyrifos, chlorpyrifosmethyl, deltamethrin, maneb group, diazinon, endosulfan, methamidophos, iprodione, metalaxyl, methidathion, thiabendazole, triazophos, permethrin, vinclozolin, lambda-cyhalothrin, pirimiphos-methyl and mercabam) by Aktar *et al.* (2009) and found that about 32% contained residues of pesticides at or below MRL, and 2% above the MRL.

Beena Kumari and Kathpal (2009) collected samples (28) of complete vegetarian diet consumed from morning till night i.e. tea, milk, breakfast, lunch, snacks, dinner, sweet dish etc from homes, hostels and hotels periodically from Hisar and analysed for detecting the residues of organochlorine, synthetic pyrethroid, organophosphate and carbamate insecticides. It was found that actual daily intake (microgram/person/day) of lindane in two and endosulfan among the four samples exceeded the acceptable daily intake. Residues of other pesticides in all the diet samples were lower than the acceptable daily intake (ADI) of the respective pesticides. All the diet samples were found contaminated with one or the other pesticide, the actual daily intake of only a few pesticides was higher than their respective ADI.

2.3 Pesticide Recommendations for Selected Crops

The term pesticide covers a wide range of compounds including insecticides, fungicides, herbicides, rodenticides, molluscicides, nematocides, and plant growth regulators. Benefits of pesticides include improving productivity, protect crop losses, and vector disease control (ICMR Bulletin, 2001).

The cropwise pesticide recommendations as prescribed in Package of Practices of the Important Horticultural Crops of Andhra Pradesh (2010) are given in the table below (2.1 to 2.3).

Table 2.1 Pests and Treatment of Tomato

Tomato

Pests	Treatment
Fruit borer	Carbaryl @ 2gm/lt or Quinalphos @ 2ml/lt
Epilachna beetle	Carbaryl @ 2gm/lt or Endosulphan @ 1.5ml/lt
Jassids	Dimethoate @ 2ml/lt or Monocrotophos @ 1.5ml/lit of water.

Table 2.2 Pests and Treatment of Brinjal

Brinjal

Pests	Treatment
Epilachana beetles	Malathion @ 3ml per water of methyl parathion @ 1ml/lt
Shoot and fruit borer	Monocrotophos @ 1.25ml/lt, Malathion @ 3ml/lt or Monocrotophos @ 1.25ml/lt
Mealy bug and mite	Wettable Sulphur 3 to 5gm/lt or Dicofol @ 2.7ml/lit

Table 2.3 Pests and Treatment of Bitter gourd

Bitter gourd

Pests	Treatment
Pumpkin beetles	Parathion @ 1ml/lt or Dimethoate @ 2ml/lt or Malathion @ 2ml/lt
Snake gourd semilooper	Endosulphan @ 2ml/lt or Monocrotophos @ 1.25ml/lt or Quinalphos @ 2ml/lt.

2.4 Monitoring of Pesticide Residues in Foods

According to European Food Safety Authority (2010) maximum residue levels are the highest levels of residues expected to be in the food when the pesticide is used according to authorized agricultural practices.

Residues of organophosphorus, carbamate, organochlorine, fungicide, pyrethroid and abamectin pesticides in the processed vegetables collected from Aga-Dhakahlia, Nobaria, Behera and Giza were analysed by gas and HPLC chromatography Gehad *et al.* (2012). Statistical analysis showed that potatoes contained the highest levels of dimethoate and diazinon as organophosphorus pesticides. Residue of pirimiphos-methyl in green bean and potatoes and residues of methomyl, abamectin and dicofol in cucumber and tomatoes were found to be higher than their corresponding MRL's.

A study was conducted by (Amengor and Tetteh, 2008), to determine the effect of application of lindane (156.0, 244.0 and 312.0 g ha⁻¹), unden (propoxur) (125.0, 187.5 and 250.0 g ha⁻¹), dithane and karate (166.6, 209.8 and 333.3 g ha⁻¹) on garden eggs, okra and tomatoes to find out if it has any advantage that makes its use attractive to farmers despite its ban. Yields of garden eggs were suppressed by all the rates of lindane applied. In tomatoes, lower rates of lindane increased yields whereas the higher rates suppressed yields lower than the control. In okra yields were higher than the control at all levels of lindane applied though yield increments were low. Unden application had the highest effect on garden egg yields followed by tomatoes and least on okro. In the garden egg and tomato treatments, increasing concentration of unden resulted in decreasing yields though yields were higher on the control plots.

Effort has been made to evaluate the residues of selected insecticides (organophosphorous and pyrethroid) and fungicides (triazoles and chloronitriles) in fruits and vegetables collected from Xiamen, China, during the October 2006 to March 2009 monitoring campaign (Chen Chen *et al.*, 2011). Gas chromatography with electron capture detector (GC-ECD) was used to determine the concentrations of 22 pesticide residues among those recommended for pest treatment. The results showed that despite a high occurrence of pesticide residues in fruits and vegetables from this region, it could not be considered a serious public health problem. Nevertheless, an investigation into continuous monitoring and tighter regulation of pesticide residues in fruits and vegetables was recommended.

A study was conducted by (Baig and Akhtera, 2009), to determine the residues of three Organophosphate pesticides (Triazophos, Profenofos and Chlorpyrifos) which were commonly used in vegetables like egg plant, pumpkins and okra that are cultivated in the Southern part of Punjab province in Pakistan. A multi-residue analysis by High-Performance Liquid Chromatography (HPLC) equipped with UV detector was used to analyze the pesticide residues. Results showed that 33.0% of the samples were contaminated with any of the above three pesticides and more than 8% of the samples tested contained residues higher than the MRLs.

Fifty nine samples of raw fruits and vegetables (banana, citrus fruits, grapes, mango, pome fruits, stone fruits, strawberry, tomato, and asparagus) and 24 samples of mixed juices (apple juice, orange juice, mango nectar, vegetable juice, prune juice, pineapple juice, grape juice and guava nectar) were analysed for Organochlorine and Organophosphorus insecticides, Triazine herbicides and fungicides, showed that 72% of fruits and vegetables (42/59) had no pesticides, 25% (15/59) had one or more pesticides detected below the MRL, and 3% (2/59) failed to comply. Of all the pesticides detected in fruits, Endosulfan was detected as the most often used pesticide (5 samples), followed by Iprodione (4), Chlorpyrifos (3), Fenthion (3), Dicofol (3), Dicloran (1), Parathion (1), Procymidone (1) and Dieldrin (1), whereas, in fruit juices 17% (4/24) of samples had detectable amounts of pesticides and all were below the respective MRL. The pesticides detected were: Endosulfan sulphate (a breakdown product of the insecticide, Endosulfan) in mango nectar; and Iprodione in tropical juice, vegetable juice and apple & kiwi juice (Andrew and Simon, 1996).

In a multi-centric study conducted by Aktar *et al.* (2009) to assess the pesticide residues in selected food commodities collected from different states of the country (Surveillance of Food Contaminants in India, 1993), DDT residues were found in about 82% of the 2205 samples of bovine milk collected from 12 states. About 37% of the samples contained DDT residue above the tolerance limit of 0.05 mg/kg (whole milk basis). The highest level of DDT residue found was 2.2 mg/kg. The proportion of the samples with residues above the tolerance limit was highest in Maharashtra (74%), followed by Gujarat (70%), Andhra Pradesh (57%), Himachal Pradesh (56%), and Punjab (51%). In the remaining states, this proportion was less than 10%

Iqbal *et al.* (2007) conducted a supervised field trial of brinjal to determine Chlorpyrifos, Acephate, Dichlorovos, Carbofuran and Imidachloprid residues by

adopting HPTLC method. These insecticides were sprayed directly on the brinjal crop and data were recorded after 0, 3 and 7th day after application. The samples were treated with organic solvent ethyl acetate and cleaned-up by activated charcoal. All the samples were found contaminated with pesticide residues except Imidachloprid. After 0 day the quantity of pesticide residues was maximum followed by 3rd day that contained lesser amount of insecticide residue and after 7th day the quantity of these residues were negligible in the brinjal, thus the brinjal was suitable for the consumption by public after three days without posing any hazard to human health.

Fardous *et al.* (2007) collected tomato samples from different regions during 2005-2006 to determine the presence of some selected Organophosphorus and carbamates pesticides residues. Analysis was done by HPLC technique. Some samples out of 18 were found to be contaminated with Chlorpyrifos, diazinon and carbaryl residues while majority of samples were found to be free from pesticide residues. Carbofuran was not found in any of the tomato samples collected. The range of chlorpyrifos, diazinon and carbaryl residues were 0.107-0.342, 0.157-0.381 and 0.1-0.32 ppm respectively in tomato samples which were within FAO/WHO Guide Line Value of Maximum Residue Limit (MRL) for tomato.

Karanth (2002) conducted a study on 514 vegetable samples collected from the markets of Mysore. Results indicate that 50-70% of vegetables were contaminated with insecticide residues. Pesticide finger printing technique together with GC analysis revealed that in most cases the residue burden was less than the maximum residue limits (MRLs) with few exceptions. Frequently DDT and HCH residues were detected and the evidence suggests that the source of these residues is from the contaminated soil from where they migrate to edible parts.

A symposium on Risk Assessment of Pesticide Residues in Water and Food (2003) suggested an action plan for the prevention of pesticide contamination in food and water and emphasized that there was a strong need to educate farmers, industry, trade and the public about good agricultural practices (GAP), choice of pesticides and their judicious use. It was recommended to make efforts to promote use of natural pesticides, biological control, and encourage integrated pest management, organic farming and use of safer pesticides. Storage, handling, transportation, spraying etc., should necessitate enough caution to minimize contact with pesticides. An effective and

credible system of surveillance and monitoring should be in place to collect data, prevent misuse of pesticides, develop priorities in the selection and use of pesticides and thereby minimize risk to health and environment.

In a cross sectional study Usman *et al.* (2009) on health hazards caused by toxic chemicals on market samples of tomato, apple and cucumber were analyzed using Liquid Chromatography- Mass Spectrometry system. The results showed that most of the samples did not contain any residues of the nine selected pesticides and only two samples of tomato had detectable level of residues with of one pesticide Imidacloprid, which were within the limits set by the WHO. The rest of the samples did not contain any pesticide residue indicating any serious threat to the health of the consumers.

Solid Phase Microextraction method (SPME-CG) was described to determine Organophosphorus pesticides in samples of fresh-water fish, water, potatoes, guava and coffee by capillary gas chromatography with nitrogen phosphorus detector. The determination of the pesticides: Co-ral, DDVP, Di-Syston, Ethion, Phorate, Phosdrin, Guthion, Malathion and Methyl-parathion in samples of fish, water and other foods with a manual SPME-CG holder using a 100 μm PDMS microfiber was used. Residues of DDVP were present in samples analyzed of fish, water, potatoes, guava and coffee. Coffee also indicated presence of Phorate. Other pesticides Co-ral, Di-Syston, Phosdrin and Malathion were detected in water. The method proposed in this work proved to be suitable for analysis of Organophosphorus pesticides in fish, showing good precision and linearity. Limits of detection ranged from 0.005 to 1.097 $\mu\text{g L}^{-1}$, depending on the compound, except for Phosdrin, whose limit of detection was 8.374 mg L^{-1} . This demonstrated that pesticides that are widely used in the agriculture and cattle breeding were leached through rains, contaminating waters and fish of the region, as well as other foods (Helena and Zenilda, 2005).

2.5 Effect of Storage and Processing on Pesticide Residues

Effect of processing in fruits and vegetables are said to be influenced by the physico-chemical properties of the pesticide as well as the processing method (Holland *et al.*, 1994).

Pesticides are widely used in fruit and vegetable production to improve the food quality, but this results in their residues being present in the harvested products.

However, food processing techniques that are normally carried out during preparation before eating often reduce the pesticide residue levels except in cases where there is concentration of the product like in juicing, frying and oil production (Keikotlhaile *et al.*, 2010).

The effects of processing and cooking on the levels of pesticide residues in rice samples were investigated for 11 pesticides in pre-harvest (9 pesticides) and post harvest (4 pesticides) samples. In the polishing process, the transfer ratio (% of total pesticide residue amount in product to that in brown rice) of rice bran ranged from 40% to 106%, and the transfer ratio of polished rice ranged from 9% to 65% in pre-harvest samples. These values varied from pesticide to pesticide. The processing factor (pf), of polished rice ranged from 0.11 to 0.73. The loss of pesticides during processing and/or cooking did not correlate to any single physical or chemical property (Saka *et al.*, 2008).

The effect of washing, storing, boiling, peeling, cooking with and without vinegar and frying on pesticide residue were investigated by Gehad *et al.* (2012) for vegetables and water/soil samples collected from Aga-Dhakalia, Nobaria, Behera and Giza. It was found that, washing process eliminated approximately 13-60% of organophosphorous, 20-50% of carbamates, 19-25% of cupermethrin, 60% of dicofol, 100% of penconazole and 18-75% of abamectin residues. Peeling of washed cucumber removed 65% of malathion, 66% of methomyl, 80% of dicofol and 83% of abamectin. It was concluded that a combination of simple washing and peeling removed 10 to 85% of insecticides if applied before consumption. Cooking and frying helped to remove 25-100% of the residual insecticides. Effect of storage on the abamectin found with vegetables and soil after spraying suggested that the residue of abamectin gradually decreased with storage time.

Angela (2004) observed in his study that peeling, blanching, cooking or a combination of these steps can significantly reduce the amount of pesticide residue that might be present on fruits and vegetables.

A research study conducted by (Beena, 2008), on effects of household processing on reduction of pesticide residues in vegetables showed that 77% reduction of organophosphates (OP) insecticides was observed in brinjal followed by 74% in cauliflower and 50% in okra by washing and the same trend was observed by boiling process where the reduction found was 100% in brinjal ,92% in cauliflower and 75% in

okra. It was suggested that boiling was more effective than washing in dislodging the residues.

Elkins (1989) reported that commercial food processing operations such as washing, blanching and cooking removed major portions of the pesticide residues that were currently permitted on the raw agricultural crop. Washing plus peeling removes 99% of Carbaryl and Malathion residues from tomatoes. Washing removed 83% of Benomyl residue from tomatoes and further processing reduced the residue by 98% in tomato puree.

Vegetable samples field-grown pepper (sweet and hot) and eggplant were collected at 1 hour to 14 days after application with Pirimiphos-methyl to determine the content and dissipation rate of Pirimiphos-methyl. The effect of different washing solutions and some kitchen processing on the removal of such residues from treated vegetables were also investigated. Analysis by GC showed the disappearance of Pirimiphos-methyl appeared to follow first order kinetics with different rates of reaction i.e. 0.31, 0.40 and 0.37 day⁻¹ for hot pepper, sweet pepper and eggplant, respectively. Results further indicated that acetic acid; potassium permanganate, sodium chloride and sodium hydroxide solution gave greater removal of Pirimiphos-methyl residues from sweet pepper and eggplant fruits, while 70.16- 76.61% removal was shown in hot pepper fruit after washing with soap and acetic acid solution, respectively (Radwan, 2004).

The effect of different processing methods was studied by (Miriam, 2011), on either reduction or increase in MRL indicated, average reduction of residue levels by blanching, boiling, canning, frying, juicing, peeling and washing of fruits and vegetables with an average processing factor (response ratio) ranging from 0.10 to 0.82. The processing factors for baking, boiling, canning and juicing ranged from 0.24 to 2.51, indicating both reduction and increases for the 95 and 99.5% confidence intervals.

According to Viju (2012) washing with tamarind water and vinegar were effective in removing the pesticide residue (up to 95%), especially in spinach, curryleaves, chillies, lady's finger, snake gourd and brinjal that were contaminated with heavy doses of pesticides.

The effect of washing with tap water and different detergent solutions, storage at different temperatures and ultrasonic cleaning on Organophosphorus pesticide (Trichlorfon, Dimethoate, Dichlorvos, Fenitrothion, and Chlorpyrifos) residue levels in raw cucumber was investigated by liquid chromatography–tandem mass spectrometry method revealed that washing with detergent solutions was more effective than with just tap water. The Organophosphorus pesticides reduced from 31.1% to 98.8% after washing with detergent solutions for 20 min. Among detergent solutions, 5% sodium carbonate solution caused the greatest loss in Trichlorfon and Dimethoate, and 5% sodium bicarbonate solution caused the greatest loss in Dichlorvos, Fenitrothion and Chlorpyrifos (Liang *et al.*, 2012).

According to Gonzalez *et al.* (2011) high moisture unprocessed foods when washed with tap water reduce the residue content by 22-60%, juicing 73-91%, wine making 70-100% and peeling 70-100% respectively.

2.6 Impact of Pesticide Residues on Human Health

Karunakaran (1958) gave the first Indian report of poisoning due to pesticides from Kerala in 1958, where over 100 people died after consuming wheat flour contaminated with parathion.

According to Aktar *et al.* (2009) the high risk groups exposed to pesticides include production workers, formulators, sprayers, mixers, loaders and agricultural farm workers.

The hazards that have been identified concerning pesticide include reproductive and endocrine disruption, neurodevelopmental delays, immune system, cancer and respiratory distress (Gilden, 2010).

As per US Council on Scientific Affairs estimates in 1988, approximately 1,10,000 cases of poisoning and 200 deaths per year were due to pesticides (Curl, 2003).

Settimi *et al.* (2003) conducted study to evaluate the association between prostate cancer and exposure to pesticides in agricultural settings in Italy. 124 new cases of prostate cancer were ascertained and interviewed, along with 659 cancer controls. The association between prostate cancer and different occupational risk factors was measured by maximum likelihood estimation of the odds ratio, controlling for potential

confounders. "Ever been employed in agriculture" was associated with a 40% increased risk. A increased risk among farmers exposed to organochlorine insecticides and acaricides more specifically to the often contemporary used compounds DDT, and dicofol, whose effects could not be well separated.

Rao (2005) reported that health effects of pesticide depend on the type of pesticide. Pesticides as the organophosphates and carbonates, affect the nervous system, others may irritate the skin or eyes. Some pesticides may be carcinogens. Other may affect the hormone or endocrine system in the body. The effects of a pesticide can be either acute or chronic. Examples of acute health effects include stinging eyes, rashes, blisters, blindness, nausea, dizziness and diarrhea. Examples of known chronic effects are cancers, birth defects, reproductive harm, neurological and developmental toxicity, immunotoxicity and disruption of the endocrine system.

Epidemiological studies were conducted on pesticide exposed agricultural workers along with an equal number of age and sex-matched controls. All the 200 exposed volunteers were suffering from fever, nausea, headache and other abnormal symptoms. Five workers (2.5%) showed decrease in RBC, hemoglobin, and increase in WBC with a large number of immature cells. These volunteers were further diagnosed as Philadelphia negative Chronic Myeloid Leukemia (CML) cases based on clinical and pathological examinations (Prabhavathy and Sudha, 2007).

Study of pesticide residue levels suggested that children under five years could rapidly build up chances of cancer from pesticide residues in food in the first few years of life as their food intake is very different from that of adults (Boobis *et al.*, 2008).

Blood samples are taken from two groups of people, one with direct exposure to pesticides (agriculturalists & public health workers) the second group with indirect exposure to pesticides through food chain (Kallidass and Jebakumar, 2006). The objective of the investigation was to analyze the blood of the respondents with minimum health complaints and skin diseases for the residue of the banned Organochlorine pesticides DDE and BHC using Gas Chromatography. High concentrations of both BHC & DDE were observed in the serum samples of the people who had direct exposure to the pesticides, namely agriculturalists and public health

workers with few exceptions. The pesticide residue concentration in serum ranged from 0.006 to 0.130ppm for BHC and 0.002 to 0.033ppm for DDE.

The relationship between environmental Endosulfan exposure and reproductive development in male children and adolescents was studied by Saiyed *et al.* (2003). One hundred and seventeen male schoolchildren (10–19 years of age) of a village situated at the foothills of cashew plantations, where Endosulfan had been aerially sprayed for more than 20 years were compared with 90 controls with no such exposure history. Mean \pm SD serum Endosulfan levels in the study group (7.47 ± 1.19 ppb) were significantly higher ($p < 0.001$) than in controls (1.37 ± 0.40 ppb). Multiple regression analysis showed that sensory motor rhythm (SMR) scoring for development of pubic hair, testes, penis, and serum testosterone level was positively related to age and negatively related to aerial exposure to Endosulfan (AEE; $p < 0.01$). Serum LH levels were significantly positively related to AEE after controlling for age ($p < 0.01$). The prevalence of congenital abnormalities related to testicular descent (congenital hydrocele, undescended testis, and congenital inguinal hernia) among study and controls subjects was 5.1% and 1.1%, respectively, but the differences were statistically nonsignificant. The results suggest that Endosulfan exposure in male children may delay sexual maturity and interfere with sex hormone synthesis.

Organophosphate exposure showed repeated association with hyperglycemia in animal models (Montgomery *et al.*, 2008; Rezg *et al.*, 2010). Dichlorvos specifically has been shown to disrupt glucose homeostasis in male Wistar rats. Applicators exposed to dichlorvos had increased risk of diabetes and this risk increased with increasing cumulative days of use (Montgomery *et al.* 2008). Organophosphates have also been shown, both in laboratory studies and epidemiological studies to increase the risk of obesity (Rezg *et al.*, 2010).

Laura *et al.* (2003) evaluated the associations of serum levels of p,p'-DDE (Dichlorodiphenyldichloroethylene) and two other persistent Organochlorine pesticides, β -HCH (Hexa Chlorocyclo Hexane) and HCB (Hexachlorobenzene) in relation to preterm birth. 233 mothers were recruited at three large maternity hospitals in Mexico City shortly after delivery. Results showed a non-significant increased risk of preterm birth in relation to serum p,p'-DDE levels. There was also a suggestion of an increased risk of preterm birth among women in the highest tertile of β -HCH (adjusted odds ratio 1.85, 95% CI= 0.94–3.66, p value for test of trend $p=0.08$) compared with the lowest

fertile. No association was found between HCB serum levels and preterm births. These findings suggest that p,p'-DDE and other Organochlorine pesticides may pose a risk of having preterm births.

Exposure to pesticides both occupationally and environmentally caused a range of human health problems (Horrigan *et al.*, 2002). It was attributed that nearly 10,000 deaths annually to use of chemical pesticide worldwide, with about three-fourths of these in developing countries.

Bonner *et al.* (2005) examined exposure to Carbofuran and several tumor sites among 49,877 licensed pesticide applicators from Iowa and North Carolina enrolled in the Agricultural Health Study. Risk of lung cancer appeared to be positively associated with exposure to Carbofuran when the less exposed were used as the reference group. The risk of lung cancer also increased when the frequency of exposure (number of days of Carbofuran use/year) and duration of exposure (number of years of Carbofuran usage) were examined separately. However, the risk was only elevated in applicators who used Carbofuran for >10 years and for >10 application days per year. Lung cancer risk was 3-fold higher for those with > 109 days of lifetime exposure to Carbofuran (RR = 3.05; 95% CI, 0.94–9.87) compared with those with < 9 lifetime exposure days, with a significant dose–response trend for both days of use per year and total years of use.

Yadav (2010) stated that environmental exposure of pesticides to human through may be cropping/ agricultural practices, consumption of food materials or air inhalation.

The association between Methyl parathion (MP) exposure and neurobehavioral development was assessed in children 6 years or younger at the time of the spraying and local comparison groups of unexposed children was done using the Pediatric Environmental Neurobehavioral Test Battery (PENTB). The PENTB evaluated cognitive, motor, sensory, and affect domains essential to neurobehavioral assessment. Children who were exposed to Methyl parathion had more difficulties with tasks involving short-term memory and attention. Additionally, parents of these children reported that their children had more behavioral and motor skill problems than did parents of children who had no exposure to pesticides. No differences between exposed and unexposed children in tests for general intelligence, the integration of visual and motor skills, and multistep processing were observed. The findings suggest that MP

might be associated with subtle changes to short-term memory and attention and contribute to problems with motor skills and some behaviors, but the results of the study are not conclusive (Ruckart *et al.*, 2004).

Hayes *et al.* (2006) found that some pesticides are suspected of being endocrine (hormone) disruptors. These chemicals affect parts of the body's hormone systems and can lead to an increase in birth defects, sexual abnormalities and reproductive failure, and may increase the risk of cancers of reproductive organs. HCH isomers, Endosulfan, Malathion, Chlorpyrifos, and methyl-parathion were monitored in human milk samples from Bhopal, Madhya Pradesh. The Endosulfan concentrations were highest and exceeded the sigma-HCH, Chlorpyrifos, and Malathion concentrations by 3.5-, 1.5-, and 8.4-fold, respectively. Infants consumed 8.6 times more Endosulfan and 4.1 times more Malathion through breast milk than the average daily intake levels recommended by the World Health Organization (Sanghi *et al.*, 2003).

2.7. Future strategies-integrated pesticide management

Singh and Dhaliwal (2000) expected organic farming to develop as an alternative ecofriendly technology for sustainable vegetable production. The bio-pesticide neemax proved equally effective to control the prevailing insect pests in all crops, compared to recommended chemical pesticides.

Garibay *et al.* (2003) reported that the net production of organic crops is more than 14,000 tonnes. Out of this production, tea and rice contribute to around 24% each, fruits and vegetables in combination makes to 17% of the total produce.

Organic farming is gaining gradual momentum in the country. In India, the National Program for Organic Production Standard is needed to maintain ecofriendly methods such as organic manure, biopesticides and bio fertilizers (Bhattacharya and Dushyant, 2004).

Pimental *et al.* (2005) carried out experiments for a period of 10 years at Rodale institute, Pennsylvania and showed that organically grown corn yields were more than the conventional ones under drought conditions.

A comparative analysis between conventional and organic agricultural farm was made on the basis of energy inputs in Turkey. It was found that organic farming was 23% energy efficient in comparison to conventional (Gundogmus *et al.*, 2006).

A review of over 300 published reports stated that out of the 18 environmental impact indicators (floral diversity, faunal diversity, habitat diversity, landscape, soil organic matter, soil biological activity, soil structure, soil erosion, nitrate leaching, pesticide residues, CO_2 , N_2O , CH_4 , NH_3 , nutrient use, water use and energy use) organic farming systems performed significantly better (Stolze *et al.*, 2006).

Chapter-III

MATERIALS AND METHODS

The study entitled “Pesticide residues and nutritional quality of selected vegetables grown in Southern Telangana Zone of Andhra Pradesh” was conducted during the year 2013-2014. The materials used and the methods followed during the course of investigation are briefly presented in this chapter.

3.1. Location of experimental work

3.2. Development of a structured questionnaire and collection of information

3.3. Procurement of samples from the selected zone

3.4. Estimation of physico-chemical parameters

3.5. Estimation of pesticide residue content in the selected whole and processed vegetables

3.6. Statistical analysis

3.1 LOCATION OF EXPERIMENTAL WORK

The study was conducted at Post Graduate & Research Centre and Quality Control Lab, ANGRAU, Rajendranagar, Hyderabad. The samples were procured from the farmers to study the physico-chemical parameters and pesticide residues.

3.2 DEVELOPMENT OF A STRUCTURED QUESTIONNAIRE AND COLLECTION OF INFORMATION

A questionnaire was developed to elicit information on package of practices in relation to pesticide usage and consumption of selected vegetables. (Appendix-A) The questionnaire included general information of the farmers such as name, age, education, main occupation, horticulture and allied activities, total land holding in acres, water facilities (irrigated / rain fed) etc and cropping patterns comprising of crops grown, land cultivated, sources for the procurement of seeds and pesticides, pesticide usage for different crops, dosage /acre, type of storage, type of treatment given to the vegetables during

storage, duration of storage period, processing techniques adopted, cooking practices employed, effect on health during the usage of pesticide, precautions taken during the usage of pesticides, yield of crops and annual expenditure on pesticides utilised for vegetables cultivation. The information in the questionnaire was collected from 30 farmers from four villages namely Thumallakunta, Timmajipeta, Marikal and Pullagiri.

3.3 PROCUREMENT OF SAMPLES FROM THE SELECTED ZONE

Four villages were covered under Southern Telangana zone of Andhra Pradesh namely Thumallakunta, Timmajipeta, Marikal and Pullagiri. The fresh vegetable samples of brinjal, bitter gourd and tomato were procured from the farmers. The samples were obtained after proper sampling was done using standard sampling techniques.

3.4. ESTIMATION OF PHYSICO-CHEMICAL PARAMETERS

The different physico-chemical parameters estimated were

3.4.1 Moisture

3.4.2 Vitamin C

3.4.3 Reducing sugars

3.4.4 β -carotene

3.4.1 Estimation of Moisture

Moisture content in vegetables was determined by the method of AOAC, (1990).

Procedure

1. The petridish with lid was weighed.
2. 10g or 10 ml of sample was weighed into the petridish and spread evenly for uniform drying.
3. Oven was set at 100 to 105⁰C and the petridish with sample was placed inside the oven with lid open for 15-17 hrs.
4. The petridish was cooled in a dessicator with lid open for 1-2 hrs.
5. The lid was closed and petridish with sample was weighed.
6. This was repeated for all samples till constant weight was achieved.

Calculations

$$\text{Moisture \%} = \frac{(W_2 - W_1) - (W_2 - W_3) \times 100}{(W_2 - W_1)}$$

Where,

W_1 = Initial weight of petridish (g)

W_2 = Weight of the petridish with sample before drying (g)

W_3 = Weight of the petridish with sample after drying (g)

3.4.2. Vitamin C

Vitamin C was determined by the method of Ranganna, (1986).

Reagents

- i. **2, 6-dichlorophenol indophenol dye solution:** In a 200 ml volumetric flask, 50 mg of sodium salt of 2, 6-dichlorophenol indophenol dye and 42 mg of sodium bicarbonate was taken and dissolved in 100 ml of hot distilled water. The volume was made upto 200 ml with distilled water.
- ii. **Metaphosphoric acid (3%):** Three grams of metaphosphoric acid was dissolved in a small quantity of distilled water and the volume was made upto 100 ml or 30 g dissolved in 1000 ml of water.
- iii. **Standard ascorbic acid:** 100 mg of L-ascorbic acid was dissolved in a small quantity of 3 per cent metaphosphoric acid in 100 ml volumetric flask and diluted to volume. From this 10 ml was taken in another 100 ml volumetric flask and volume was made up with 3 per cent metaphosphoric acid (1 ml = 0.1 mg of ascorbic acid).

Standardization of dye

In a 100 ml conical flask, 5 ml of standard ascorbic acid solution was taken and 5 ml of 3 per cent metaphosphoric acid was added. The dye solution was filled in a burette and

standard ascorbic acid solution was titrated. The titre value was noted at the end point where pink colour persisted for about 10 seconds. This was done in triplicate.

$$\text{Dye factor} = \frac{0.5}{\text{Titre value}}$$

Preparation of sample

In a 100 ml volumetric flask 10 ml of sample was taken and the volume was made up with 3 per cent metaphosphoric acid.

Procedure

Five ml of 3 per cent metaphosphoric acid extract of the sample was taken in a conical flask and titrated with standard dye. The titre value was noted at the end point where pink colour existed for 15 seconds. The titre value was noted.

Calculations

$$\text{Mg ascorbic acid per ml} = \frac{\text{Titre value} \times \text{Dye factor} \times \text{Volume made} \times 100}{\text{Aliquot taken} \times \text{Volume of sample taken}}$$

3.4.3. Reducing Sugars

Reducing sugars were determined by the method of Nelson, (1944).

Principle

The sample extract or the test solution is heated with alkaline copper reagent and the reduced copper formed then reacts with arsenomolybdate reagent to produce molybdenum blue resulting in the formation of a violet colored complex which is estimated colorimetrically at 500nm. Sodium sulphate is included in the copper reagent to prevent reoxidation of cuprous oxide by atmospheric oxygen.

Chemicals

- a) **45% neutral lead acetate:** 45g of lead acetate was dissolved in 100ml of distilled water.
- b) **Potassium oxalate:** 3 spatulas full of potassium oxalate was taken
- c) **Standard glucose solution:** 50mg of glucose dissolved in 50 ml of distilled water.

d) Copper reagent A: 2.5g anhydrous sodium carbonate, 25g sodium potassium tartarate, 20g sodium bicarbonate and 200g anhydrous sodium sulphate were dissolved in 1000ml of distilled water.

e) Copper reagent B: 15g copper sulphate was dissolved in 100ml of distilled water, with 1 to 2 drops of H_2SO_4

f) Alkaline copper reagent: 25 parts of reagent A + 1 part of reagent B were mixed before use.

g) Arsenomolybdate reagent: 25g of ammonium molybdate was dissolved in 450ml of water + 21ml of Conc. H_2SO_4 . The solution was mixed well. 3g of sodium arsenate was dissolved in 25ml of water and added to the above solution, mixed and incubated at 37°C

Procedure

1. 1 gram of the food sample was weighed to which 25ml of water was added and simmered for 20 minutes
2. After cooling, 5ml of 45% lead acetate was added and kept for 30 minutes.
3. 3 small spatulas of potassium oxalate was added and the solution was made up to 50ml with distilled water and filter. This is the reducing sugar extract.
4. 0.2ml of the reducing sugar extract was taken for different range of standards (0.05, 0.1, 0.2, 0.3, 0.4 and 0.5ml) in test tubes, to which 1ml of alkaline copper reagent was added and all the test tubes were kept in a boiling water bath for 20 minutes.
5. The tubes were cooled and 1ml of arsenomolybdate solution was added.
6. All the solutions were made up to 25ml in a 25ml volumetric flask.
7. Reading the solutions when taken in a spectrophotometer at 520nm wavelength.
8. A standard graph was plotted or obtained directly from the spectrophotometer, from which the sample values are extrapolated.

Observations to be taken: OD values from the standard graph in μg (**X**)

Calculations:

$$\begin{array}{l} \text{Reducing sugars} = \quad \text{X} \times \frac{50}{0.2} \times \frac{100}{1} \times \frac{1}{1000} = \\ \text{mg/ 100g sample.} \end{array}$$

3.4.4 Estimation of β - carotene

β -carotene was determined by the method of Ranganna, (1986).

Reagents

1. Stock KOH: 40g of KOH was dissolved in 100ml of water.

2. Working alcoholic KOH solution (12%): The above solution was diluted 3.3times i.e., 3ml was made up to 9.9ml with alcohol for each sample to be analysed.
3. Petroleum ether, analytical grade 60-80 °C.
4. Anhydrous Sodium Sulphate.

Procedure

Five grams of pulp was taken and ground in a mortar and pestle with acetone. Carotene was extracted repeatedly from the sample by using petroleum ether. Supernant liquid was decanted. Then the colour was measured in a spectrophotometer at 452 nm using 3 percent acetone in petroleum ether and taken as a blank, concentration of carotene were read from standard curve and calculated using the following formula (Ranganna, 1986).

Calculations

$$\mu\text{g carotene} / 100 \text{ grams} =$$

$$\frac{\text{Concentration of carotene in a solution as read from standard curve (mg l}^{-1}\text{) X final volume X dilution}}{\text{Weight of the sample}}$$

3.5 ESTIMATION OF PESTICIDE RESIDUE

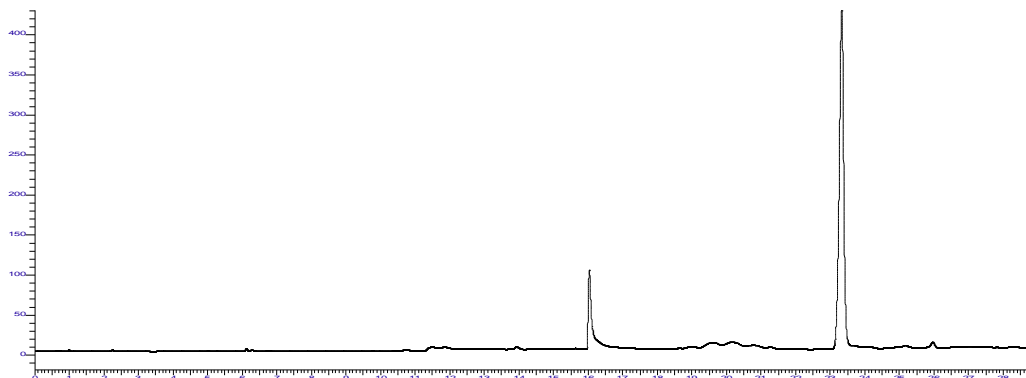
Pesticide residue determined by the method of Sharma, (2007).

Principle: Non-ionic residues were extracted with acetone / water, and the residues were seperated from aqueous acetone to dichloromethane / hexane phase. The traces of dichloromethane were removed and made up to the final extract with acetone/ hexane. Organophosphate residues were determined directly by a gas chromatography with ECD/NPD Detector.

3.1 STANDARD CHROMATOGRAM TABLE

S.No	Name of the Pesticide standard	Retention time (in minutes)
1	Chlorophyriphos	
2	Quinalphos	

3.1.1 Standard Chromatograms of Chlorophyriphos, Quinalphos



Method used

Oven programme

3.1.2 Method used for selected crops

	Ramp rate (°C/min)	Set point (°C)	Hold time (Min)
Initial	-----	50.00	2.00
Step1	10.00	200.00	5.00
Step2	10.00	250	2.00
Injector Temperature - 260 °C			
Detector Temperature - 325 °C			
Total Run Time 29.00 min			

Scope

The following pesticides were extracted by this method.

Organophosphates:

Acephate, azinphos-methyl, azinphos-ethyl, bromophos, bromophos-ethyl, chlorfenvinphos, chlorpyrifos, chlorpyrifos-methyl, demeton-O, demeton-O sulfone, demeton-O, sulfoxide, demeton-S, diazinon, dichlorvos, dimethoate, EPN, ethion, ethion oxygen analog, fenamiphos, fenamiphos sulfone, fenamiphos sulfoxide, fenitrothion, fenthion, malathion, methamidophos, monocrotophos, parathion-methyl, phorate, phosalone, phosphamidon, profenophos, quinalphos, triazophos.

Apparatus:

High-speed blender, Buchner funnel, porcelain 12cm diameter separating funnel, 1litre chromatographic column (10mm I.D. x 300mm), vacuum rotary evaporator, Gas chromatography unit.

Reagents:

Acetone, dichloromethane, hexane, acetonitrile, hexane (distilled using all-glass apparatus), sodium chloride (reagent grade), sodium sulphate (anhydrous- granular reagent grade), florisil(GR grade)..

Extraction:

- 25gms of sample was taken in a reagent bottle.
- The dry sample was moistened with water and 1:1 Acetone: n-hexane solvent was added.
- The sample was completely dipped in Acetone and hexane, occasionally shake was added and kept aside overnight.
- After extraction, the sample extract was to dry transferred to 1litre separating funnel and extract residues with 100ml mixture of hexane: dichloromethane (1:1, v/v) by vigorous shaking for 1min. The lower aqueous phase was to transfer another 1litre separating funnel.
- The organic phase of the first separating funnel was dried by passing through approximately 1.5” sodium sulphate supported on pre-washed cotton in 4”funnel.
- 10ml saturated sodium chloride solution was added to the separating funnel containing aqueous phase and shake vigorously for 30sec.
- To this, 100ml dichloromethane was added and shaken vigorously and dried at lower organic phase through the same sodium sulphate.
- The extraction was separated once more with 100ml dichloromethane and dried as above. Sodium sulphate was rinsed with about 50ml dichloromethane.
- The extract was concentrated using vacuum rotary evaporator.
- Concentration step was repeated in hexane to remove all trace of dichloromethane, and then repeated again to produce final extract in hexane/acetone solution.
- Solution was not allowed to go to dryness during any of the concentration steps
- Volume of extract was adjusted to suitable definite volume with hexane/acetone (2ml).
- The extract is suitable for determination of organophosphate residues by the GC on ECD/NPD

Clean-up:

- To a chromatographic column, 4g activated florisil was added followed by 2cm layer of sodium sulphate. Column was pre-wetted with 15ml hexane and column was not allowed to go dry.
- One ml of the extract was diluted to 10ml with 10% acetone in hexane. Container was rinsed with 2x3 ml portions of hexane.
- Column was eluted at about 5ml/min with 50ml eluant. Florisil eluate was concentrated to definite volume (2-5ml).

Operating conditions for Gas Chromatography:

Instrument	Gas chromatography with ECD / NPD
Column	Col-Elite-1 HT-30M, 0.250mm x 0.10um film Temperature
Oven Temperature	Initial 150 °C with hold time-10.00 min Ramp1: 2 °C / min to 200 °C hold for 1.00 min Ramp 2: 10.0 °C / min to 250 °C hold for 5.00 min Total run time for 46.00 min.
Injector temperature	225 °C
Detector temperature	275 °C

Gas Chromatography:

- The residue was dissolved to certain volume of hexane / acetone(2 ml)
- An aliquot (20 µl) of the solution was injected in to the GC.
- Retention time depends on the type of pesticide determined.
- 0.1mg in 10 ml of the solvent Hexane was dissolved to get 10ppm solution
- From this 1 PPM concentrated solution of the standard mix was prepared and injected into Gas chromatography to ascertain the peak values.
- The concentration of the pesticide present in test powder with reference to the standard mix was calculated.

Calculations:

Residue in test mix (mg/kg) =

$$\frac{\text{Peak area of the sample}}{\text{Peak area of the standard}} \times \frac{\text{Standard Concentration (1ng)}}{\text{Sample injected (1 } \mu\text{l)}} \times \frac{\text{Final Volume of the extract}}{\text{Weight of the sample (g)}}$$

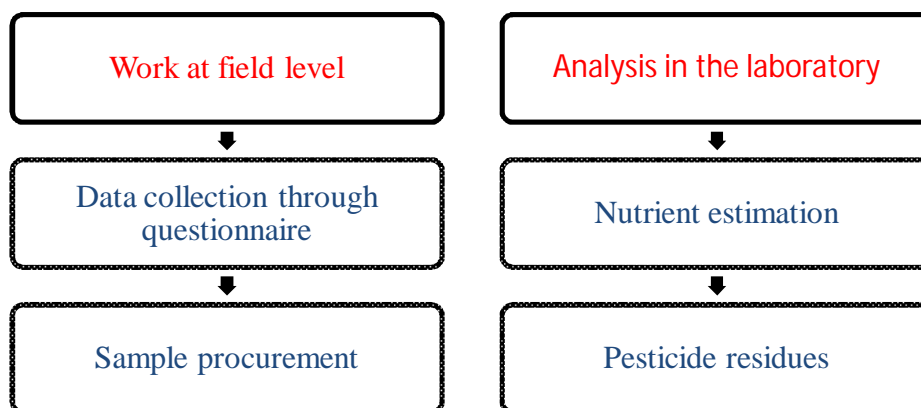
3.6 STATISTICAL ANALYSIS

Data collected from the farmers were tabulated using frequencies. The physico-chemical parameters and pesticide residues were subjected for mean and standard deviation.

Chapter IV

RESULTS AND DISCUSSION

The study titled “Pesticide Residues and Nutritional Quality of Selected Vegetables Grown in Southern Telangana Zone of Andhra Pradesh” focused on the pesticide residue levels and the nutrient analysis of the selected vegetable crops, tomato, bitter gourd and brinjal. The study was carried out in two phases i.e., field experiment and laboratory experiment



The most commonly grown vegetable samples tomato, bitter gourd and brinjal were collected from four villages namely Thumallakunta, Timmajipeta, Marikal and Pullagiri of Southern Telangana Zone of Andhra Pradesh. Data regarding package of practices in relation to the time, mode and quantity of pesticide application and vegetable consumption practices of selected vegetables was obtained through the questionnaire developed.

All the selected vegetables were analyzed for moisture, β carotene, vitamin C and reducing sugars and for pesticide residues of Chlorophyriphos, Quinalphos, Monocrotophos, Prophinophos, Carbofuran and Copperoxichloride.

. The results are presented under the following headings.

4.1 PRACTICES OF FARMERS ON USAGE OF PESTICIDES

4.1.1 General information of the farmers

4.1.2 Information on horticulture & allied activities

4.1.3 Information on pesticides usage by the farmers

4.2 NUTRITIONAL QUALITY OF VEGETABLES

4.2.1 Moisture

4.2.2 Vitamin-C

4.2.3 β -carotene

4.2.4 Reducing sugars

4.3 PESTICIDE RESIDUES IN VEGETABLES

4.3.1 Chlorophyrphos

4.3.2 Quinalphos

4.3.3 Prophinophos

4.3.4 Monocrotophos

4.3.5 Carbofuran

4.3.6 Copperoxichloride

4.1 PRACTICES OF FARMERS ON USAGE OF PESTICIDES

Information was collected from 30 farmers using purposive sampling with the help of a structured questionnaire prepared for this study. The purpose of the survey was to know the information on cultivation practices of vegetables with special reference to application of pesticides by the farmers. The information of the questionnaire was statistically analyzed by frequency distribution. The detailed questionnaire is given in the Appendix A.

4.1.1 General information

The general information of the farmers such as age, sex, educational status and occupation (main and allied activities) was collected, using a structured questionnaire. The general information is summarized in table 4.1.

Majority of the farmers were under classified into two different age groups of 41-50yrs and 51-60years. About 34% and 46% of the selected farmers were falling under the age group of 41-50years and 51-60years respectively. Only 20% of the farmers interviewed were within the age group of 61 years and above. All the farmers selected were males.

The educational status of the farmers showed that only 10% were illiterate. About 33% of them had education below 5th standard, while 40% of the farmers studied up to 5th - 7th standard. About 17% of the farmers were educated up to 8th standard & above.

Poor education and lack of awareness was reported to result in excessive use of pesticide by farmers who had also neglected safety rules and hygienic practices while using pesticides (Akeem and Nurudeen, 2012).

Table 4.1 General information of the farmers

General information	Details	Number	Percentage of farmers (%)
Age in years (n= 30)	41-50years	10	34
	51-60years	14	46
	61 and above years	6	20
Educational status (n= 30)	Illiterate	3	10
	Below 5th standard	10	33
	5th - 7th standard	12	40
	8th standard and above	5	17
Occupation (n= 30)	Horticulture	30	100

n- Number of farmers interviewed

4.1.2 Information on horticulture & allied activities

The main occupation of all farmers was horticulture. Besides horticulture, farmers were exploring ancillary occupation such as poultry (57%) and dairy (43%).

Majority of the farmers (40%) were marginal farmers having < 2.5 acres of land for cultivation. About 34% of farmers were small farmers having 2.5-5 acres of land for cultivation, 13% of farmers were medium farmers having of 5-10 acres of land for cultivation and 13% of farmers were large farmers with >10 acres of land for cultivation.

With regard to irrigation of the land it was observed that 63% of the farmers had only irrigated land, 33.33% of the farmers had both rain fed and irrigated farms and 3.33% of the farmers had only rain fed farms for cultivation (table 4.2).

Table 4.2 Percentage distribution of the farmers based on horticulture & allied activities

Horticulture allied activities	Details	Number	Percentage of farmers (%)
Horticulture allied activities (n= 30)	Dairy	13	43
	Poultry	17	57
Land cultivated (n= 30)	Up to 2.5 acres (Marginal farmers)	12	40
	2.5-5 acres (Small farmers)	10	34
	5-10 acres (Medium farmers)	4	13
	> 10 acres (large farmers)	4	13
	Irrigated	19	63.33

Irrigation facility (n= 30)	Rain fed	1	3.33
	Both	10	33.33

4.1.3 Information on pesticides usage by the farmers

The reasons given by farmers for application of pesticides was to increase the yield of the crop and to prevent or kill the insects and pests. About 60% of the farmers used pesticides to increase the yield of the crop, whereas 40% of the farmers used pesticides to kill the insects and pests. The definition of pesticides itself supports the reasons for usage of pesticides. The farmers took information from various sources for the usage of pesticides such as, horticulture scientists (10%), AO/AEO (27%), books or vyavasaya panchangam (7%), shopkeepers (87%), head of the family (40%), labels on pesticides (20%), and neighbours (67%) table 4.3 and fig 4.1.

More than one-sixth of the pesticides used on commercial vegetable crops in Dhading district of Nepal were extremely hazardous which were banned for general agriculture use, the reasons being no official training on pesticides for majority of the farmers and not reading the information available on the label by one- third of the farmers interviewed (Shrestha and Koirala, 2010).

Mode of usage of pesticides

Application of pesticides in the farm was done by two methods such as spraying and granular sprinkling. All the farmers were following both the methods of application (table 4.3).

Application of pesticides with manual equipment using knapsack by 70% farmers and tractor-mounted sprayer by 5% farmers was reported. Tasks like mixing chemicals and washing equipment exposed 66% and 65% of farmers respectively to the pesticides and majority of the workers/applicators used no personal protection measures or used them defectively/partially (Singh, 2009).

William and Peter (2006), found knapsack sprayers and motorised sprayers being the most widely used equipment for spraying pesticides in Ghana. It was reported that

various inappropriate practices in handling and use of pesticides caused possible poisoning symptoms among those farmers who generally did not wear protective clothing. Younger farmers (<45 years of age) were the most affected, probably because they did more spraying than older farmers (>45 years of age).

Precautions to be taken during the application of pesticides

While applying pesticides 67% of the farmers did not take any precautions, 13% of farmers used mask, 17% of farmers used gloves and up to 3% of the farmers used both mask and gloves (table 4.3 and fig 4.2).

Health effects

Majority of the farmers (67%) did not take proper precautions, due to negligence or lack of knowledge about the ill health effects caused by usage of pesticides such as headache (40%), nausea (13%), vomiting (17%), diarrhoea (3%), fever (7%), burning eyes (23%), itchy skin (7%) and skin rashes (10%) (table 4.3).

Shrestha and Koirala (2010) also reported that fifteen out of thirty commercial vegetable growers of Dhading district of Nepal were not using personal protective equipment during pesticide application and experienced symptoms of health hazards.

Mohiuddin (2009) also found that very few farmers (21%) used protective measures or safety measures during pesticide application, only 39% of the respondents did not use any safety measures where 21% of the vegetable growers covered their body and faces. Eight percent covered their face and 32% covered their body at the time of spraying.

Study on direct exposure to pesticides and indirect exposure to pesticides through food chain on agriculturists and public health workers respectively showed high concentrations of both BHC & DDE in the serum samples of both the groups indicating that any human being is liable to be exposed to pesticides either directly or indirectly leading to health problems (Kallidass and Jebakumar, 2006).

Application of pesticides before harvesting of the crop

The holding period after the usage of pesticides by farmers was seven days (30%), fifteen days (50%) and twenty days (20%) before the harvest of the crop (table 4.3).

Year by year almost about (100%) of the farmers increased the quantity of pesticide usage and 100% of the farmers increased the frequency of application of pesticides (table 4.3).

Annual expenditure on pesticides

Annual expenditure on pesticides was Rs. 4000-5000 by 50% of farmers whereas 33.33% of the farmers spent Rs.5000-6000 and 16.66% of the farmers spent Rs.6000-7000. The expenditure on pesticides was large by the farmers as they were very particular about getting a high yield of healthy crop. The awareness of health problems caused by the consumption of foods cultivated using pesticides was present in 60% of the farmers (table 4.3).

Table 4.3 Percentage distribution of the farmers based on the package of practices of pesticide usage

Package of practices of pesticide usage	Details	Number	Percentage of farmers (%)
Reasons for application of pesticides	Increase in yield	18	60
	Killing insects and pests	12	40
Advise taken by the farmers about the application of pesticides	Horticulture scientists	3	10
	AEO/AO	8	27
	Books / Vyavasaya panchangam	2	7
	Shopkeeper	26	87
	Labels on pack of pesticides	6	20
	Head of the family	12	40
	Neighbours	20	67
Mode of usage of pesticides	Knapsack Sprayers	30	100
	Sprinkling	30	100

Precautions taken during the application of pesticides	No precautions	20	67
	Wearing mask	4	13
	Wearing gloves	5	17
	Wearing both mask and gloves	1	3
Health effects	Headache	12	40
	Nausea	4	13
	Vomiting	5	17
	Diarrhoea	1	3
	Fever	2	7
	Burning eyes	7	23
	Itchy skin	2	7
	Skin rashes	3	10
Holding period of pesticides before harvesting	7 days	9	30
	15 days	15	50
	20 days	6	20
Change in the usage of pesticides over the years	Increase in the quantity of pesticides used over years	30	100
	Increase in the frequency of application of pesticides over years	30	100
Annual expenditure on pesticides	Rs. 4000-5000	15	50
	Rs. 5000-6000	10	33.33
	Rs. 6000-7000	5	16.66

Awareness of health problems caused by the consumption of foods cultivated using pesticides	Yes	18	60
	No	12	40

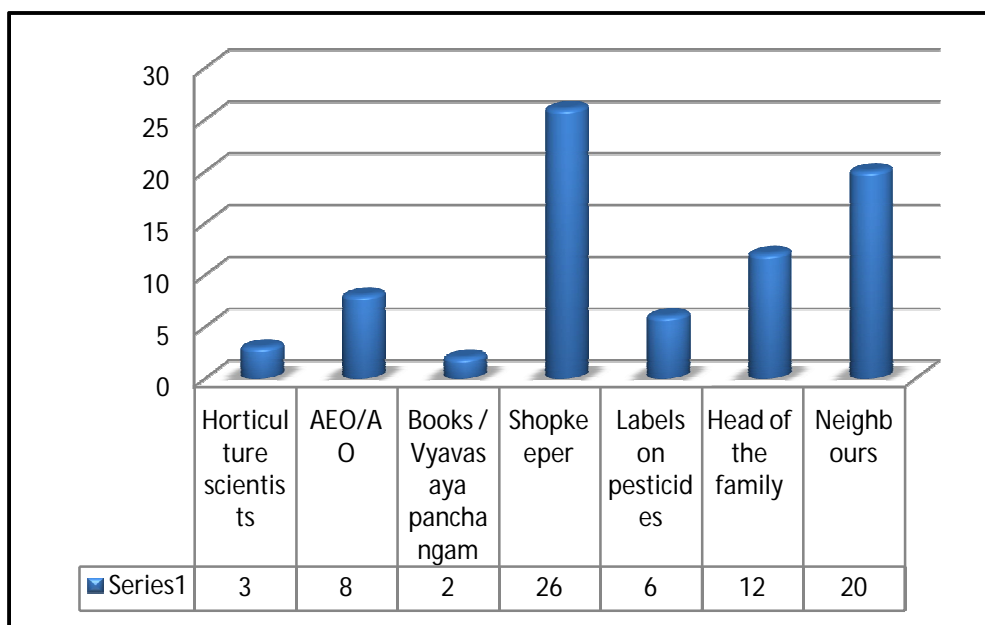


Fig 4.1 Sources of advice taken by the farmers about the application of pesticides

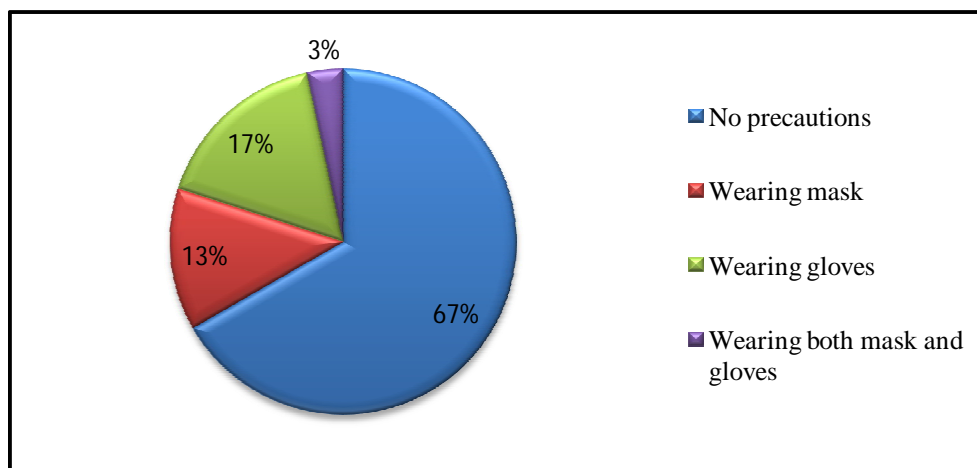


Fig 4.2 Precautions taken during the application of pesticides

Comparison between recommended and practiced pesticide dosage

The most commonly used pesticides were Monocrotophos, Prophinophos Chlorophyriphos, Quinalphos, Carbofuran and Copperoxichloride. The recommended dosage of pesticides (Vyavasaya panchangam 2010-2011, 2011-12 and 2013-14) per acre was 320ml for monocrotophos, 375ml for chlorophyriphos, 300ml for quinalphos, 4000gms for carbofuran and 450ml for copperoxichloride. More than the half of the farmers (55%) was using the recommended dosage of pesticide / acre, whereas 45% of the farmers were using greater than the recommended dosage of pesticide / acre. No farmer was found to be using less than the recommended dosage of the pesticide / acre (table 4.4).

Table 4.4 Percentage distribution of the farmers based on the recommended dosage of Pesticides

Pesticides	Recommended dosage/acre(RD/acre)	Percentage of farmers		
		< RD / acre	Equal to RD / acre	> RD / acre
Monocrotophos	320 ml	0 (0%)	12 (40%)	18 (60%)
Prophinophos	300ml	0 (0%)	17 (57%)	13 (43%)
Chlorophyriphos	375ml	0 (0%)	14 (47%)	16 (53%)
Quinalphos	300ml	0 (0%)	17 (57%)	13 (43%)
Carbofuran	4000gms	0 (0%)	16 (53%)	14 (47%)
Copperoxichloride	450ml	0(0%)	25 (83.33%)	5 (16.66%)

Source for recommended dosage/acre Vyavasaya panchangam 2010-2011, 2011-12, 2013-14.

Banerji and Dixit (2003) conducted a study to determine the residues of carbamates, organophosphorus and some organochlorines in summer fruits and vegetables collected from different places near Ganga river at Kanpur, Uttar Pradesh, India, using a multiresidue method. Selection of fruits and vegetables, i.e. cucumber, snake cucumber (*Cucumis melo* var. *utilissimus*), muskmelon (*Cucumis melo*), watermelon, white gourd (*Benincasa hispida*), pumpkin (*Cucurbita pepo*), bottle gourd (*Lagenaria siceraria*) and ribbed gourd (*Luffa acutangula*). The residues of organophosphorus insecticides were below detectable limits in the vegetables and fruits. However, a few samples showed residues of these insecticides in very low quantities, which were below the tolerance limits.

Similar study was conducted by Srivastava *et al.* (2011) on 20 vegetables including leafy, root, modified stem, and fruity vegetables like bitter gourd, jack fruit, french-bean, onion, colocassia, pointed gourd, capsicum, spinach, potato, fenugreek seeds, carrot, radish, cucumber, beetroot, brinjal, cauliflower, cabbage, tomato, okra, and bottle gourd. Forty-eight pesticides including 13 organochlorines (OCs), 17 organophosphates (OPs), 10 synthetic pyrethroids (SPs), and eight herbicides (H) pesticides were analyzed with a total number of 60 samples, each in triplicates. The limit of detection ranged from 0.001-0.009 mg kg⁻¹ for OCs, SPs, OPs, and H, respectively. Twenty-three pesticides were detected from total 48 analyzed pesticides in the samples with the range of 0.005-12.35 mg kg⁻¹. The detected pesticides were: Sigma -HCH, Dicofol, Sigma -Endosulfan, Fenprothrin, Permethrin-II, beta -cyfluthrin-II, Fenvalerate-I, Dichlorvos, Dimethoate, Diazinon, Malathion, Chlorofenvinfos, Anilophos, and Dimethachlor. In some vegetables like radish, cucumber, cauliflower, cabbage, and okra, the detected pesticides (Sigma -HCH, Permethrin-II, Dichlorvos, and Chlorofenvinfos) were above maximum residue limit (MRL) (PFA 1954). However, in other vegetables the level of pesticide residues was either below detection limit or MRL.

4.2 NUTRITIONAL QUALITY OF VEGETABLES

The samples of bitter gourd, brinjal and tomato were collected exclusively from Southern Telangana zone. The collected samples were subjected to preprocessing technique (soaking for 15 minutes in 3% salt water). These samples were analyzed for physico-chemical parameters including moisture, vitamin-C, β -carotene and reducing sugars. Besides the analysis of physico-chemical parameters, the selected whole and processed vegetables were assessed for pesticide residues for chloropyriphos and quinalphos.

The analysis of nutritional quality was done in triplicate for the selected vegetables to study the physico-chemical parameters such as moisture, vitamin-C, β -carotene and reducing sugars. The mean and standard deviation of the physico-chemical parameters of selected vegetables is given in table 4.5.

Temperature during analytical process, the storage time of vegetable and extraction process of the samples were kept in control because all these will have a tremendous effect on the results (Marcela and Amaya, 2003).

Table 4.5 Physico-chemical parameters of selected vegetables grown in Southern Telangana zone

Physico-chemical parameters	Vegetable sample		
	Bitter gourd	Brinjal	Tomato
Moisture	92.15±0.12	91.13±0.02	94.51± 0.03
Vitamin C	55.21±0.68	10±0	23.44±1.27
β-carotene	1340.11±0	1150±0.01	250± 0.008
Reducing sugars	0.6±0	2.46±0.008	3.13±1.01

4.2.1 Bitter gourd

Moisture

The moisture content of bitter gourd was analyzed by AOAC (1990) method. The result of the selected vegetables are tabulated in the table 4.5 and shown in Fig 4.3.

The moisture content of bitter gourd was 92.15 g per 100 g which was comparable with the moisture content of bitter gourd was 92.1 g per 100 g reported by Naseem *et al.* (2011). The moisture content of bitter gourd (92 g/100 g) reported by Gopalan *et al.* (2004). The moisture content of bitter gourd was 83.20 % reported by Gopalan *et al.* (1993). Wills *et al.* (1984) reported that the moisture content of bitter gourd was 93.8%. Anita Kochhar *et al.* (2006) reported that the moisture content of bitter gourd was 93.43 %.

Vitamin C

Vitamin C, being the most sensitive vitamin, is used as an indicator to measure the degree of changes owing to processing (Giannakourou and Taoukis, 2003).

Vitamin C is the most labile of the nutrients, so its degradation is used as an indicator of quality (Smith and Hui, 2004).

The losses of ascorbic acid is probably attributable to oxidation of ascorbic acid to dehydroascorbic acid followed by hydrolysis of the latter to 2,3-diketogluconic acid, which then undergoes polymerization to other nutritionally inactive products (Dewanto *et al.*, 2002).

The vitamin C content of bitter gourd was analyzed by the method of (Ranganna, 1986). The result of vitamin C in selected vegetables are tabulated in the table 4.5 and shown in fig 4.4.

The vitamin C content of the bitter gourd was 55.21 mg %. This value was nearly equal to the vitamin C content of bitter gourd (50 mg %) reported by Wills *et al.* (1984). The vitamin C content of the bitter gourd was 88 mg % reported by (Shakuntala Manay & Shadaksharaswamy, 2008).

β-Carotene

Vegetables were analyzed for their beta carotene content because it is the precursor of vitamin A and eaten in both raw and cooked form by humans in daily life (Bendich and Higdon, 2004).

It is evident that dark green vegetables contained more β-carotene as compared to other vegetables

Many fruits and vegetables have high concentration of carotenoid compounds. Carotenoids are known to have antioxidant activity by quenching free radicals and singlet oxygen. Antioxidant functions are associated with lowering DNA damage, malignant transformations, and other parameters causing cell damage in vitro (Sies *et al.*, 1992).

The ability of carotenoids to function as antioxidants may contribute to a reduction in disease risk (Sies *et al.*, 1992).

Researchers have found that bitter melon is full of antioxidants such as carotenoids, including alpha and beta-carotene, lycopene and zeaxanthin (Rodriguez *et al.*, 1976).

The β-carotene content of bitter gourd was analyzed by the method of (Ranganna, 1986). The β-carotene content of the selected vegetables can be observed in table 4.5 and shown in fig 4.5.

The β-carotene content of bitter gourd was 1070.11 μg %. This value was nearly comparable with the β-carotene content of bitter gourd (1078 μg %) reported by (Nauman *et al.*, 2007).

Reducing sugars

Sugars contribute 65-70% of the total soluble solids (TSS) in tomato fruit (Hobson and Kilby 1985).

The reducing sugar content of bitter gourd was determined by the method of Nelson (1944).

The reducing sugar content of bitter gourd was 0.6 % (table 4.5 and fig 4.6). This value was nearly comparable with the reducing sugar content of bitter gourd (1%) reported by Anita Kochhar *et al.* (2006).

4.2.2 Brinjal

Moisture

The moisture content of the brinjal was analyzed by AOAC (1990) method. The result of the selected vegetables are tabulated in the table 4.5 and shown in Fig 4.3.

The moisture content of brinjal was 91.13 g per 100 g which was comparable with the moisture content of brinjal (91.7 g/100g) reported by Naseem *et al.* (2011). The moisture content of brinjal was (93 g/100 g) reported by Gopalan *et al.* (2004). The moisture content of brinjal was 92.7 % reported by Gopalan *et al.* (2007).

Vitamin C

Miller and Rice (1997) reported that phenolic substances have been found to play a protective effect on the ascorbic acid. The presence of phenolics in the fruit cells may help to maintain the ascorbic acid content.

The vitamin C content of brinjal was analyzed by the method of (Ranganna, 1986). The result of vitamin C in selected vegetables are tabulated in the table 4.5 and shown in fig 4.4.

The vitamin C content of the brinjal was 10 mg %. This value was nearly equal to the vitamin C content of brinjal was 12.0 % mg reported by Gopalan *et al.* (2007). The vitamin C content of the brinjal was 2 mg % reported by Shakuntala Manay &

Shadaksharaswamy, (2008). The vitamin C content of brinjal was 4.8 mg % reported by Shahnaz *et al.* (2003).

β-carotene

The β-carotene content of brinjal was analyzed by the method of (Ranganna, 1986). The β-carotene content of the selected vegetables can be observed in table 4.5 and shown in fig 4.5.

The β-carotene content of brinjal was 2000 μg %. This value was nearly comparable with the β-carotene content of brinjal (2100 μg %) reported by Nauman *et al.* (2007).

The β-carotene content of brinjal was 0.74 μg reported by (Gopalan *et al.*, 2007).

Reducing sugars

The reducing sugar content of brinjal was determined by the method of Nelson (1944).

The reducing sugar content of brinjal was 2.65% (table 4.5 and fig 4.6). This value was nearly comparable with the reducing sugar content of brinjal (2.78-4.58 %) reported by (Khorsheduzzaman *et al.*, 2010).

4.2.3 Tomato

Tomatoes contain modest to high amounts of vitamin C, vitamin E, folates, phenolic compounds, and other carotenoids such as β-carotene (Beecher, 1998). All of them are relevant to the prevention of chronic diseases (Gerber, 1999).

Tomato is a major contributor of carotenoids (especially lycopene), phenolics, vitamin C and small amounts of vitamin E in daily diets (Khachik *et al.*, 2002).

The epidemiological studies showed that tomatoes and tomato products may have a protective effect against various forms of cancer, especially prostate cancer and cardiovascular diseases (Barber and Barber, 2002).

The bitter melon has considerable amount of potassium, calcium, magnesium, vitamin C, protein, and dietary fiber as compared with other commercial vegetables (Wills *et al.*, 1984).

Moisture

The moisture content of tomato was analyzed by AOAC (1990) method. The result of the selected vegetables are tabulated in the table 4.5 and shown in Fig 4.3.

The moisture content of tomato was 94.51 g per 100 g. This value was nearly equal to the moisture content of tomato (93.3 g/100 g) reported by Naseem *et al.* (2011). Gopalan *et al.* (2004) reported that the moisture content in tomato was 93 g per 100 g. Rumeza *et al.* (2006) reported that the moisture content in tomato was 93.5 %. Rumeza *et al.* (2006) reported that the moisture content in all vegetables was very high ranging from 77% to 94.5%.

Vitamin C

The ascorbic acid content of tomatoes is positively influenced by light intensity during growth (Dumas *et al.*, 2003).

Gahler *et al.* (2003) mentioned that the ascorbic acid content of tomatoes depend highly on the varieties and the conditions of cultivation. The decrease in ascorbic acid is an important loss for tomato products, tomato being a significant dietary source of this antioxidant vitamin (Davies and Hobson, 1981).

Ascorbic acid in tomatoes increases to a maximum level and then decreases with ripening (Malewski and Markakis, 1971).

The vitamin C content of tomato was analyzed by the method of Ranganna, 1986. The result of vitamin C in selected vegetables are tabulated in the table 4.5 and shown in fig 4.4.

The vitamin C content of the tomato was 23.44 mg %. This value was nearly equal to the vitamin C content of tomato is 22.52 mg % reported by Shahnaz *et al.* (2003). The vitamin C content of tomato is 20 mg % reported by A. V. Rao and L. G. Rao (2007). The vitamin C content of tomato (31 mg %) reported by Shakuntala Manay and Shadaksharaswamy, 2008. Rumeza *et al.* (2006) reported that the vitamin C content of the tomato was 26 mg %. A fresh, ripe, tomato fruit weighing 100g supplies 10.4-44.6 mg ascorbic acid (vitamin C) and

0.21-0.80 mg /l- of carotene (provitamin A) (Lincoln *et al.*, 1943). George *et al.* (2004) reported that the ascorbic acid content ranged from 8 to 56 mg/100 g in the skin, and from 8 to 32 mg/100 g in the pulp of Indian tomatoes.

β-carotene

β-carotene approaches maximum level by the time the tomato are partially ripe (Lampe and Watada, 1971).

McCollum (1954) showed that tomato fruit exposed to sunlight during ripening contained more β-carotene than those ripened in shade.

The β-carotene content of tomato was analyzed by the method of Ranganna, 1986. The β-carotene content of the selected vegetables can be observed in table 4.5 and shown in fig 4.5.

The β-carotene content of tomato was 250 µg %. This value was nearly comparable with the β-carotene content of tomato (200 µg %) reported by Demissie *et al.* (2009). The β-carotene content of tomato was 590 µg % reported by Gopalan *et al.* (2004). The β-carotene content of tomato was (1610 µg %) reported by Nauman *et al.* (2007). The variation could be attributed to varietal difference, time of harvesting and degree of ripeness.

Consumption of β-carotene and lycopene, has been related to lower incidence of cardiovascular disease and prostate, gastrointestinal and epithelial cell cancer (Ishida and Chapman, 2004; Rao and Rao, 2007).

Reducing sugars

The reducing sugar content of tomato was determined by the method of Nelson (1944).

The reducing sugar content of tomato was 3.13 % (table 4.5 and fig 4.6). This value was equal to the reducing sugar content of tomato (3.73 %) reported by Kovvuri vijaya bhanu (2004).

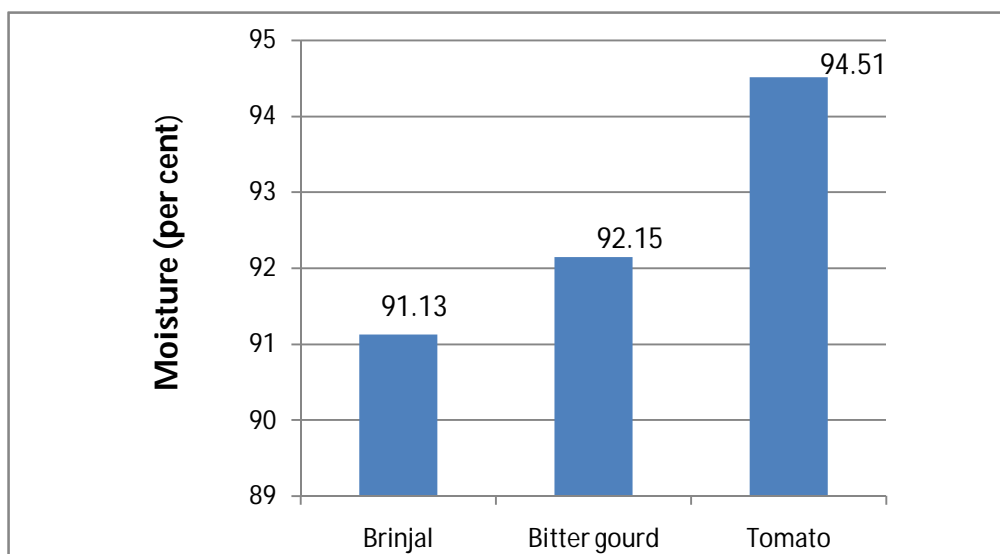


Figure 4.3 Moisture content of selected vegetables

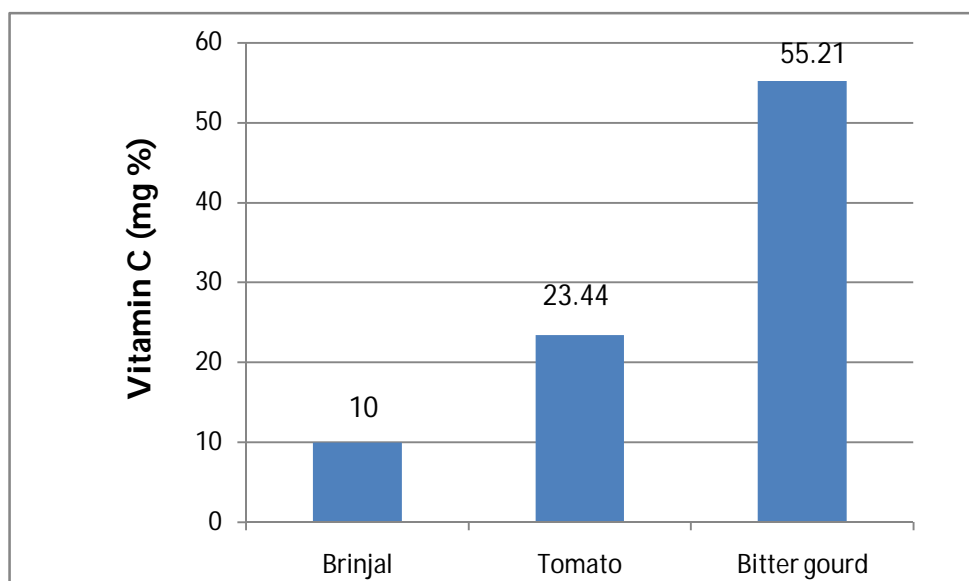


Figure 4.4 Vitamin C content of selected vegetables

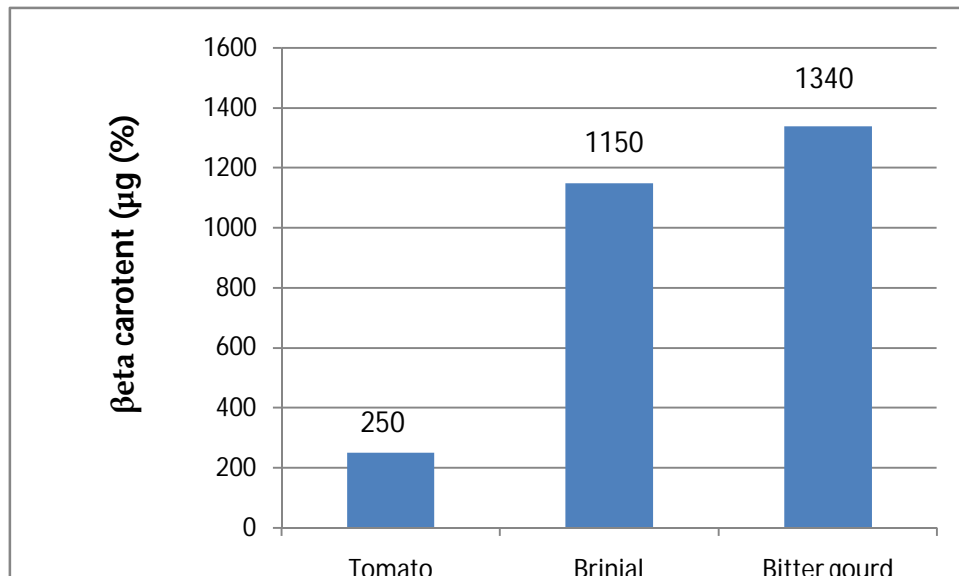


Figure 4.5 Beta carotene content of selected vegetables

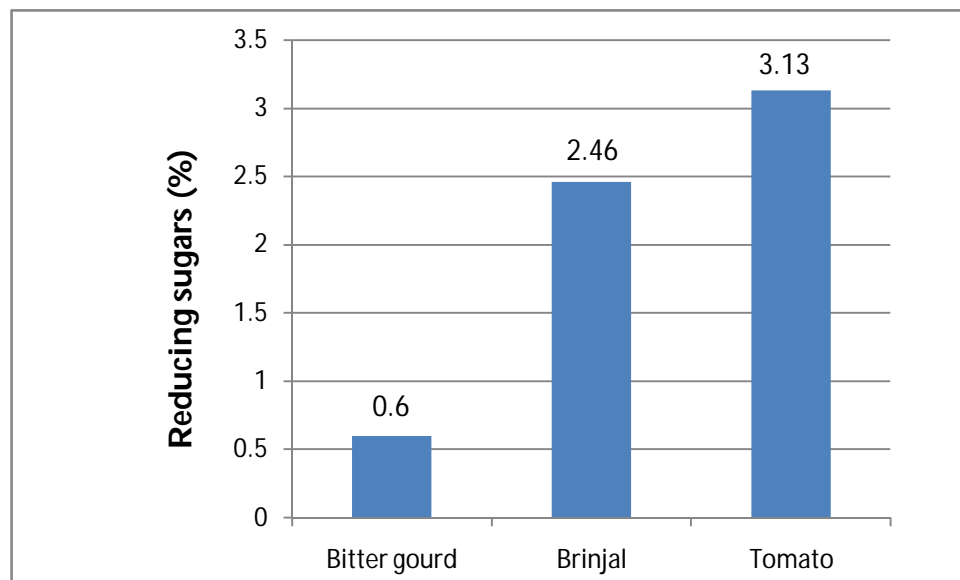


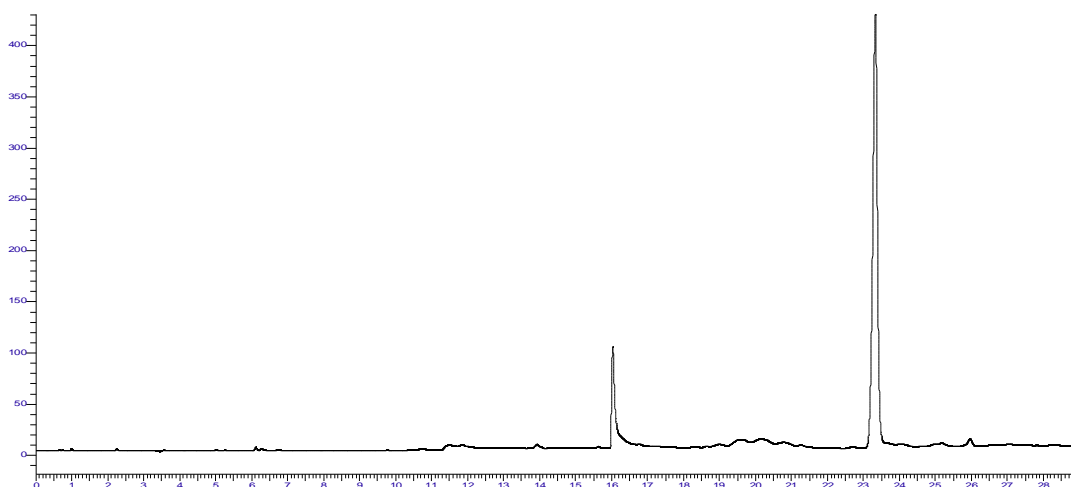
Figure 4.6 Reducing sugars content of selected vegetables

4.3 PESTICIDE RESIDUES IN VEGETABLES

The data collected through the structured questionnaire revealed that five pesticides were being used on all the three selected vegetable crops i.e. Monocrotophos, Chlorpyriphos, Quinalphos, Prophinophos, Copperoxichloride and Carbofuran belonging to

Organophosphate and Carbamate group of pesticides. The crops were tested for two pesticides, viz., Chlorpyrifos and Quinalphos.

The pesticide residue content of the selected whole and processed vegetables (soaking in 3% salt water for 15 min) were analyzed for the following pesticides namely chlorophyriphos and quinalphos simultaneously by a gas chromatography with ECD/NPD detector. Initially, the two standard pesticides were run on the gas chromatographic equipment to develop the standard graphs and obtain the retention times and the same are shown in Figure 4.7



S.No	Name of the Pesticide standard	Retention time (in minutes)
1	Chlorophyriphos	16.136
2	Quinolphos	23.322

Figure 4.7. Standard chromatographs of Chlorpyrifos and Quinalphos

These peaks were taken as reference chromatographs against which the peaks obtained for pesticide residues of all the selected vegetables were compared. Both fresh and washed vegetables were treated with the solvents as per the procedures for extraction of the sample and were run simultaneously on the gas chromatographic equipment. The chromatographs thus obtained are illustrated in figures 4.8 to 4.13.

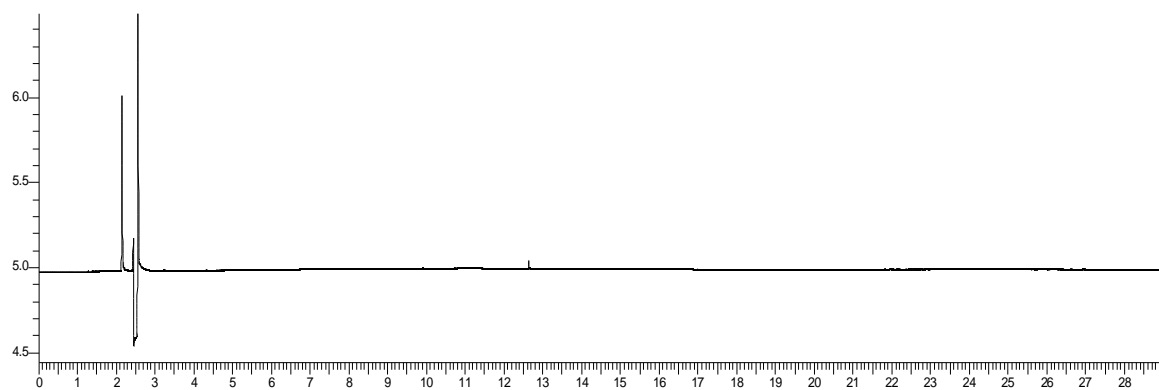


Figure 4.8. Chromatogram for pesticide residues in brinjal (fresh)

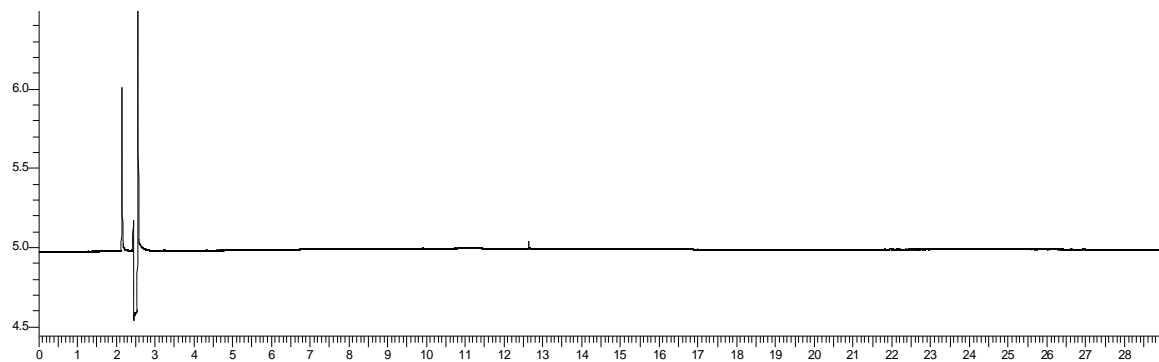


Figure 4.9. Chromatogram for pesticide residues in brinjal (after washing in 3% salt solution).

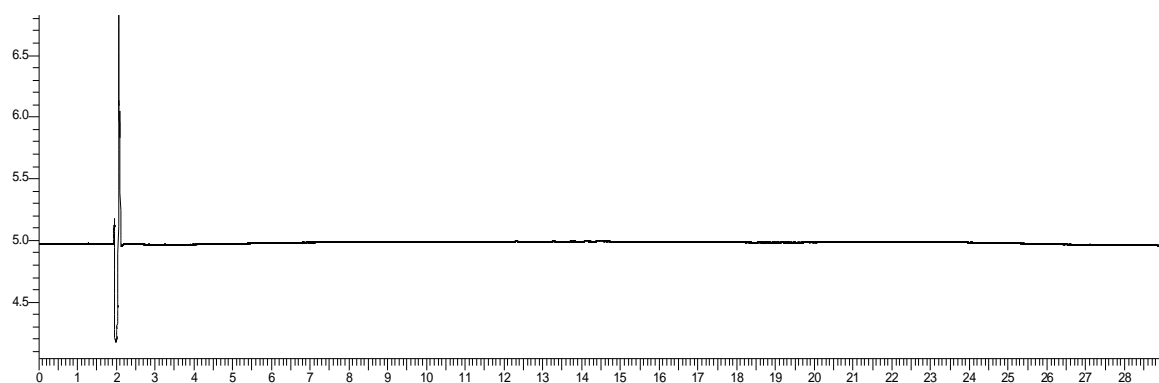


Figure 4.10. Chromatogram for pesticide residues in bitter melon (fresh).

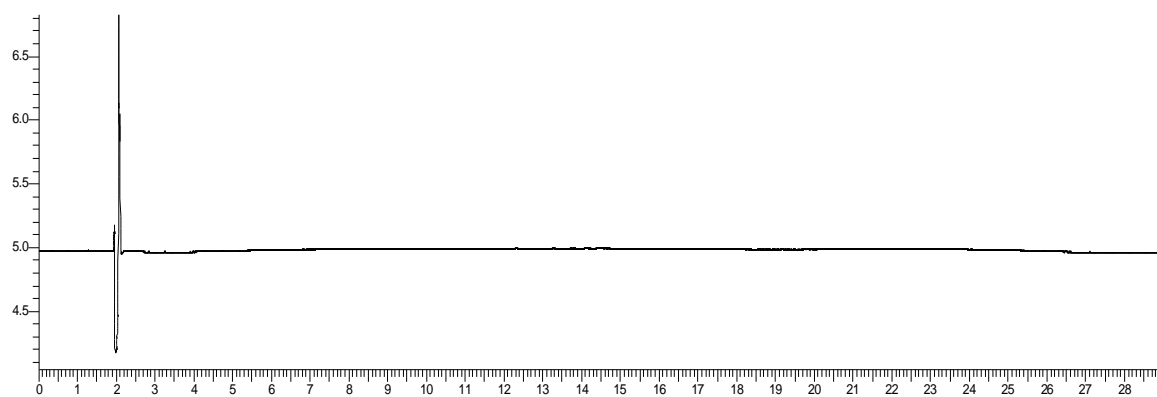


Figure 4.11. Chromatogram for pesticide residues in bitter melon (after washing in 3% salt solution).

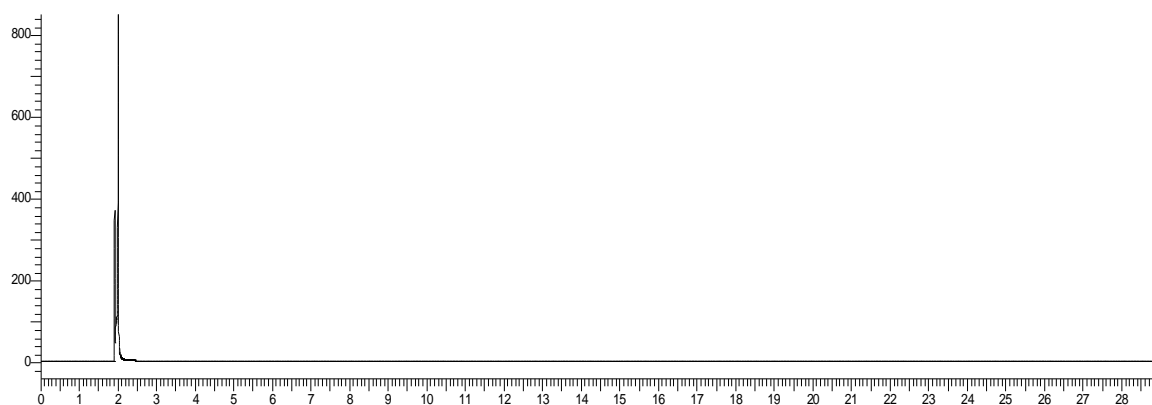


Figure 4.12. Chromatogram for pesticide residues in tomato (fresh)

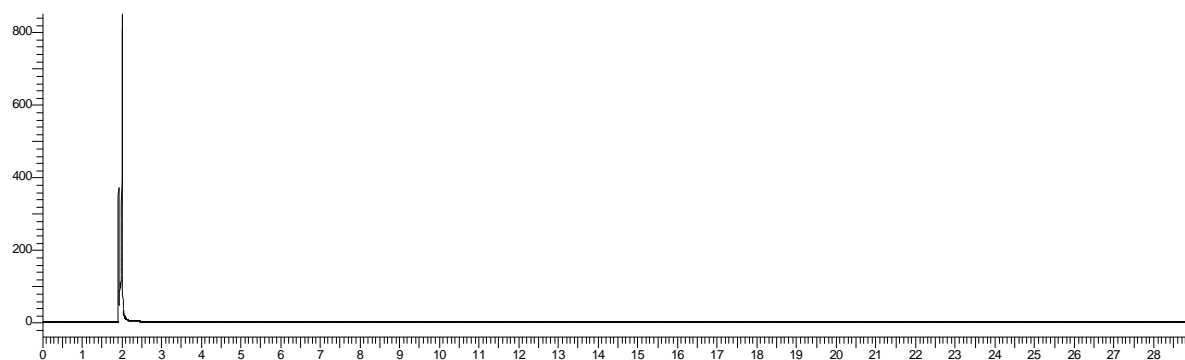


Figure 4.13. Chromatogram for pesticide residues in tomato (after washing in 3% salt solution).

It was observed that none of the chromatograms of the selected vegetable samples matched with the peak time of the standard chromatograms of the tested pesticides.

Therefore, it can be derived that the pesticide residues in all the selected vegetables analyzed in fresh and processed forms had negligible quantities i.e., they were Below Detectable Limits (BDL).

Similar results coincide with the results of Rao *et al.*(2009) on the pesticide residues in vegetable (brinjal, cucumber, okra, ridge gourd and tomato) and water samples collected from Kothapally Adarsha watershed in Rangareddy district, Andhra Pradesh, India during 2007. The results revealed the presence of monocrotophos (range 0.001-0.044 mg kg⁻¹), chlorpyrifos (0.001 to 5.154 mg kg⁻¹), cypermethrin (0.001 to 0.352 mg kg⁻¹) and endosulfan (0.001 to 0.784 mg kg⁻¹). The residues of monocrotophos and endosulfan were below maximum residue limit (MRL) in all the 59 vegetable samples. The water samples also revealed the presence of pesticide residues, but were below MRLs.

In field studies conducted in India, the maximum initial deposits of cypermethrin, fenvalerate and deltamethrin applied to cabbage at 50, 50 and 12 g a.i./ha were 0.34, 0.96 and 0.25 mg/kg, respectively, on heads, and 1.34, 0.08 and 0.30 mg/ kg, respectively, on leaves. These values were within the maximum residue limits of 2 mg/ kg for cypermethrin and fenvalerate on lettuce heads, and 0.5 mg/ kg for deltamethrin on leafy vegetables. Most of the insecticide residues were found on the outer leaves and it was concluded that the residue levels found do not constitute any health hazard to consumers (Singh *et al.*, 1992).

Persistence of fluvalinate and the safe interval between the last application and the harvest of brinjal, okra, cauliflower and cabbage were determined at two dose rates in field experiments in India. Residues at 30 g a.i./ha persisted for 7, 10 and 15 days on brinjal, okra, cabbage and cauliflower respectively. A post application holding period before harvest of one day was suggested for these crops after treatment with fluvalinate (Agnihotri *et al.*, 1992). Bordia and Gupta (1992) reported initial deposits of 1.17, 12.80, 29.27 and 3.23 mg/kg when 0.05% monocrotophos, 0.1% carbaryl, 0.07% endosulfan and 0.05% fenitrothion, respectively, were applied in foliar application to cauliflowers in the field. Residues of monocrotophos, endosulfan and fenitrothion fell to about 50% in 3 days and those of carbaryl in 7 days. Residues of monocrotophos, carbaryl and fenitrothion were below the level of detection in 15 days, and those of endosulfan in 21 days indicating that they have to be harvested only after 15 days / 21 days respectively after application of pesticides.

Fields of tomato were sprayed (Egypt) with recommended doses of profenofos, pirimiphos-methyl and methamidophos in 1992. Fruit samples were taken on 1, 4, 8, 11 and 15 days after treatment and analysed for residues. After 4 days tomatoes contained 1.02, 0.41 and 1.46 ppm profenofos, pirimiphos-methyl and methamidophos, respectively. Tomatoes were considered to be safe for human consumption 1 day after treatment with pirimiphos-methyl and 8 days after treatment with profenofos or methamidophos (Abdalla *et al.*, 1993).

The residues of most commonly used pesticides (endosulfan, cypermethrin, dimethoate, monocrotophos and mancozeb) on vegetables grown in India was observed by Dethé *et al.* (1995). Detectable levels of residues of endosulfan, cypermethrin, dimethoate, monocrotophos and mancozeb were observed in 33.3% samples of tomatoes (endosulfan, dimethoate and monocrotophos), 73.3% samples of brinjal (endosulfan, cypermethrin, fenvalerate, quinalphos, dimethoate and monocrotophos), 14.3% samples of okra (endosulfan), 88.9% samples of cabbage (endosulfan, fenvalerate and dimethoate) and 100% of cauliflower (endosulfan, fenvalerate, dimethoate, cypermethrin, and monocrotophos). However, the levels of pesticide residues were below the prescribed Maximum residue levels (MRL).

Therefore, from the results and discussion it could be concluded that the majority of farmers were taking advice from fertilizer's shopkeepers and using more than recommended dosage of pesticides/ acre. The farmers had awareness of health problems caused by the consumption of foods cultivated using pesticides, but they were very particular about getting more yield of the crop. Therefore they were not following the recommended dosage, application frequency of pesticides usage. However they should be educated on the dosage and application of pesticides.

The pesticide residues in all the selected vegetables were below detectable level (BDL) in both whole and processed vegetables which reflects that the pesticides used were highly volatile. Therefore, it could be concluded from the present study that it is safe to consume vegetables where pesticides are applied in recommended dosage and harvested after the required holding period as the pesticide residues come down to BDL (below detectable level).

Chapter V

SUMMARY AND CONCLUSIONS

Pesticides have become an integral part of crop cultivation practices. This may be attributed to rapidly increasing population which has posed a stress on the food production industry. Despite resulting in bountiful production, pesticides also pose a serious threat to the mankind. Fruits and Vegetables have a pivotal role in the diet for maintenance of health and prevention of disease. Therefore, to meet the consumer demands for safe food and to serve the trade related obligations becomes essential to analyze their pesticide residue content and also ensure timely supply.

The study entitled “**Pesticide Residues and Nutritional Quality of Selected Vegetables Grown in Southern Telangana Zone of Andhra Pradesh**” was conducted to estimate the pesticide residues in tomato, brinjal and bitter gourd in fresh form and after washing and the nutrient composition in fresh vegetables. The other objective was to determine the pesticide residue levels, of the selected vegetables before and after washing. Comparison was made between recommended package of practices versus farmers’ practices in the usage of pesticides and nutrient composition of fresh sample against reference values.

The farmers in Southern Telangana zone were selected using the random sampling technique and interviewed using the structured questionnaire. The questionnaire included information on the package of practices in relation to pesticide usage for different fruit crops, dosage/hectare, mode of application, sources for the procurement of seeds and pesticides, processing techniques which includes washing fruits before consumption and affect of pesticides on health.

The educational status of the farmers showed nearly 90 % of the farmers were literates while only 10 % of the sample farmers were illiterates. In terms of age, the farmers were on either side of middle age which shows there was a good mix of young and old farmers. All the interviewed respondents had agriculture as their main occupation. Among the entire vegetables tomato was the most widely cultivated crop in the selected zone and majority of the farmers (40%) interviewed had land holdings in the range of up to 2.5 acres with tube wells as the major source of irrigation. Most of the farmers used low doses of pesticides on their crops when compared to the recommended doses and highest percentage of farmers depended on pesticide shopkeepers for

knowledge on which pesticide to use, how much to spray as they were easily accessible compared to other sources.

Burning eyes (23%), vomiting (17%) and headache (40%) were the most common immediate symptoms experienced and few of them used masks or gloves, very few used both. Nearly 67 per cent did not use any precautionary measure while pesticide application. The reasons for application of pesticides were quoted as higher yield and prevention of pests. The farmers were aware of the use of natural pesticides like neem oil instead of chemicals but were not practicing. Most of them did not feel the need for washing the vegetables before consumption as they were unaware of the serious health effects of pesticides.

The nutrient compositions of selected fresh vegetables (moisture, reducing sugars, β -carotene and ascorbic acid) were comparable with the reference values. The moisture content of bitter gourd, brinjal and tomato were 92.15%, 91.13%, 94.51% and ascorbic acid content were 55.21mg%, 10mg%, 23.44mg% and β -carotene content were 1340 μ g%, 1150 μ g%, 250 μ g% and reducing sugars content were 0.6%, 2.46% and 3.13% respectively.

Pesticide residue levels in all the selected vegetables before and after washing were below detectable limits (BDL). Pesticide residues in vegetables were influenced by the time lag between pesticide spray and fruit harvesting, storage, transport, handling and processing techniques like washing and peeling before consumption of vegetables. The lower dosages of pesticides applied by the farmers than recommended could have also contributed to the non-detectable levels of pesticide residues in the vegetable samples.

Thus, the results of the present study indicate that the pesticide residue levels in the selected vegetables tomato, bitter gourd and brinjal of Southern Telangana Zone are within the MRL levels and are therefore safe for human consumption. Pre-processing techniques like washing with water or 3% salt water and peeling for obtaining edible portion reduced the residual burden as the residues fell below detectable levels.

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