

**Residue Dynamics of Flusilazole and Tebuconazole on
Chilli (*Capsicum annum* L.) in Kashmir**

Vinod Kumar
(MSH-2019-279)



Division of Plant Pathology

Faculty of Horticulture

**Sher-e-Kashmir University of Agricultural Sciences and
Technology of Kashmir**

2022

**Residue Dynamics of Flusilazole and Tebuconazole on
Chilli (*Capsicum annum* L.) in Kashmir**

**Vinod Kumar
(MSH-2019-279)**



Thesis

**Submitted to
Faculty of Horticulture**

**Sher-e-Kashmir
University of Agricultural Sciences and Technology of Kashmir
in partial fulfilment of requirements for the award of the degree of**

**Master of Science in Horticulture
(Plant Pathology)**

2022

*Affectionately Dedicated to my
Beloved Parents*

Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
Faculty of Horticulture, Division of Plant Pathology

Certificate – I

This is to certify that the thesis entitled “**Residue Dynamics of Flusilazole and Tebuconazole on Chilli (*Capsicum annuum* L.) in Kashmir**” submitted in partial fulfilment of the requirements for the award of the degree of **Master of Science in Horticulture (Plant Pathology)**, to the **Faculty of Horticulture, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir** is a record of bonafide research work carried out by **Mr. Vinod Kumar (Regd. No. MSH-2019-279)** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that any help or information received during the course of investigation has duly been acknowledged.

(Dr. Ashraf Alam Wani)
Chairman
Advisory Committee

Endorsed

Professor & Head,
Division of Plant Pathology

Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
Faculty of Horticulture, Division of Plant Pathology

Certificate – II

We, the members of the Advisory Committee of **Mr. Vinod Kumar (Regd. No. MSH-2019-279)** a candidate for the degree of **Master of Science in Horticulture (Plant Pathology)** have gone through the manuscript of the thesis entitled, “**Residue Dynamics of Flusilazole and Tebuconazole on Chilli (*Capsicum annuum* L.) in Kashmir**” and recommend that it may be submitted by the student in partial fulfilment of the requirements for the award of the degree.

Advisory Committee

Chairman

Dr. Ashraf Alam Wani
Professor and Head,
Division of Plant Pathology

Members

Dr. Ali Anwar
Professor,
Division of Plant Pathology

Dr. Ajaz Ahmed Malik
Assistant Professor,
Division of Vegetable Science

Dr. Imran Khan
Associate Professor,
Division of Agricultural Statistics

Dr. G. M. Lone
Professor & Head
Division of Entomology
(Dean’s Nominee)

Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
Division of Plant Pathology, Faculty of Horticulture

Certificate – III

This is to certify that the thesis entitled, “**Residue Dynamics of Flusilazole and Tebuconazole on Chilli (*Capsicum annuum* L.) in Kashmir**” submitted by **Mr. Vinod Kumar (Regd. No. MSH-2019-279)** to the **Faculty of Horticulture, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir** in partial fulfilment of the requirements for the award of the degree of **Master of Science in Horticulture (Plant Pathology)** was examined and approved by the Advisory Committee and External Examiner on

Chairman
Advisory Committee

External Examiner

Professor & Head,
Division of Plant Pathology

Dean,
Faculty of Horticulture

Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
Faculty of Horticulture, Division of Plant Pathology

Name of the student : **Vinod Kumar**

Registration No. : **MSH-2019-279**

Major Subject : Plant Pathology

Minor Subject : Entomology/Vegetable Science

Major Advisor : **Dr. Ashraf Alam Wani**
Professor and Head,
Division of Plant Pathology

Title of the Thesis : **“Residue Dynamics of Flusilazole and Tebuconazole on Chilli (*Capsicum annuum* L.) in Kashmir”**

ABSTRACT

The use of pesticides in large quantities and remarkable biological persistence in environment cause their widespread presence in all elements of food chain, which particularly involves water, vegetation and fish etc. Human beings being at the top of food chain, are exposed to hazardous effects of pesticides from every corner. Contaminated diet even with traces of these harmful chemicals is a chronic unavoidable source of exposure. Besides health benefits, the chillies can also have adversative effects on health if they hold pesticides over the MRLs and are hazardous for consumption. The priority is to detect the pesticide residues in the food in order to avoid the possible risks to human health. It was, therefore thought appropriate to undertake the study on the determination of fungicide residues in chilli and to study the dissipation behaviour of the two fungicides in chilli. The present investigation was, therefore, carried out to study “Residue Dynamics of Flusilazole and Tebuconazole on Chilli (*Capsicum annuum* L.) in Kashmir” in 2020 at Research Centre for Residue and Quality Analysis (RCRQA), Directorate of Research, SKUAST-K. Flusilazole 40 EC at recommended dosage (0.02%) and double the recommended dosage (0.04%) and tebuconazole 29.5EC at recommended dosage (0.05%) and double the recommended dosage (0.10%) were applied on chilli variety KL-1 (Kashmir

long-1) in the field of Division of Plant Pathology, SKUAST-K Shalimar campus. The sampling period for chilli fruit comprised 0, 1, 3, 5, 7, 10, 15, 20, 25 and 30 after application. Flusilazole and Tebuconazole was analyzed by Gas Chromatography and Mass Spectroscopy (GC-MS). The linearity was achieved, with determination coefficients (R^2) of 0.998 and 0.9974 for flusilazole and tebuconazole respectively. Mean percent recovery of flusilazole and tebuconazole when fortified at concentrations *viz.*, 0.1, 0.2 and 0.5 mg kg⁻¹ and 0.1, 0.2, and 0.5 mg kg⁻¹ respectively, was found to be 88.26 percent for flusilazole and 87.70 percent for tebuconazole. The studies on dissipation of pesticides in chilli fruit revealed that flusilazole at recommended dose (0.02%) left an initial deposit of 1.713 ±0.004 ppm which dissipated by 99.35 per cent in 15 days and was non-detectable beyond this period. The half-life period ($T_{1/2}$) and waiting period ($T_{tol.}$) of 1.55 days and 15.19 days were recorded. Flusilazole at double the recommended dose (0.04%) left an initial deposit of 2.634±0.004 ppm which dissipated by 99.46 percent in 25 days and was non-detectable beyond this period. The half-life period ($T_{1/2}$) and waiting period ($T_{tol.}$) of 3.01 days and 19.83 days were recorded. Tebuconazole at recommended dose (0.05%) left an initial deposit of 1.909±0.004 ppm which dissipated by 88.26 percent in 15 days and was non-detectable beyond this period. The half-life period ($T_{1/2}$) and waiting period ($T_{tol.}$) of 3.37 days and 4.07 days were recorded. Tebuconazole at double the recommended dose (0.10%) left an initial deposit of 2.666±0.020 ppm which dissipated by 90.05 per cent in 30 days and was non-detectable beyond this period. The half-life period ($T_{1/2}$) and waiting period ($T_{tol.}$) of 7.76 days and 8.13 days were recorded.

Keywords: Dynamics, dissipation, GC-MS, flusilazole, half-life, tebuconazole, pesticide residue, waiting period.

Signature of Student
Dated : _____

Signature of Major Advisor
Dated: _____

ACKNOWLEDGEMENT

I am one among the few fortunate to be a part of SKUAST-K, the seat of learning, which with good grace provided me the most constructive atmosphere for study and overall improvement. No human effort could climb the ladder without being given right direction. Perseverance, diligence and inspiration are the basic principles to climb towards excellence and reach the top. An individual is neither wise, nor rich or great at once. But by softly treading the path and firmly planting the steps of the climb triumph will be certain and advance sure.

Indeed, the words in my lesson are not sufficient either in form or in thought to elucidate my profound sense of reverence and indebtedness to Dr. Ashraf Alam Wani, Professor and Head Division of Plant Pathology, SKUAST-Kashmir and the chairman of my advisory committee, whose scientific acumen, lasting contribution with expert insights, supervision, prolific discussion, healthy criticism from time to time, outstanding cooperation, judicious technical support and infinite priceless suggestions from the very conception became an impetus behind my efforts for the completion of the present work and I will always remain proud of having had the chance to work under his tutelage.

I take this opportunity to extend sincere thanks to my Co-Advisor, Dr. Ali Anwar Professor, Division of Plant Pathology for his full support, scientific criticism, probing questions and timely help during the course of my research.

I feel privilege in taking this opportunity to mention here that indeed the words at my command are not adequate in form or in spirit to meet the ends of justices in the matter of expression of deep sense of gratitude to the head of my advisory committee Dr. G. M. Lone, Professor, Division of Entomology (Dean's Nominee) for his proficient suggestions, unrelenting help and critical advice in my calculations which led to a profound improvement in the composing of this document.

I owe a deep sense of gratitude to Dr. Imran Khan, Associate Professor, Division of Statistics, FOH, Shalimar and Dr. Ajaz Ahmed Malik, Assistant Professor, Division of Vegetable Science for their sincere guidance, generous help, affectionate encouragement, close supervision, constant inspiration, valuable suggestions and keen interest for the completion of present study.

With profound sense of gratitude, I beseech the entire array of words of thanks and reverence to mark personal regards to all the faculty members- Dr. N. A. Khan, Dr. T. A. Shah, Dr. Z. A. Bhat, Dr. M. A. Bhat, Dr. B.A. Padder, Dr. T. A. Sofi, Dr. Efath Shehnaz, Dr. Najeeb Mughal and Dr. Saba Bandy for their help, encouragement and for inspiring me to touch the levels of understanding and real sense of pedagogy.

With highest degree of veneration, I express deep gratitude and thanks to Dr. Amit Khokher, Assistant Professor, Division of Fruit Science whose painstaking advices, masterly inputs, meticulous guidance, moral support, scholarly suggestions, diligence and valuable guidance from time to time shall remain a lifelong gifted memory for me.

I express deep gratitude and thanks to Ms. Ishrat Jan, Residue Analyst, RCRQA and Dr. Alamgir, Residue Analyst, RCRQA for their sincere guidance, generous help, affectionate encouragement, close supervision, constant inspiration, valuable suggestions and keen interest for the completion of present study.

Parents are the most precious gifts of God to mankind who care, love, cooperate, protect and encourage their children. Words would never be able to fathom the depth of feelings for my beloved parents Mr. Ghan Shyam Sharma and Mrs. Sunita Sharma and I take pride on myself in being their son whose everlasting desire, selfless sacrifice and untiring struggle paved my way to pink of perfection. Though it is beyond the worth of my pen but today, I record my everlasting indebtedness to them and salute their generosity, greatness, their blessings in my favour, best wishes and ceaseless inspirations throughout my academic career.

From the profundity of my inner heart, I take the privilege to express my deep humility and devotion to my siblings Mrs. Anjali Shandil and Manish Sharma for their measureless prayers, everlasting love, constant encouragement and whole hearted support throughout the period of my study with immense patience, courage, sincerity and dedication.

With a deep sense of gratitude and zeal, I extend my heartfelt thanks to all the non-teaching staff members of RCRQA and Division of Plant Pathology SKUAST-K especially to Mr. Naseer, Mr Ramzan, Mr. Ishfaq and Mr. Bilal for their solemn cooperation, moral support and generous help during the research work,

I take this privilege with great pleasure to express profound and sincere sense of gratitude to the galaxy of my friends, seniors and class mates– Mr Harish Kumar, Mr. Ishant Dutta, Mr Rohit Godara, Mr. Ghulam Jeelani, Mr. Fasil Fayaz, Mr. Rishabh Dhiman, Mr. Avinash Pilla , Mr. Nikhil Chandel, Mr, Ajay Kumar, Mr. Shahjahan Rashid, Mr. Mehraj Yattoo, Mr Deoshish Attri, Mr. Faruk, Mr. Brahamam, Mr Vikas Kumar, Mr. Raj Meena, Mr. Ram Ambiya, Mr. Ram Babu, Mr. Haldhar Chandra, Mr. Murshid, Mr. Ashiq, Ms. Neha Sharma, Ms Shivali Sharma, Ms. Gazala, Ms. Nawreen and Ms. Arifa for their cooperation, appreciation, meticulous suggestions, everlasting moral support and constant encouragement during the course of this study.

I am highly grateful to members of Library staff of SKUAST-K for their constant encouragement, valuable suggestions and generous help during preparation of this manuscript.

Also, I express my gratitude to all those who care for me, are in my heart and mind and may not have got a mention, but none shall ever be forgotten.

Vinod Kumar

Place: Shalimar, Srinagar

Dated:

CONTENTS

Chapter	Particulars	Page No.
1.	INTRODUCTION	1-3
2.	REVIEW OF LITERATURE	4-23
3.	MATERIALS AND METHODS	24-33
3.1	Field trial	24
3.2	Pesticide Application	24
3.3	Meteorological data	26
3.4	Laboratory materials	26
3.5	Sampling schedule	28
3.6	Extraction and clean-up using QuEChERS method	28
3.7	Preparation of standard curves	29
3.8	Recovery	31
3.9	GC-MS parameters	31
3.10	Residue assay	32
4.	EXPERIMENTAL FINDINGS	34-39
4.1	Recovery studies	34

4.2	Initial deposits and persistence of fungicides(s) deposits	35
5.	DISCUSSION	40-45
5.1	Recovery studies	40
5.2	Initial deposits and persistence of fungicides(s) deposits	41
5.3	Statistical constant of pesticides	44
6.	SUMMARY AND CONCLUSION	46-47
	LITERATURE CITED	i-x
	APPENDICES	

LIST OF TABLES

Table No.	Particulars	Page No.
1	Fungicides application doses and dates of application on the chilli crop	25
2	Fungicides used for determination of residues in chilli	25
3	Standard curve of flusilazole	30
4	Standard curve of tebuconazole	30
5	Oven Programming	32
6	Recovery of flusilazole from chilli fruit	34
7	Recovery of tebuconazole from chilli fruit	34
8	Dissipation of flusilazole 40 EC in chilli fruit <i>cv.</i> Kashmir Long-1 at recommended concentration of 0.02 percent	35
9	Dissipation of flusilazole 40 EC in chilli fruit <i>cv.</i> Kashmir Long-1 at double the recommended concentration of 0.04 percent	36
10	Dissipation of tebuconazole 25.9 EC in chilli fruit <i>cv.</i> Kashmir Long-1 at recommended concentration of 0.05 percent	37
11	Dissipation of tebuconazole 25.9EC in chilli fruit <i>cv.</i> Kashmir Long-1 at recommended concentration of 0.10 per cent	38
12	Statistical constants of test pesticides in green chilli fruits	39

LIST OF FIGURES

Fig. No.	Particulars	After page No.
1	The temporal change in weather parameters during the course of experiment	26
2	Linearity curve for the estimation of flusilazole	30
3	Linearity curve for the estimation of tebuconazole	30
4	Average flusilazole 40EC residues in KL-1 with days after treatment at the recommended concentration of 0.02 percent and at a double concentration of 0.04 percent	36
5	Average tebuconazole 25.9EC residues in KL-1 with days after treatment at the recommended concentration of 0.05 percent and at a double concentration of 0.10 percent	38

LIST OF PLATES

Plate No.	Particulars	After page No.
1	General view of research trial	25
2	Sample extraction and clean-up	29

LIST OF ABBREVIATIONS

%	:	percent
/ or ⁻¹	:	per or per unit
@	:	At the rate
°C	:	Degree celcius
µml	:	Micro millilitre
µg	:	Micro gram
µV	:	Micro volt
a.i	:	Active ingredient
ADI	:	Acceptable Daily Intake
ARfD	:	Acute Reference Dose
b	:	Slope of regression equation
BDL	:	Below Detectable Limit
BQL	:	Below Quantification Limit
cm	:	centimetre
Conc.	:	Concentration
DAD	:	Diode-Array-Detection
DMI	:	Demethylation Inhibitor
D-SPE	:	Dispersive Solid Phase Extraction
EBI	:	Ergosterol biosynthesis inhibitors
EC	:	Emulsified Concentrate
EU	:	European- Union
Evap.	:	Evaporation
<i>et al.</i>	:	et alia (Co - workers)
Fig.	:	Figure
FAO	:	Food and Agriculture Organisation
FASSAI	:	Food Safety Standard Authority of India
g	:	gram
GC-MS	:	Gas Chromatography- Mass Spectroscopy
ha	:	Hectare
HPLC	:	High Performance Liquid Chromatography
Kg	:	Kilogram
L	:	Litre

LOD	:	Limit of Detection
LOQ	:	Limit of Quantification
m	:	metre
m ²	:	Square meter
mg	:	milligram
min	:	minute
ml	:	Millilitre
mm	:	Millimetre
MRL	:	Maximum Residue Level
ND	:	Non Detectable
N-P D	:	Nitrogen Phosphorous Detector
PDI	:	Percent Disease Incidence
PHI	:	Pre Harvest Interval
ppm	:	parts per million
PSA	:	Primary Secondary Amine
PTFE	:	Poly Tetra Fluoro Ethylene
q/ha	:	Quintal per Hectare
QuEChERS	:	Quick, Easy, Cheap, Effective, Rugged and Safe
R ²	:	Coefficient of Determination
RBD	:	Randomized Block Design
RH1	:	Relative Humidity 1
RH2	:	Relative Humidity 2
rpm	:	revolution per minute
SC	:	Suspension concentrate
SD	:	Standard Deviation
t _{1/2}	:	Half-life
T _{tol.}	:	Waiting Period
UV	:	Ultra-Violet
WG	:	Water dispersible granules
WP	:	Wettable Powder
X	:	Single Dose/Recommended dose
2X	:	Double Dose/ Double Recommended Concentration

Chapter-1

INTRODUCTION

Chilli (*Capsicum annuum* L.) is grown in India, China, Mexico, Thailand, the United States, the United Kingdom, Germany and Sweden (Velayutham and Damodaran, 2015). Chili is produced and exported in large quantities from India, the "Land of Spices" (Prabhavathi *et al.*, 2013). Red chilli production in the world is estimated to be about 21 lakh tonnes, with India producing 45 percent of it. India ranks first in production, consumption and export by 86 percent contribution. Due to their high colour value and pungency, Indian chillies have gained worldwide popularity (Mehta, 2017). In India, chilli is grown on 309 thousand hectares, with a total output of 3592 thousand MT out of which Karnataka contributes 14.85 percent of land with a production of 18.75 percent (NHB, 2019). Jammu and Kashmir has a total area of 2.15 thousand hectares under chilli cultivation, with an output of 15.76 thousand MT (Anonymous, 2019). The state of Andhra Pradesh has the highest chilli crop productivity. Guntur produces 30 percent of all Indian chillies. Moreover, due to its super quality Guntur chilli contribute 75 percent of total quantity of Indian export (Velayutham and Damodaran, 2015). Thus, chilli is one of the important spices on India. Besides meeting domestic demand also aids in earning of foreign exchange.

The low production of chilli is attributed to several factors, the most important being diseases and insect-pest. The major disease responsible for losses in chilli yield are powdery mildew *Leveillula taurica*, fruit rot *Colletotrichum capsici*, bacterial wilt *Pseudomonas solanacearum*, soft rot *Erwinia carotovora* and fusarium wilt *Fusarium oxysporum* f.sp *solani* etc. For managing diseases and insect pests of green chilli, farmers rely mainly on the application of pesticides. Flusilazole and tebuconazole both are effective against fruit rot caused by *Colletotrichum capsici* (Tomlin, 1994) (Manjunath, 2015).

Flusilazole and tebuconazole are systemic pesticides. Regardless of the manner of application and route of entry to the plant, they translocate throughout all plant tissues making them toxic to any fungal pathogen that attack the plant. Flusilazole and tebuconazole are fungicides that belong to triazole group and acts as a demethylation inhibitor (DMI) of fungal sterol biosynthesis (Bartlett, 1995).

Flusilazole and tebuconazole belong to a category of triazole derivatives that are applied on a wide range of crops because of their curative and preventive mode of action against a variety of fungal pathogens, including ascomycetes, basidiomycetes and deuteromycetes. Besides antifungal activity tebuconazole also act as plant growth regulator (Lewis *et al.*, 2016).

Despite the benefits of pesticides in agriculture and public health, their use often has negative environmental and public health consequences. Owing to their high biological activity and toxicity, they may occupy a unique place among environmental pollutants. Their indiscriminate use, may be harmful to humans, livestock, other living organisms and overall environment. Pesticides are believed to have killed 5000–20, 000 people and infected 0.5–1.0 million people per year (Yadav *et al.*, 2015; FAO/WHO, 2000). Agricultural employees account for at least half of those who are intoxicated and 75 percent of those who suffer from pesticide poisoning. The rest are being killed as a result of consuming contaminated food.

Pesticide residues in edible commodities, as well as their accumulation in the human system and the environment, are a major source of concern for human health. Anemia, dizziness, nephritis, convulsions and other illnesses occur in humans as a result of the chronic and acute effects of pesticide residues (Hallenbeck and Cunningham, 1985). Pesticides have been connected to a variety of human health issues, including acute symptoms such as headaches and nausea, as well as long-term impacts such as cancer, reproductive damage and endocrine system disruption (Blasco *et al.*, 2006). Pesticides exposure can occur through both direct routes (oral, inhalation and dermal) and indirect (drinking water, food,

residential and occupational exposure). Pesticide laden food crops, on the other hand, are the main cause of concern (Boobis *et al.*, 2008). In addition, improper pesticide application can damage the environment, increase resistance in target pest species and have negative consequences for non-target organisms.

When good agricultural practices are not followed by vegetable farmers, for e.g. if they do not allow enough time for pesticides to disperse from the fruits before they are harvested and shipped to market, pesticide residues may become a severe concern. Consumers may easily be exposed to pesticide residue ingestion when they eat the fruit since they are unaware of the level of pesticide contamination. It is important to actively track pesticide residue levels associated with various food crops in order to ensure consumer protection. Since new molecules are constantly added to our spray schedule, determining their residues on a regular basis has become extremely important. Furthermore, due to the fact that very limited research has been done on this aspect under the agro-climatic conditions of the Kashmir valley, the current investigation, entitled “Residue Dynamics of Flusilazole and Tebuconazole on Chilli (*Capsicum annum* L.) in Kashmir” was undertaken. The research was undertaken during 2020 with the following objectives:

1. To study the dissipation of flusilazole and tebuconazole residues on chilli fruits.
2. To determine the waiting period and half-life of flusilazole and tebuconazole on chilli fruits.

Chapter-2

REVIEW OF LITERATURE

Pesticides used in pest control programmes on various crop varieties have often been challenged because of their long persistence and slow degradation rate. Pesticide persistence and degradation rates are affected by the crop as well as the regions agro-climatic conditions (Teotia and Dahm, 1950; Kolkat *et al.*, 1961; Kavadia and Lal, 1967; Srivastava *et al.*, 1969). This necessitates a comprehensive investigation of pesticide residues on vegetables, fruits and other food products in order to ensure their safety. Therefore, some of the literature pertaining to pesticide residues of commonly used fungicides on chilli are summarised below:

Clean-up:

Chen *et al.* (2002) used HPLC method for determining flusilazole residue in grapes and soil. Fruit & soil samples were processed with acetone, further cleaned up with petroleum ether and determined by HPLC.

Anastassiades *et al.* (2003) developed a method for determining pesticide residues in fruits and vegetables that was easy, fast and inexpensive. The procedure involved initial single-phase extraction of 10 g of sample in 10 ml acetonitrile, followed by liquid-liquid partitioning with 4g anhydrous MgSO₄ and 1g NaCl. Using a quick technique called dispersive solid phase extraction (dispersive-SPE), 150 mg anhydrous MgSO₄ and 25 mg primary secondary amine (PSA) were mixed with 1ml acetonitrile extract to remove residual water and clean up. The quantitative and confirmatory study of GC-amenable pesticides was then carried out using gas chromatography/mass spectrometry (GC/MS).

Wu *et al.* (2014) tested multi-analyte selectivity nano-fibrous molecularly imprinted membranes (nano-MIMs) made by encapsulating two forms of molecularly imprinted polymer nanoparticles (MIP-NPs) into electrospun polyvinyl alcohol nanofibers. Each of the MIP-NPs provided high molecular

selectivity in the nano-MIMs obtained. The highest binding selectivity for acid bisphenol A (BPA) and basic tebuconazole (TBZ) was found using Nano-MIM embedding BPA-imprinted nanoparticles and TBZ-imprinted nanoparticles together (TBZ). This nano-MIM was used as an affinity material of membrane-based molecularly imprinted solid-phase extraction (m-MISPE) to extract trace BPA and TBZ in vegetables and juices simultaneously. The recoveries of BPA and TBZ from different samples were higher than 70.33 percent.

Persistence and Dissipation:

Garland *et al.* (1999) investigated the dissipation of tebuconazole and propiconazole in peppermint, finding that three applications each at 125 and 250 g a.i. ha⁻¹ resulted in residues of 0.26 and 0.80 mg kg⁻¹ respectively at harvest 64 days after the last application. Tebuconazole residues were also found in peppermint oil, with concentrations ranging from 0.011 to 0.041 mg kg⁻¹.

Angioni *et al.* (2003) studied the degradation of five triazole fungicides (cyproconazole, hexaconazole, penconazole, propiconazole and tebuconazole) in peach to assess whether the residue levels were below the legal limit. Because of their high stability, all fungicides showed higher residues than the legal limits, with the exception of propiconazole. In reality, some didn't degrade at all (cyproconazole, penconazole and tebuconazole) while others only changed in part. The key factor contributing to an apparent decrease in pesticide levels on a weight for weight basis was the increasing weight of the fruit during development. Co-distillation and sunlight photo-degradation were found to be the key mechanisms leading to a decrease in triazole levels in field. Despite the fact that these fungicides are systemic, they did not move into the fruit, leaving residues only in the peel.

Flusilazole residue dynamics in cucumber and soil were studied by Li and Leng (2004). Acetone and petroleum ether were used to extract cucumber and soil samples, respectively. After that, the extracts were cleaned using a florisil column

and partitioned with 35 percent acetone in petroleum ether. The material was evaluated using a Bpx-50 capillary column and gas chromatography with a N-P detector. Flusilazole had half-lives of 11-13 days in soil and 2-3 days in cucumber, respectively.

Rajabaskar *et al.* (2004) studied the dissipation and waiting times of propiconazole on marketable green chillies in the field. The initial fungicide deposits varied from 3.2-6.3 ppm at 125 kg a.i. ha⁻¹ to 7.6-9.5 ppm at 250 kg a.i. ha⁻¹, respectively. The healthy waiting periods were found to vary between 7.3-9.1 and 11.5-13.5 days at 125 and 250 kg a.i. ha⁻¹, respectively

Flusilazole and triflumizole residues in unwashed, washed and peeled apple fruits were analysed by HPLC at different periods, i.e. one hour, 1, 3, 5, 7, 14 and 21 days after application under field conditions in Egypt in 2004. Flusilazole (Punch 40% EC) was used at 3 ml/100 L water, while triflumizole 15% EC was used at 17.5 ml/100L water. After seven days of flusilazole application, unwashed apple fruits contained higher residues (0.322 ppm) than the allowed tolerance level (0.2 ppm), while washed and peeled fruits contained lower residues (0.149 and 0.087 ppm, respectively). The amount of residues rapidly decreased by elapse of time, so unwashed, washed and peeled apple fruits contained levels below the allowed maximum residue limit, *i.e.* 0.092, 0.008 and <0 ppm, respectively, after 14 days of flusilazole application. In the case of triflumizole, unwashed apple fruits contained 2.030 ppm after 5 days of application. Which was higher than the tolerance value (2.0 ppm). Washed and peeled fruits contained residues below the tolerance permissive values, *i.e.* 1.010 and 1.970 ppm after 3 and one day of application, respectively. These values rapidly decreased by time prolongation, so unwashed, washed and peeled apple fruits contained below the allowed tolerance values, *i.e.* 1.280, 0.490 and 0.160 ppm at 7 days after triflumizole application, respectively. Accordingly, unwashed apple fruits could be marketed safely 7 days after triflumizole application, while washed and peeled fruits could be used safely 3 and one day after application, respectively. (Ahmed, 2005).

Jyot *et al.* (2010) investigated four foliar applications of Nativo 75 WG (trifloxystrobin 25% + tebuconazole 50%) on grape at 175 and 350 g a.i. ha⁻¹ respectively, resulting in active applications of trifloxystrobin 43.75 and 87.5 g a.i. ha⁻¹ and that of tebuconazole 87.5 and 175 g a.i. ha⁻¹ respectively. At single and double application rates, the initial half-lives of trifloxystrobin on leaves was 2.92 and 3.48 days and on tebuconazole was 2.68 and 3.96 days, respectively. There were no traces in ripe grape berries or in soil samples obtained at harvest, 34 days after the last application.

With three spray applications, Mohapatra *et al.* (2011) evaluated tebuconazole and quinalphos residue persistence in spring onion, mature onion bulb and soil. The treatment included untreated control and application of tebuconazole at 187.5 and 375 g a.i. ha⁻¹ and quinalphos at 300 and 600 g a.i. ha⁻¹, respectively. Initial residue deposits of tebuconazole in immature onion bulbs along with leaves from the two treatments were 0.628 and 1.228 mg kg⁻¹, respectively. Tebuconazole residues dissipated with a half-life of 5-7.7 days. For consumption of immature onion bulb with leaves, the healthy Pre-Harvest Intervals (PHI) were 16 and 35 days, respectively. In immature onion bulbs with leaves from the two treatments, initial residual deposits of quinalphos were 0.864 and 2.283 mg kg⁻¹, respectively. The residues had a half-life of 1.7 and 2.6 days, respectively and the required PHI was 5 and 11 days. At harvest, the mature onion bulbs were found to be free of tebuconazole and quinalphos residues.

Shuang *et al.* (2011) conducted an experiment to develop a flusilazole residue analytical method that was clear, quick and reliable in apple and soil. Acetonitrile was used to extract the samples, which were then analysed using liquid chromatography with UV detection. The LOQ (Limit of Quantification) of method was 0.02 mg kg⁻¹. The dissipation dynamics and final residues of flusilazole in apple and soil were investigated in field trial. The half-life value of flusilazole in apple and soil were 4.23-7.77 days and 3.04-5.14 days, respectively, after the apple was treated with flusilazole at 3 times of the prescribed dose (3.75g

kg⁻¹ H₂O) in a residual dynamics experiment. At harvest time, flusilazole residues in apples were all less than 0.05 mg kg⁻¹ at both the 3 times and 1.5 times the recommended dosage. Flusilazole had a half-life of less than 8 days in apple and soil, according to the findings. The final residues in apples after 21 days of flusilazole treatment were all less than 0.2 mg kg⁻¹. According to the findings, it is safe to use the flusilazole formulation (5 percent WP) on apple trees in the prescribed dose and for a maximum of 5 applications with a 14-day interval between each application. Between the last application of the formulation and the apple harvest, the Pre-Harvest Period (PHI) may be 21 days.

Kundu *et al.* (2011) conducted a field trial to determine the harvest residue of Tebuconazole (25.9% EC) in paddy and ground nut at two application rates (750 and 1500 ml ha⁻¹) using LC-MS/MS. For various groundnut substrates, the average recovery was found to be between 86.33 percent and 91.87 percent. The average paddy recovery ranged from 86.40 percent to 90.86 percent depending on the substrate. Regardless of doses in different paddy and groundnut substrates, the residues of fungicide remained below the detection limit of the equipment (0.01 ppm) in all situations.

Liu *et al.* (2011) carried out the estimation of tebuconazole, trifloxystrobin and its metabolite trifloxystrobin acid by gas chromatography with a Nitrogen-Phosphorus Detector (GC-NPD) and Ion Trap Mass Spectrometry (GC-IT-MS). Acetonitrile was used as an extracting agent and subsequently cleaned up using Primary Secondary Amines (PSA) or Octadecylsilane (C18) as sorbent prior to GC analysis. The method provided sufficient sensitivity as reflected by the values of Limit of Detection (LOD) and Limit of Quantification (LOQ) of 0.4-7 and 1.2-20 µg kg⁻¹ for GC-IT-MS/MS and GC-NPD. The recoveries were, on average, 68-117 and 68-121 percent, respectively, for three compounds by GC-NPD and GC-IT-MS/MS with intra-day precision achieved with an RSD of 2.7-19.1 percent. The inter-day precision was better than 15.1 percent as determined by GC-NPD. The QuEChERS procedure, by using two sorbents (PSA and C18) and the matrix-

matched standards, gave satisfactory recoveries and RSD values in different matrices.

Kaur *et al.* (2012) conducted a study for residue analysis of Nativo 75 WG (trifloxystrobin 25% + tebuconazole 50%) at single application on wheat crops at 300 and 600 g a.i. ha⁻¹, trifloxystrobin applied at 75.0 and 150.0 g a.i. and tebuconazole at 150 and 300 g a.i. ha⁻¹. At recommended dose trifloxystrobin and tebuconazole initial deposits on wheat leaves were 5.54 and 8.30 mg kg⁻¹, respectively, while at double the recommended dose trifloxystrobin and tebuconazole initial deposits were 14.66 and 27.94 mg kg⁻¹, respectively. At recommended and double the recommended dosages, half-life values for trifloxystrobin were found to be 2.80 and 2.51 days, respectively, while those for tebuconazole were 2.46 and 1.85 days. At their detection limit of 0.01 mg kg⁻¹, trifloxystrobin and tebuconazole were not found in wheat grain samples harvested after a pre-harvest period of 140 days.

Sahoo *et al.* (2012) conducted study on dissipation of trifloxystrobin and tebuconazole were studied following two applications of a combination formulation of Nativo 75WG (trifloxystrobin 25%+tebuconazole 50%) @ 250 and 500 g ha⁻¹ at 10 days interval. Samples of chili were collected at 0, 1, 3, 5, 7, 10 and 15 days after the last application. Red chili and soil samples were collected after 20 days of last application. Half-life period for trifloxystrobin were found to be 1.81 and 1.58 days and for tebuconazole these values were observed to be 1.37 and 1.41 days, respectively, at single and double the application rates. Trifloxystrobin residues dissipated below its LOQ of 0.01 mg kg⁻¹ after 5 and 7 days, respectively, at single and double the application dosages whereas tebuconazole residues took 7 and 10 days, respectively. Red chili & soil samples collected after 20 days did not reveal the presence of trifloxystrobin and tebuconazole at their determination limit of 0.01 mg kg⁻¹.

Patyal *et al.* (2013) studied the dissipation behaviour of trifloxystrobin 25% + tebuconazole 50% (Nativo 75WG) on apple fruits at four different locations in

the North West Himalayan region of India. Trifloxystrobin was sprayed twice at the rate of 400 g and 800 g a.i. ha⁻¹ and tebuconazole at application rate of 200 and 400 g a.i. ha⁻¹. The half-life value of the tebuconazole ranged between 19.38-24.93 days and 19.84-28.86 days at the respective single and double doses.

In order to better understand the dissipation dynamic and terminal residual effects of flusilazole in mandarin and soil, Wang *et al.* (2013) conducted research at three locations in China. Mandarin peel, pulp, whole mandarins and soil samples were extracted with acetonitrile, washed with dispersive solid-phase extraction and analysed using GC-MS. With the exception of the soil dissipation at the Hunan site, which showed an increase-decrease process, the flusilazole dissipation half-lives in mandarin and soil were 6.3-8.4 days and 5.5-13.4 days, respectively, at all three experiment sites. Fourteen days after the last application, flusilazole residue levels in whole mandarin were below 0.1 mg kg⁻¹. Flusilazole was found to be mostly distributed in mandarin peel in a terminal residue sample, indicating that consuming mandarin pulp posed little risk.

Dong and Hu (2014) devised a sensitive and selective method for measuring fluopyram and tebuconazole in watermelon and soil at the same time. Watermelon and soil samples spiked at 0.01-0.1 and 0.5 mg kg⁻¹, respectively, were used to verify the procedure. The average fluopyram and tebuconazole recovery rates were found to be between 88.2-106 percent. For both compounds, a quantification limit of 0.01 mg kg⁻¹ was obtained. Fluopyram and tebuconazole had half-lives of 6.48-6.60 days and 5.87-6.93 days in watermelon, respectively and 15.8-24.8 and 11.2-14.4 days in soil, respectively.

After spraying the combined formulation, Nativo 75 WG, at the regular dose of 87.5+175 and the double dose of 175+350 g a.i. ha⁻¹, Mohapatra and Kumar (2014) evaluated residue persistence of the fungicides trifloxystrobin and tebuconazole on banana fruit, other edible parts and soil. For both fungicides, the limit of quantification of the method was 0.05 mg kg⁻¹. The half-life of trifloxystrobin and tebuconazole was around 11 days. The fruit pulp did not

contain trifloxystrobin or its metabolite. Tebuconazole, which is systemic, transferred to the fruit pulp on the third day (0.103 and 0.147 mg kg⁻¹ at single and double dose) and remained on it for 15 days.

Risk assessments of the fungicides pyraclostrobin, iprodione, tebuconazole and cyprodinil were carried after determining their persistence on strawberries following two applications by spraying in a greenhouse. The range for the average recoveries of the four fungicides was 86.2-105.4 percent and the relative standard deviation range was 2.7-6.1 percent. The half-lives of pyraclostrobin, iprodione, tebuconazole and cyprodinil after a single application were 3.7, 3.6, 3.3 and 2.8 days, respectively (Wang *et al.*, 2015).

Saha *et al.* (2016) conducted *in-vitro* and *in-vivo* bio-efficacy of different doses of a combination of fungicide, fluopyram 20%+tebuconazole 20% 40SC against anthracnose disease of chilli (*Capsicum annuum* L.) and their pre-harvest intervals pertaining to two different application rates. *In-vitro* bio-efficacy studies revealed the best control (79.3%) at 250 mg kg⁻¹ against 68.4 percent control under field conditions. A sample preparation method based on ethyl acetate extraction and estimation by LC-MS multiple reaction monitoring was validated in chilli fruits. Residue dissipation studies were conducted in field at single and double doses. The residues of both fluopyram and tebuconazole on all the sampling days were below the maximum residue limit (European Union) and maximum permissible intake estimated on the basis of the prescribed acceptable daily intakes. The combined bio-efficacy and residue dynamics information will support label claim of this combination of fungicide for the management of anthracnose in chilli and facilitate its inclusion in good agricultural package of practices.

To ensure consumer protection, Singh and Singh (2014) calculated the dissipation kinetics of trifloxystrobin and tebuconazole residues on tomato in field conditions. Three applications of Nativo 75% WG (trifloxystrobin 25%+tebuconazole 50%) at 350 and 700g ha⁻¹ were made at 7 days' interval

and tomato samples collected at 0 (1 hour), 1, 3, 5, 7, 10 and 15 days after the last application. At single and double doses, trifloxystrobin had half-lives of 1.39 and 1.94 days respectively, while tebuconazole had half-lives of 0.93 and 0.78 days.

Malhat *et al.* (2015) developed a High-Performance Liquid Chromatography with Photodiode Array Detector (HPLC-DAD) analytical method to determine flusilazole residue levels in grape and investigate the dissipation pattern and protection. The average recoveries were in the range of 86-90 percent, according to the findings. The identification and quantification limits (LOD and LOQ) were 0.008 and 0.02 mg kg⁻¹, respectively. First order rate kinetics best identified flusilazole residue levels, with half-lives ranging from 4.2 (recommended dose) to 4.6 days (double the recommended dose).

Mohapatra *et al.* (2015) conducted residue study of trifloxystrobin and tebuconazole on gherkin carried out at two locations (Bangalore and Gouribiddunur, India) after applications at the standard and double doses of 75+150 and of 150+300 g ha⁻¹ of the formulated product, trifloxystrobin (25 %) + tebuconazole (50 %) (Nativo 75 WG). The fungicides were determined by Gas Chromatography (GC) and confirmed by GC-MS. Initial residues of trifloxystrobin on gherkin fruits were 0.335 and 0.65 mg kg⁻¹ at Bangalore and 0.34 and 0.615 mg kg⁻¹ at Gouribiddunur. Tebuconazole residues were 0.842 and 1.682 mg kg⁻¹ at Bangalore and 0.71 and 1.34 mg kg⁻¹ at Gouribiddunur. Residue dissipation of the fungicides followed first-order rate kinetics. Trifloxystrobin residues dissipated at half-life of 2.9–3.7 days and tebuconazole at 3.2 days. At the standard dose treatment, trifloxystrobin residues dissipated to below the MRL of 0.2 mg kg⁻¹ (European Union) within 3 days at both the locations. Tebuconazole residues dissipated to below its MRL (0.05 mg kg⁻¹) within 14 and 11 days, at Bangalore and Gouribiddunur, respectively. From the two trials, it was concluded that the required Pre-Harvest Interval (PHI) for the combination formulation was 14 days. Application of Navio 75WG should be given before flowering to allow the residues to dissipate below the MRLs at harvest.

A rapid, simple and selective analytical method for the simultaneous determination of tebuconazole, trifloxystrobin and its metabolite trifloxystrobin acid residues in gherkin and soil was developed and validated by GC-MS. The samples were extracted with acetonitrile and cleaned up by dispersive solid-phase extraction with primary secondary amine sorbent. The LOQ of the method was 0.05 mg kg^{-1} for all three compounds. The method was validated using blank samples spiked at three levels and recoveries ranged from 83.5 to 103.8 percent with a relative standard deviation of 1.2 to 4.8 percent. The developed method was validated and applied for the analysis of a degradation study sample. The residues of trifloxystrobin and tebuconazole were found to dissipate following first-order kinetics with half-life ranging between 3.31–3.38 and 3.0–3.04 days, respectively, for two different dosages. Pesticide residues were below the European Union maximum residue level after seven days for trifloxystrobin (0.2 mg kg^{-1}) and ten days for tebuconazole (0.05 mg kg^{-1}), which suggested the use of this fungicide mixture to be safe for humans. These results can be utilized in formulating the spray schedule and safety evaluation on trifloxystrobin and tebuconazole in gherkin crop (Paramasivam *et al.*, 2015).

Dhanker (2017) investigated tebuconazole persistence in chilli and soil. On 0 day, the average initial tebuconazole deposits were 0.21 and 0.44 mg kg^{-1} for single and double doses, respectively (1hr after spray). At single and double doses, tebuconazole residues fell Below the Detectable Limit (BDL) of 0.01 mg kg^{-1} after 5 and 7 days, respectively. BDL was observed in soil samples taken after harvest.

According to Kyung *et al.* (2017) during greenhouse cultivation and processing, the dissipation of fluquinconazole and flusilazole residues in tomatoes was evaluated. The test pesticide fluquinconazole+flusilazole (7+1.5%) 8.5SC, was sprayed on tomatoes growing in a greenhouse according to Korea pre-harvest intervals and samples were collected for decline testing at 0 (3 hours after spraying), 1, 3, 5 and 7 days after the last application. Tomatoes harvested 5 days

after the last application were pureed and juiced for the processing examination. In both tomatoes and their finished components, the quantification limits for fluquinconazole and flusilazole were 0.005 mg kg^{-1} . For validation of analytical procedures, the recovery rates for fluquinconazole and flusilazole in tomatoes and their processed products ranged from 74.8 to 97.5 percent. The biological half-lives of fluquinconazole and flusilazole in tomatoes were reported to be 5.2 and 6.4 days, respectively, under greenhouse conditions. After washing, fluquinconazole and flusilazole had 37.34 and 79.53 percent continuous residual levels, 8.95 and 28.75 percent in boiling filtrates, 3.58 and 14.66 percent in puree and 3.34 and 13.52 percent in juice, respectively. According to these findings, pesticide residues on tomatoes could be largely eliminated by washing and boiling.

Saha (2017) studied the dissipation and residue dynamics of tebuconazole in peanut plants and soil, as well as the final residue in peanut plants, kernels and soil. Two foliar applications of tebuconazole (at an interval of 15 days, between 40 and 60 days after sowing of peanut in two consecutive years) were done at recommended ($187.5 \text{ g a.i. ha}^{-1}$) and double the recommended dose. During the seasons, tebuconazole degradation was faster in plants (half-life: 3.2-5.5 days; degradation rate constant: $0.1267\text{-}0.2142 \text{ day}^{-1}$) than in soil (half-life: 9.6–12.5 days; degradation rate constant: $0.0553\text{-}0.0725 \text{ day}^{-1}$). Regardless of doses or season, no tebuconazole residues were detected in soil, plant or peanut kernel samples at harvest, suggesting that tebuconazole is safe for human and do not cause environmental contamination.

Huatao *et al.* (2018) developed an updated QuEChERS system combining Liquid Chromatography-Tandem Triple Quadrupole Mass Spectrometry (HPLC-MS/MS) for three novel pesticides, *viz.*, albendazole, flusilazole and imazalil used to combat tobacco leaf spot disease. The three pesticides were recovered at 84.9-104.4 percent, 75.8-103.0 percent and 83.7-92.5 percent respectively, in tobacco leaves. Albendazole, flusilazole and imazalil had half-lives of 5.4-16.1, 4.3-7.7

and 4.4-6.9 days in fresh tobacco leaves, respectively. The terminal residues cured tobacco leaves were 0.33-1.33 mg/kg, 0.17-1.00 mg/kg and 0.76-2.00 mg kg⁻¹, respectively, when the fungicide was applied three times at the prescribed maximum dose and 1.5-fold dose with a PHI of 14 days. Based on these test results, potential MRLs for albendazole, flusilazole and imazalil in tobacco were estimated to be 2 mg kg⁻¹, 2 mg kg⁻¹ and 5 mg kg⁻¹ respectively, based on the same concepts used to set MRLs for food and feed.

Mai *et al.* (2018) estimated tebuconazole residues on grapes treated with minimal and maximal recommended concentrations. The grape samples were collected randomly after 1, 5, 10 and 15 days after last application, extracted using the ethyl acetate method and finally analysed with a gas chromatograph instrument. The method was validated and performance criteria complied with legislation requirements. Recovery data was over 90 percent, the limit of quantification Limit of Quantification was smaller than maximum residue limit and linearity ≤ 20 percent. According to the MRLs, the degradation of tebuconazole for the recommended concentrations applied dissipated between 10 and 15 days after the last treatment.

In 2016, a supervised field trial was conducted to determine the residues and dissipation of fluopyram, its metabolite and tebuconazole on pomegranate and in soil as a result of spray application of fluopyram+tebuconazole (Luna Experience 400SC). The fungicide was applied at 0.075 percent and 0.15 percent concentration (75+75 and 150+150 g a.i. ha⁻¹) by spraying two times. On days 0, 1, 3, 5, 7, 10, 15 and 20, samples were obtained after the second spray. A third spray was sprayed 15 days before harvest and mature fruits and soil samples were again collected at harvest. At the prescribed and double doses, fluopyram residues dissipated after 4.90 and 5.29 days respectively. The half-life values for tebuconazole were 5.02 and 4.86 days respectively. On the 15th and 20th days, residues in both fungicides at recommended and double the recommended doses fell Below Quantification Limit (BQL). When applied at 75 g a.i. ha⁻¹ and 150 g

a.i. ha⁻¹, the results showed the PHI was 13.74 and 12.59 days at recommended dose and 21.92 and 18.97 days at double the recommended dose for fluopyram and tebuconazole, respectively (Patil *et al.*, 2018).

Chau *et al.* (2020) designed study to understand the dissipation of fipronil, cypermethrin and tebuconazole in green onions and mustard greens grown in Central Vietnam. A field trial was implemented for two months with three different cropping regimes (natural plot fully exposed to natural conditions, bed plot fenced in by plastic sheets with an open roof and protected plot fully covered by plastic sheets) for each vegetable. A first-order kinetic model was successfully employed to interpret the dissipation data of each studied pesticide. A comparing the dissipation rate constants obtained under the same crop and growing regime between chemicals suggested that, among the chemicals examined, fipronil dissipated most readily. The pesticides were also proven to be more persistent in crops grown in protected plots than those grown in natural plots. The half-lives for fipronil, cypermethrin and tebuconazole fluctuated, occurring at 0.4-2.2 days, 2.0-6.0 days and 0.9-3.3 days, respectively.

Dubey *et al.* (2020) investigated the dissipation kinetics and persistence of tebuconazole on apple, tomato, chilli and onion after three foliar applications of the formulation Folicur 430SC at normal (X) doses (322, 268.75, 215 and 215 g a.i. ha⁻¹) and double (2X) doses (645, 537.5, 430 and 430 g a.i. ha⁻¹), respectively, to determine half-life and healthy waiting periods of tebuconazole on each of these four crops. GC-MS was used to determine residues of tebuconazole. With a LOQ of 0.05 mg kg⁻¹, tebuconazole recovery in fortified matrix was greater than 90 percent. For both the study locations, initial deposits of tebuconazole on apple ranged from 1.986-2.011 mg kg⁻¹ at X dose to 3.698-3.843 mg kg⁻¹ at 2X dose. The initial deposits in tomato, chilli and onion were 1.129, 1.760, 1.169 mg kg⁻¹ and 2.213, 2.784, 2.340 mg kg⁻¹, at the X dose and 2X dose, respectively. The half-lives ranged from 1.30-2.25 days at X dose to 1.40-2.62 days at 2X days. The residue declined below LOQ after 10 and 15 days in apple, 7 and 10 days in

tomato, 10 and 15 days in chilli and onion at X and 2X doses, respectively. Also, the waiting periods of 5, 2, 7 and 12 days were recommended for apple, tomato, chilli and onion, respectively at 2X dosage.

Disease management:

Determination of biological characteristics of *Colletotrichum gloeosporioides* was done by using crossing method and haemocytometer method. The mycelial growth method was used to test the toxicity of seven fungicides against *C. gloeosporioides*. The control efficacy of five fungicides against *C. gloeosporioides* was then investigated in field trials. The optimum temperature for mycelium growth was 20-30 °C, the optimum temperature for sporulation was 25-30 °C and the optimum pH value was 5-6, according to the findings. The inhibitory activities of carbendazim, difenoconazole, tebuconazole and flusilazole were all very high, with EC50 values of 0.118, 0.138, 0.159 and 1.297 g/mL, respectively, among the fungicides tested. The control efficacy of prochloraz 250EC (600), difenoconazole 100WG (1000) and tebuconazole 250EW (1500) against *C. gloeosporioides* was 89.60, 88.56 and 87.82 percent, respectively, according to field trials (Jianlu *et al.*, 2012).

Field experiments were conducted for three consecutive *Kharif* seasons of 2009, 2010 and 2011 at the experimental farm of Zonal Agricultural Research Station (Sub-montane Zone), Shenda Park, Kolhapur, to study the efficacy of five fungicides of triazole group *viz.*, tebuconazole (25.9%) EC, difenconazole (25%) EC, hexaconazole (5%) SC, tricyclazole (75%) WP and propiconazole (25%) EC against fruit rot disease of chilli caused by *Colletotrichum capsici*. Performance of these triazole fungicides was compared with mancozeb 75% WP, propineb 70% WP, copper oxychloride 50% WP and carbendazim 50% WP. Fungicide, tebuconazole appeared the most effective amongst the fungicides tested, with reduction in fruit rot incidence and intensity to the tune of 69.96 percent and 73.56 percent, respectively over unsprayed control, followed immediately with similar efficacy by other two triazoles *viz.*, difenconazole and hexaconazole. Highest dry

fruit yield of 25.91 q ha⁻¹ was recorded in the plots sprayed with tebuconazole and was at par with that obtained in difenconazole, hexaconazole, tricyclazole, propineb and mancozeb sprayed plots (Kumbhar and More, 2013).

Begum *et al.* (2015) tested different botanicals, fungicides and bioagents and evaluated *in-vitro* and *in-vivo* condition against *Colletotrichum capsici*. Amongst fungicides it was found that at 150 µg ml⁻¹ concentration 100 percent inhibition in mycelial growth was recorded in tebuconazole, mancozeb and trifloxystrobin+tebuconazole and minimum inhibition in mycelial growth was recorded in carbendazim (6.71%). Two isolates each of three bioagents *viz.*, *Trichoderma harzianum*, *T. viride* and *Pseudomonas fluorescens* were evaluated for their antagonistic properties against *Colletotrichum capsici*. *T. harzianum* isolate Th-2 was found most effective giving 77.78 percent inhibition on the mycelial growth followed by *T. harzianum* isolate Th-1 inhibiting 74.00 percent. Among four botanicals Garlic oil, Neem, Polyalthia and Citronella tested for *in-vitro* efficacy against *C. capsici* at concentrations of 0.05 percent, 0.1 percent and 0.2 percent, Garlic oil at all concentrations and neem oil at 0.1 percent gave cent per cent inhibition of mycelial growth of the fungus followed by Polyalthia at 0.20 percent (84.45%). Under field condition, significant minimum disease severity of 4.85 percent was found in plot receiving seed treatment with garlic oil at 0.5 ml kg⁻¹ seed followed by three sprays of Trifloxystrobin+Tebuconazole (50%) at 1g L⁻¹ water.

Jat (2015) evaluated *in-vitro* and *in-vivo* efficacy of propiconazole, tebuconazole, copper oxychloride, carbendazim+mancozeb and hexaconazole against chilli anthracnose (*Colletotrichum capsici*). Among fungicides tebuconazole inhibited mycelial growth of the pathogen completely at 300 and 500 ppm concentration. Fungicides were used as seed application, foliar application and seed-cum-foliar application against die-back of chilli under pot condition. Tebuconazole was found most effective in reducing the disease intensity followed by propiconazole.

Pandey and Gupta in 2015 conducted field trials on chilli variety LCA-355 for management of anthracnose (*Colletotrichum capsici*) through fungicides, bioagents and hand picking of initial set of green fruits. The results clearly indicated that highest total yield (1947.95 g plot⁻¹) as well as healthy red chilli fruits (1286.66 g plot⁻¹) was recorded in foliar sprays with Mancozeb at 0.3 percent followed by Azoxystrobin @0.1 percent (1125.00 g plot⁻¹), Flusilazole @ 0.1 percent (975 g plot⁻¹) and lowest yield in control (550 g plot⁻¹).

Sreeja (2014) carried out an investigation on integrated management of Fusarium wilt and anthracnose of vegetable cowpea (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdcourt) using new generation fungicides with the aim of testing the efficacy of new generation fungicides for the management of Fusarium wilt and anthracnose of vegetable cowpea. The pot culture experiments revealed that soil drenching with flusilazole (0.1%), tebuconazole (0.1%) or carbendazim (0.1%) completely suppressed the incidence of wilt, whereas foliar application with flusilazole (0.1%) reported the lowest index (5.60) of a wilt. Considering the overall efficacy of fungicides against two diseases, fusarium wilt and anthracnose of cowpea following integrated management package was developed for the control of Fusarium wilt and anthracnose of cowpea in disease prone areas as: 1) Seed treatment with carbendazim (2 g kg⁻¹ seed); 2) Soil solarization for a period of 45 days using transparent polythene sheets during warm season; 3) Application of *Trichoderma* enriched neem cake organic manure mixture @ 1 kg pit⁻¹ 15 days after seed emergence and; 4) Application of tebuconazole (0.1%) at 30, 45 and 60 days after seed emergence. The contact fungicide copper oxychloride (0.2%) can be used in rotation to prevent resistance from developing due to repeated use of the triazole fungicide.

Manjunath *et al.* (2015) evaluated nine fungicides (Penconazole 10 EC, Tebuconazole 25.9 EC, Thiophanate methyl 70 WP, Flusilazole 40 EC, Copper hydroxide 77 WP, Azoxystrobin 23SC, Mancozeb 75WP, Propiconazole 25 EC and Difenconazole 25 EC) and two biocontrol agents (tal

formulation/*Pseudomonas fluorescens* Pfl and *Trichoderma* BATF39- I) for three consecutive years under field trial conditions (2009-2012) against anthracnose disease of chilli. Flusilazole 40EC showed the highest disease control (51.007%), followed by *Pseudomonas fluorescens* Pfl (42.997%), Tebuconazole 25.9EC (42.997%), *Trichoderma* BATF39-1 (35.957%), Propiconazole 25EC (35.457%), Azoxystrobin 23SC (35.177%) and others (28.27-33.23%) as compared to untreated control. Similarly, flusilazole recorded the highest yield (31.1 q ha⁻¹) followed by tebuconazole (25.3q ha⁻¹), difenoconazole (24.3 q ha⁻¹), Pfl (22.5 q ha⁻¹), BATF 39-1 (21.6 q ha⁻¹) and others (16.1- 19.6 q ha⁻¹) as compared to control (15.9 q ha⁻¹). Hence, Flusilazole 40EC is an excellent alternative chemical for controlling chilli anthracnose.

Hafiz (2016) investigated efficacy of bioagents and fungitoxicant against *Colletotrichum capsaci* causing fruit rot and die back of chilli. Among systemic fungitoxicants, flusilazole 40EC proved to be the most effective with 86.01 percent inhibition of mycelial growth and among non-systemic fungitoxicants metiram (55%)+pyraclostrobin (5%) was found most effective with 92.80 percent inhibition of mycelial growth of the test fungus.

Sarkar *et al.* (2016) evaluated bioefficacy of different fungicides *viz.*, Thiophanatemethyl, Flusilazole, Copperhydroxide, Azoxystrobin, Mancozeb, Propiconazole, Difenconazole, Penconazole, Tebuconazole and two bioagents *viz.*, *Pseudomonas fluorescens*, *Trichoderma harzianum* were evaluated against anthracnose of chilli at AICRP on Vegetable Crops under Horticulture Research Station, OUAT, Bhubaneswar during rabi season of 2012-13 and 2013-14. Among the twelve tested treatments including control, all the treatments significantly reduced the anthracnose incidence as compared to control. Difenconazole (T7) was found to be most effective in reducing the disease intensity (mean PDI-5.0) which significantly varied from all other treatments. The same treatments also registered maximum disease control of (85.2%) over control corresponding to the highest mean ripe fruit yield (74.5 q ha⁻¹). However, the

control plot recorded maximum mean disease intensity (PDI-33.9%) with lowest mean ripe fruit yield (32.3 q ha⁻¹). The bioagents viz., *Pseudomonas fluorescens*, *Trichoderma harzianum* also significantly reduced the disease intensity as compared to control but both the treatments were at par with each other in reducing the disease intensity. No phytotoxic effects such as stunting, chlorosis, necrosis, discolouration, blackening and burning/malformation were observed.

Muzzafar (2017) studied the etiology and treatment of chilli wilt complex disease. *Fusarium pallidoroseum* (Cooke) Sacc. and *Sclerotium rolfsii* Sacc. were identified as the pathogen responsible for the disease. *F. pallidoroseum* was most common pathogen associated with disease with isolation frequency of 77.08 percent, followed by *S. rolfsii* with isolation frequency of 15.42 percent and a combined association of *F. pallidoroseum* and *S. rolfsii* with an isolation frequency of 7.50 percent, respectively. Carbendazim (25%) + Flusilazole (12.5%) 37.5SC was the most effective against both *F. pallidoroseum* and *S. rolfsii* among the five fungitoxicants tested at different concentrations under *in-vitro* conditions, with 100 percent mycelial growth inhibition at 40g ml⁻¹ concentration. Similarly, among the four botanical aqueous extracts tested, *Allium sativum* extract was the most successful, inhibiting mycelial growth by 100 percent at a 10 percent concentration of both test fungi. In pot experiment fungitoxicant and botanical extract were found more effective by seedling dip and soil drench method in reducing wilt incidence. When applied by seedling dip and soil drench methods, Carbendazim (25%) + Flusilazole (12.5%) 37.5 SC at 0.05 percent and *A. Sativum* extract at 10.0 percent provided 73.85 to 86.27 and 55.39 to 70.59 percent disease control, respectively.

Comparative performance of seven fungicides belonging to triazole, dithiocarbamate and chloronitrile was evaluated against *Colletotrichum capsaci*, causal agent of chilli fruit rot under *in-vitro* and *in-vivo* condition for two seasons, under *in-vitro*, tebuconazole (ED₅₀ 24 µg ml⁻¹) was found to be most effective followed by myclobutanil (ED₅₀ 43 µg ml⁻¹), hexaconazole (ED₅₀ 59 µg ml⁻¹),

chlorothalonil (ED_{50} 115 $\mu\text{g ml}^{-1}$) and difenconazole (ED_{50} 135 $\mu\text{g ml}^{-1}$). Dithiocarbamate fungicides propineb (ED_{50} 310 $\mu\text{g ml}^{-1}$) and mancozeb (ED_{50} 473 $\mu\text{g ml}^{-1}$) were relatively inferior among the test fungicides (Jain *et al.*, 2017).

Golakiya *et al.* (2018) studied the most common fungal disease chickpea wilt caused by *Fusarium oxysporum* f. sp. *ciceri*. To determine the most effective fungicides for the treatment of chickpea wilt, three fungicides *viz.*, Systemic, non-systemic and fungicide mixture were all screened against *Fusarium oxysporum* f. sp. *ciceri*. In laboratory testing of various fungicides, Tebuconazole 25.9EC was found to be very successful in inhibiting the radial growth of the test pathogen among systemic fungicides, while Copper Oxychloride 50 percent WP and fungicide combinations Carbendazim (12%) + Mancozeb (63%) WP were found to significantly inhibit the growth of the test pathogen among non-systemic fungicides. The combination of Carbendazim 12%+Mancozeb 63% WP had the lowest PDI of the six fungicides tested, followed by Carbendazim 50 percent WP.

Akhtar *et al.* (2018) evaluated the efficacy of five different fungicides *viz.*, Kasugamycin (Kasu B 3% SL), Pyraclostrobin+Metaram (Cabrio Top 60% WG), Azoxystrobin (Onestar 23% SC), Flusilazole (Cursor 40% EC) and Carbendazim (Dhanustin 50% WP) and tested at 0.1 percent concentration against Anthracnose disease of chilli caused by *Colletotrichum capsaci*. All the test fungicides efficiently reduced the disease intensity, thus increased the yield of chilli significantly. Cabrio top 60% WG (Pyraclostrobin + Metaram) resulted in highest percentage increase in yield over control followed by Onestar 23% SC (Azoxystrobin), Cursor 40% EC (Flusilazole), Dhanustin 50% WP (Carbendazim) and Kasu B 35% SL (Kasugamycin).

Kumar and Jakhar (2019) conducted an experiment to investigate the influence of fungicides and packaging materials on longevity of chilli seed (variety: RCH-1). The seeds were treated with 15 fungicides and were kept in three containers *viz.*, Metal box, Cloth bag and Plastic zipling bag up to 12 months under ambient conditions in seed pathology laboratory of Department of Seed

Science & Technology, CCSHAU, Hisar, India. The samples were drawn at quarterly intervals for ascertaining the seed quality parameters. The seeds treated with flusilazole (2 g kg⁻¹ seed) and stored in metal-box were found better for maintenance of higher seed quality parameters (germination, root length, shoot length, mean seedling dry weight, vigour indices) during the study period. The study suggested that use of appropriate packaging material and seed treatment could be useful to prolong the storage life of chilli seeds.

Anand *et al.* (2020) evaluated eight chemical and botanical fungicides against *Colletotrichum capsici in-vitro* at T.M. Bhagalpur University, Bhagalpur. Results revealed that the fungicides tebuconazole, hexaconazole, trifloxystrobin, mancozeb and saponin at 0.05 and 0.10 percent concentrations were effective in inhibiting the mycelial growth and sporulation *in-vitro* over control, while carbendazim, neem oil and azadirachtin were least effective. When evaluated by fruit dip method for uninjured and injured inoculated fruits under laboratory condition, tebuconazole, hexaconazole and saponin significantly reduced fruit anthracnose.

Badgujar *et al.* (2020) conducted an experiment to study the field efficacy of fungicides against anthracnose disease of chilli. Average disease intensity recorded during *in-vivo* efficacy of fungicides ranged from propineb (46.63%) to propiconazole (70.23%). However, significantly maximum average disease intensity was recorded with Propineb fungicide. This was followed by the treatments *viz.*, Copper oxychloride Hexaconazole, Difenconazole, Tricyclazole, Tebuconazole, Azoxystrobin, Carbendazim (12%)+Mancozeb (63%) and Propiconazole.

Chapter-3

MATERIALS AND METHODS

An investigation was carried out on the determination of fungicide residues in chilli (*Capsicum annuum* L.) cultivar Kashmir long-1 in the Research Centre for Residue and Quality Analysis Laboratory of Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir (SKUAST-K), Shalimar, Srinagar during the year 2020. The experimental details related to study sites, materials used and methodology adopted for the studies are presented in the chapter under different heads.

3.1 Field trial

The seeds of chilli *Capsicum annuum*, variety Kashmir Long-1 were procured from Division of Vegetable Science Shalimar, SKUAST-Kashmir. Seeds were sown on 24th of April 2020 in nursery bed under poly-house condition. Before transplanting of seedlings, the field was ploughed and sufficient quantity of well decomposed farm yard manure and fertilisers were added into the soil. Plots of size 3×3 m were prepared and then seedlings were transplanted on 15th of June, 2020 at a spacing of 60×60 cm between rows and plants, respectively, as per good agronomic practices. The experiment was conducted in Randomised Complete Block Design (Plate-1).

3.2 Pesticide Application

Fungicides were applied at single and double dose on the chilli crop as mentioned in Table 1. Fungicides were sprayed on the crop thrice at 30 days interval. Control plots (no fungicides treatment) were treated with water only. The fungicides were sprayed using low volume hand sprayer to avoid the drifting of pesticides to adjoining plots. Both foliage and fruit of the plants were thoroughly covered with spray fluid to run-off stage. Spraying of fungicides were done on a clear day to avoid drifting of fungicides to adjoining plots. Also, application of lower concentration of fungicide prior to their respective higher concentration was

considered. The sprayer and measuring cylinder were washed thoroughly after each application to avoid carryover of fungicides from one treatment to another.

Table 1: Fungicides application doses and dates of application on the chilli crop

Fungicides	Single dose(%)	Double dose(%)	Date of 1 st application	Date of 2 nd application	Date of 3 rd application*
Flusilazole	0.02	0.04	17/07/2020	10/08/2020	14/09/2020
Tebuconazole	0.05	0.10	17/07/2020	10/08/2020	03/09/2020

(* Fruit sampling was done after 3rd application when plants were at full bearing stage.)

Table 2: Fungicides used for determination of residues in chilli

Common name	Trade name	Conc.	IUPAC name	Source	CRM (Certified Reference Material)
Flusilazole	Governer 40EC	0.02%	1- [Bis (4-fluorophenyl) (methylsilyl methyl)] 1H-1, 2, 4-triazole	Division Plant Pathology SKUAST-K	Sigma Aldrich
		0.04%			
Tebuconazole	Folicur 25.9EC	0.05%	1-p-chlorophenyl-4, 4-dimethyl-3-(1H-1, 2, 4-triazol-1-ylmethyl) pentan-3-ol	Division Plant Pathology SKUAST-K	Sigma Aldrich
		0.10%			



A. Experimental field layout



B. Field view at fruiting stage

Plate 1 : General view of Research Trial

3.3 Meteorological data

The dissipation of pesticide residues is largely depending upon the prevalent weather conditions of the region. Meteorological data on humidity, rainfall, temperature and evaporation during the field experiments were obtained from the division of Agronomy, SKUAST-Kashmir. The average maximum and minimum temperatures recorded during the sampling period (3rd September to 30th October) were $27.00 \pm 0.33^\circ\text{C}$ and $6.49 \pm 0.54^\circ\text{C}$. The average maximum and minimum relative humidity (%) recorded was 77.50 ± 0.83 and 41.68 ± 1.12 , respectively. Meteorological parameters from the day of pesticide application up to final sampling for the year 2020 is presented in appendix I. The temporal change in meteorological parameters during the investigation are depicted in Fig. 1.

3.4 Laboratory materials

The following chemicals (analytical grade), glassware and instruments were used to carry out the study:

3.4.1 Chemicals

1. Acetone (CH_3COCH_3), Genetix Biotech Asia Pvt. Ltd. Najafgarh Road, New Delhi
2. Acetonitrile (CH_3CN), Genetix Biotech Asia Pvt. Ltd. Najafgarh Road, New Delhi
3. Magnesium Sulphate (MgSO_4), Merck Specialities Pvt. Ltd. Worli, Mumbai
4. n-Hexane (C_6H_{14}), Genetix Biotech Asia Pvt. Ltd. Najafgarh Road, New Delhi
5. Primary Secondary Amines (PSA), Agilent Technologies, USA
6. Reference standards, Sigma Aldrich
7. Sodium Chloride (NaCl), Merck Specialities Pvt. Ltd. Worli, Mumbai

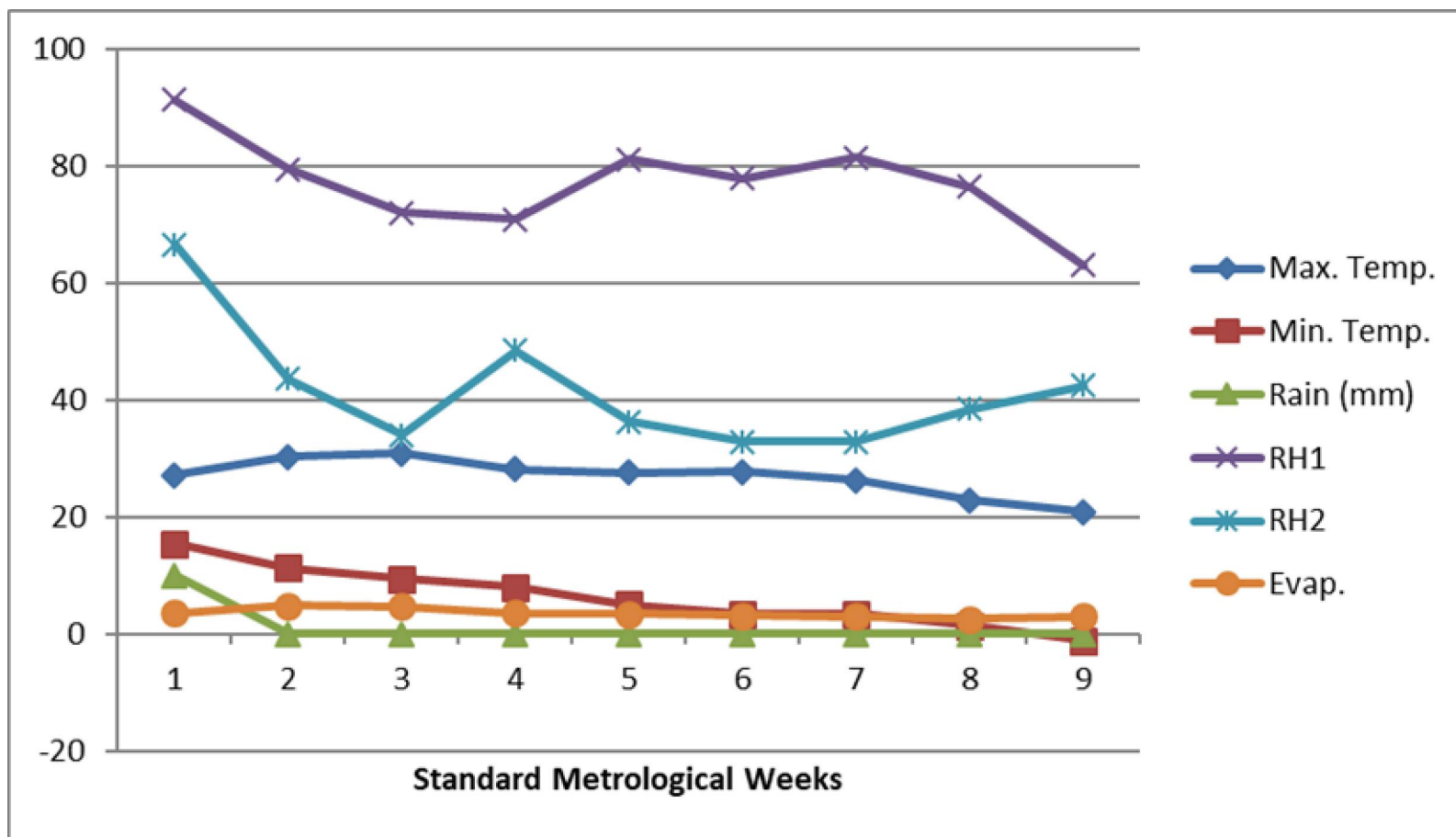


Fig. 1: The temporal change in weather parameters during the course of experiment.

8. Sodium Sulphate (Na_2SO_4), Merck Specialities Pvt. Ltd. Worli, Mumbai
9. Teepol, Merck Specialities Pvt. Ltd. Worli, Mumbai

3.4.2 Glassware and plastic wares

1. Beakers of 50 and 100 ml capacity, Borosil Glassworks Ltd., Delhi
2. Graduated test tubes with stoppers of 5 ml capacity, Borosil Glassworks Ltd., Delhi
3. Injection vials (2 ml), Agilent Technologies, USA
4. Ivory PTFE / red silicone rubber septa, Agilent Technologies, USA
5. Plastic stands for holding tubes, Tarson Products Pvt. Ltd., Kolkata
6. Polypropylene centrifuge tubes (50 and 15 ml), Tarson Products Pvt. Ltd., Kolkata
7. Tips of 1 and 5 ml capacity, Tarson Products Pvt. Ltd., Kolkata
8. Turbo tubes of 30 ml capacity, Borosil Glassworks Ltd., Delhi

3.4.2 Instruments

1. Auto Pipettes (1 and 5 ml): Tarson Products Pvt. Ltd., Subhash Road, Kolkata
2. Q-Sep 3000 centrifuge
3. Electronic balance: Mettler Toledo India Pvt. Ltd., Mumbai
4. Agilent gas chromatogram with ECD: Agilent technologies, USA.
5. Low volume high speed homogenizer: Heidolph, Germany
6. Robot Coupe high volume homogenize (Pro Scientific PRO 250): Heidolph, Germany
7. Philips mixer grinder: Philips India Ltd., Nalagarh, Baddi

8. Refrigerator: Godrej India Ltd., Mumbai
9. Spinix Test Tube Shaker: Tarson Products Pvt. Ltd., Subhash Road, Kolkata
10. Turbo Evaporator (Turbo Vap® LV), Caliper Life Sciences
11. Sonicator, PCI India Pvt. Ltd., Bhiwandi, Mumbai.

3.5 Sampling schedule

Samples of the fruit weighing 1 kg each were collected from all the treated plots in transparent polythene bags and taken to the laboratory for analysis. Samples were collected at the intervals of 0, 1, 3, 7, 10, 15, 30, 40, 50, 55, 60, 65 and 70 days after spray. Day zero samples were collected within 1 hours of spraying after the spray solution had properly dried up.

3.6 Extraction and clean-up using QuEChERS method

- Extraction and clean-up of chilli samples has been carried by QuEChERS method as described by Wani *et al.* (2019) for the estimation of flusilazole and tebuconazole residues in chilli fruits.
- Whole chilli fruit (1kg) was chopped and blended in a high-volume mixer grinder (Robotcoupe Blixer 6 V.V) for 3 minutes at 1500 rpm in the QuEChERS method.
- A 15g test sample was weighed in a 50-ml centrifuge tube before 30 ml of HPLC grade acetonitrile was poured into it.
- The samples were homogenized using high speed low volume homogenizer (Pro Scientific PRO 250) for 2-3 min at 15000-16000 rpm.
- To homogenise the sample and improve phase separation, around 3.0 ± 0.1 g sodium chloride (NaCl) was added. The contents were

centrifuged for 3 minutes at 2500-3000 rpm on a Q-Sep 3000 centrifuge.

- To remove moisture, an aliquot of 18 mL acetonitrile layer was transferred over 9.0 ± 0.1 g anhydrous sodium sulphate (Na_2SO_4) in a test tube.
- Dispersive solid phase extraction was used to clean and purify the acetonitrile extract (D-SPE). Precisely 11 mL acetonitrile aliquot was added to a test tube containing 1.15 g MgSO_4 and 0.4 g primary secondary amine (PSA), vortexed for 30.0 seconds, then centrifuged for 5.0 minutes at 3000 rpm.
- An aliquot of acetonitrile (6 mL) was evaporated to dryness at 30°C using a low-volume evaporator. For GC-MS analysis, the final volume was reconstituted with 3 mL hexane.
- The flusilazole and tebuconazole residues were identified and estimated using GC-MS (Plate 2).

3.7 Preparation of standard curves

Flusilazole and tebuconazole analytical grade reference standards were obtained from Sigma-Aldrich to prepare the standard curve. First, a stock solution of 400 ppm was prepared from certified reference material. From each stock solution, respective working solution of 40 ppm was prepared in 25 ml volumetric flask by transferring 6.25 ml from 400 ppm stock and then volume made-up by adding 18.75 ml of n-hexane. Then, 10 ppm solution was prepared by transferring 6.25ml from 40ppm and then volume made-up by adding 18.75ml of n-hexane. Then, 1ppm from 10ppm by transferring 2.5 ml from 10 ppm in 25 ml volumetric flask and volume made-up by adding 22.5 ml of n-hexane. From 1 ppm different concentrations *viz.*, 0.01, 0.02, 0.04, 0.06, 0.1 and 0.2 ppm were made to prepare the standard curve. Regression analysis on the data from Table 3 and 4 was done and depicted in Figure No. 2 and 3 for flusilazole and tebuconazole, respectively.

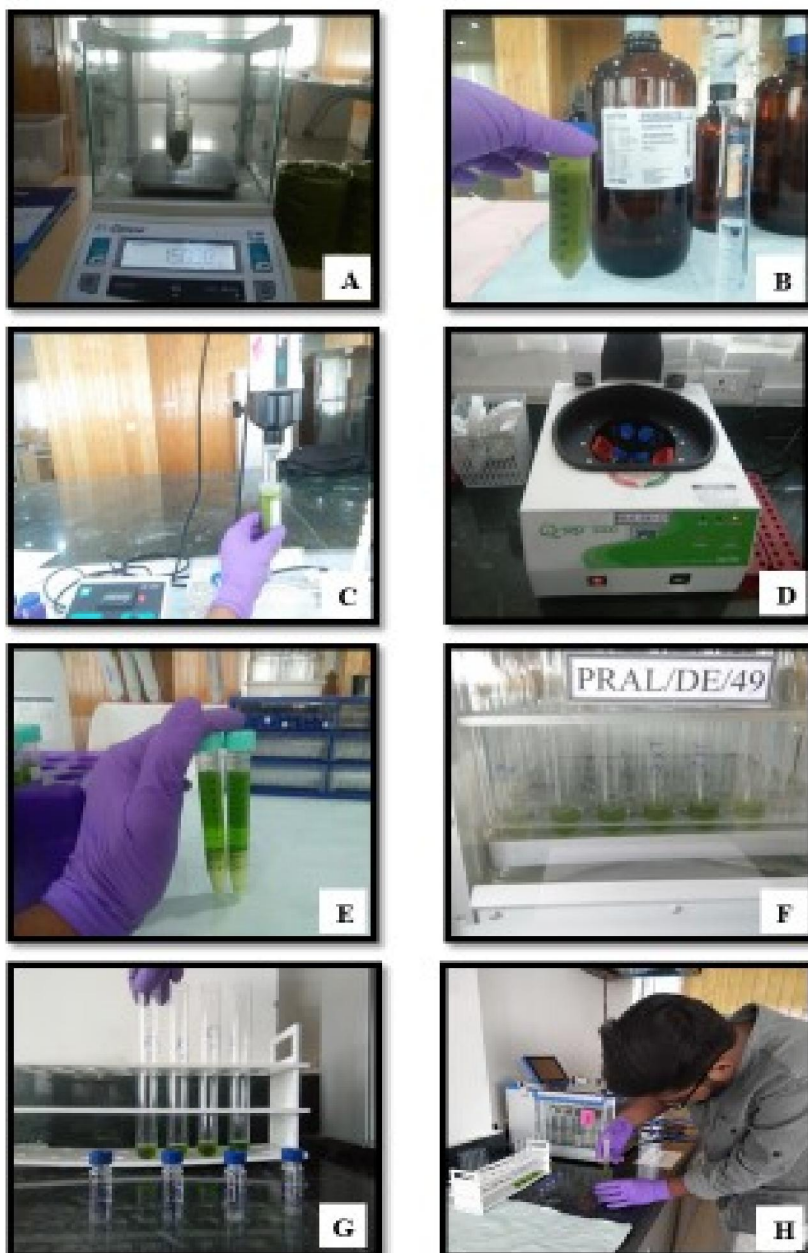


Plate 2: Sample extraction and clean-up. A) Ground Samples, B) Addition of ACN; C) Homogenising; D) Centrifuge the Sample; E) Addition of PSA & MgSO₄; F) Turbo-vap; G) After reconstitution, and H) Aliquot Transferring

Both the fungicides followed a linear relationship showing corresponding increase in respective fungicide concentrations. The coefficient of determination (R^2) for flusilazole and tebuconazole were 0.998 and 0.9974, respectively. Chromatogram of standard fungicide flusilazole and tebuconazole are shown in appendix II and III, respectively. (Percent Purity of Certified Reference Material: 99.9%)

Table 3: Standard curve of flusilazole

S. No.	Concentration (ppb)	Response
1	10	12770
2	20	21690
3	40	54714
4	60	162509
5	100	240384
6	200	308586

Table 4: Standard curve of tebuconazole

S. No.	Concentration (ppb)	Response
1	10	24537
2	20	46358
3	40	118556
4	60	183530
5	100	397804
6	200	485742

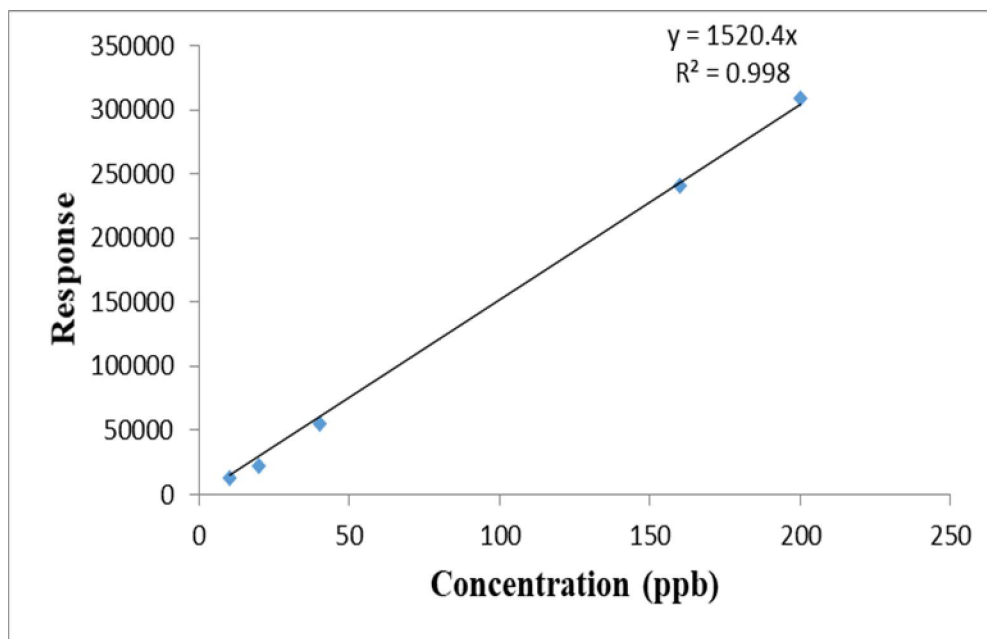


Fig. 2: Linearity curve for the estimation of flusilazole

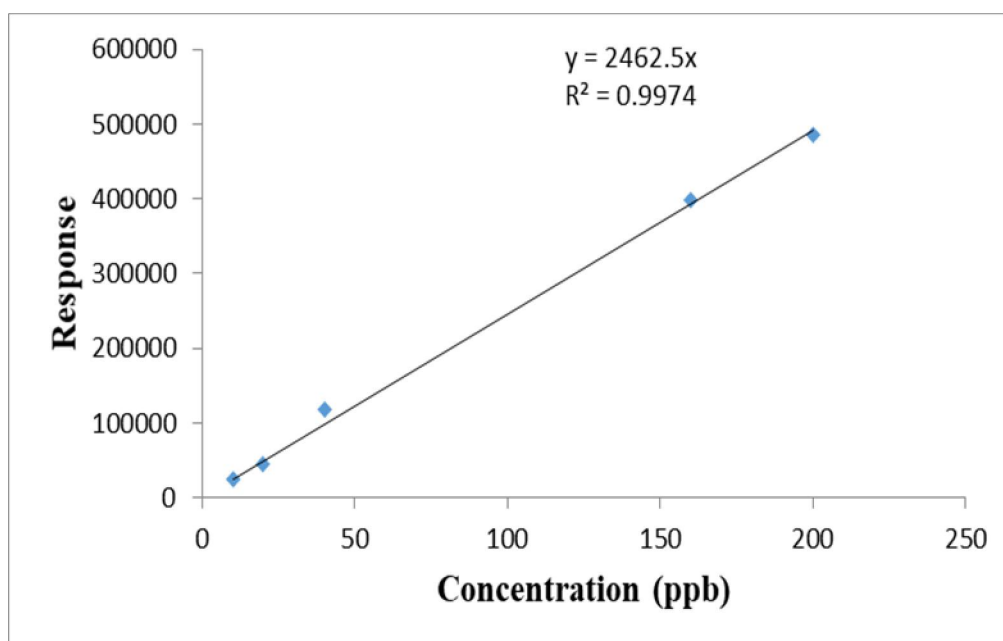


Fig. 3: Linearity curve for the estimation of tebuconazole

3.8 Recovery

The residues of flusilazole and tebuconazole in chilli fruits were analysed using Gas Chromatography Mass Spectroscopy (GC-MS). Before analysing the actual samples of fruits, the method's efficacy was tested in recovery experiments by spiking flusilazole and tebuconazole into untreated samples of fruits (taken from control plots). A 15 g well homogenized sample of chilli was spiked with known amount of Standard fungicide flusilazole at 0.1, 0.2, and 0.5mg kg⁻¹ and tebuconazole at 0.1, 0.2, and 0.5 mg kg⁻¹ concentrations. The samples were extracted, cleaned and assayed for each pesticide using the procedure previously described after 1hr. Tables 6 and 7 showed the recovery of pesticide residues of flusilazole and tebuconazole, respectively.

$$\text{Percent Recovery} = \frac{\text{Residue X 100}}{\text{Concentration of Standard}}$$

3.9 GC-MS parameters

Instrument	: Agilent 7890-GC, MS-7000D
Column	: Agilent HP-5 MS (30m × 0.25mm × 0.25µm)
Carrier gas	: Helium
Inlet temperature	: 280 °C
Injection mode	: Splitless
Injection volume	: 1 µml
Detector	: MS
Detector temperature	: 300 °C
Oven Programming	: (See Table 5)

3.9.1 Retention time and Limit of Determination

Name of pesticide	Retention time (minutes)	Limit of Determination (mg kg ⁻¹)
Flusilazole	23.591	0.02
Tebuconazole	26.469	0.05

Table 5: Oven Programming

Rate (°C)	Temperature (°C)	Hold time(min)
-	70 °C	2 min
25 °C	150 °C	0 min
3 °C	200 °C	0 min
8 °C	280 °C	10 min
Total run time- 41.867 min		

3.10 Residue assay

The residues of flusilazole and tebuconazole in ppm were computed by using following formula:

$$\text{Residue (ppm)} = \frac{\text{Peak Area (sample)} \times \mu\text{l of std injected} \times \text{Conc. of std} \times \text{final volume}}{\text{Peak Area (std.)} \times \mu\text{l of sample injected} \times \text{Wt. of sample}}$$

3.10.1 Residues Dissipation Rate

The per cent dissipation of the residue over the initial deposit was calculated for various sampling intervals as per the following mathematical formula.

$$\text{Percent dissipation (\%)} = 100 - \text{Residue (mg kg}^{-1}\text{) / Initial deposit (mg kg}^{-1}\text{)}$$

3.10.2 Half-life or T_{1/2}

Time needed for half of the applied pesticide to disappear from the substrate. The half-life value of fungicides as indices of the rates of residue dissipation was calculated as per Hoskins (1961).

$$T_{1/2} = (\mathbf{Log\ 2}) / \mathbf{b}$$

Where,

b = slope of regression equation

3.10.3 Waiting Period (T_{tol}):

Withholding periods (T_{tol}) based on the prescribed maximum residue limits (MRLs) by FASSAI, 2020 was worked out as indices of the safety to consumers.

$$T_{Tol} = \frac{\mathbf{Log\ K_2 - Log\ K_1}}{\mathbf{b}}$$

Where,

K₂ = Average Initial Residues (ppm)

K₁ = MRL

b = slope of regression equation

Chapter-4

EXPERIMENTAL FINDINGS

The findings of the study on "Residue Dynamics of Flusilazole and Tebuconazole on Chilli (*Capsicum annuum* L.) in Kashmir" are presented in this chapter.

4.1 Recovery studies

4.1.1 Recovery of flusilazole from fortified chilli fruits

The recovery of flusilazole was evaluated by spiking the chilli fruits at three fortification levels viz., 0.10, 0.20 and 0.50 mg kg⁻¹.

Table 6: Recovery of flusilazole from chilli fruit

Fortification Levels (mg/kg)	Average recovery (%) ± SD
0.10	77.00±0.002
0.20	99.5±0.004
0.50	90.4±0.007

4.1.2 Recovery of Tebuconazole from fortified chilli fruits

The recovery of tebuconazole was evaluated by spiking the chilli fruits at three fortification levels viz., 0.10, 0.20, 0.04 and 0.50 mg kg⁻¹.

Table 7: Recovery of tebuconazole from chilli fruit

Fortification Level (mg/kg)	Average recovery (%) ± SD
0.20	104.5±0.006
0.4	98.25±0.02
0.50	86.6±0.007

4.2 Initial deposits and persistence of fungicides(s) deposits

4.2.1 Flusilazole

The data on flusilazole (40EC) persistence and dissipation at 0.02 percent concentration is presented in Table 8 and depicted in Fig.4.

The initial deposit of flusilazole on chilli fruit at day zero (1hr) after application was found 1.713 ± 0.004 ppm. The appendix IV-VI shows chromatograms of Flusilazole 40 EC @ 0.02 percent at various intervals after treatment. With the passage of time, the residue of flusilazole decreased gradually and reduced to 0.937 ± 0.006 , 0.588 ± 0.007 , 0.372 ± 0.002 , 0.203 ± 0.005 and 0.011 ± 0.002 ppm on 1, 3, 5, 7 and 10 days, after application respectively. While the dissipation percentage of 45.30, 65.67, 78.28, 88.14 and 99.35 was observed in 1, 3, 5, 7 and 10 days, respectively. It further observed that the pesticide was not detectable after 15 days of application, indicating that it had either degraded below the detection limit or had completely dissipated in that time. The residues dissipated with a half-life period ($T_{1/2}$) of 1.55 days. The waiting period ($T_{tol.}$) of 15.19 days was worked out on the basis of MRL of 0.01 ppm for flusilazole on chilli.

Table 8: Dissipation of flusilazole 40 EC in chilli fruit variety Kashmir Long-1 at recommended concentration of 0.02 percent

Days after treatment (X)	Residues (ppm) \pm S.D.	Dissipation (%)	$T_{1/2}$	T_{tol}
0	1.713 ± 0.004	0.00	1.55	8.39
1	0.937 ± 0.006	45.30		
3	0.588 ± 0.007	65.67		
5	0.372 ± 0.002	78.28		
7	0.203 ± 0.005	88.14		
10	0.011 ± 0.002	99.35		
15	BDL	-		
$Y = 1.273 - 0.147X$				
$R^2 = 0.809$				

The data on flusilazole (40EC) persistence and dissipation at 0.04 percent concentration is presented in Table 9 and depicted in Fig.4.

The initial deposit of flusilazole on chilli fruit at day zero (1hr) after application was found 2.634±0.004 ppm. The appendix VII-X shows chromatograms of Flusilazole 40 EC @ 0.04 percent at various intervals after treatment. With the passage of time, the residue decreased gradually and reduced to 2.164±0.004, 1.723±0.002, 0.987±0.006, 0.765±0.004, 0.578±0.007, 0.249±0.006, 0.014±0.003 ppm on 1, 3, 5, 7, 10, 15 and 20 days after application, respectively. The dissipation percentage of 17.84, 34.58, 62.52, 76.95, 78.05, 90.54 and 99.46 was observed on 1, 3, 5, 7, 10, 15 and 20 days, respectively. The pesticide was not detectable after 25 days, indicating that it had either degraded below the detection limit or had completely dissipated in that time. The residues when applied at a concentration of 0.04 percent dissipated with a half-life period ($T_{1/2}$) of 3.01 days. The waiting period (T_{tol}) of 19.83 days was worked out on the basis of MRL of 0.01 ppm for flusilazole on chilli.

Table 9: Dissipation of flusilazole 40 EC in chilli fruit variety Kashmir Long-1 at double the recommended concentration of 0.04 percent

Days after treatment (2X)	Residues (ppm) ± S.D.	Dissipation (%)	$T_{1/2}$	T_{tol}
0	2.634±0.004	0.00	3.01	11.61
1	2.164±0.004	17.84		
3	1.723±0.002	34.58		
5	0.987±0.006	62.52		
7	0.765±0.004	70.95		
10	0.578±0.007	78.05		
15	0.249±0.006	90.54		
20	0.014±0.003	99.46		
25	BDL	BDL		
Y=2.07 – 0.122X				
R²= 0.835				

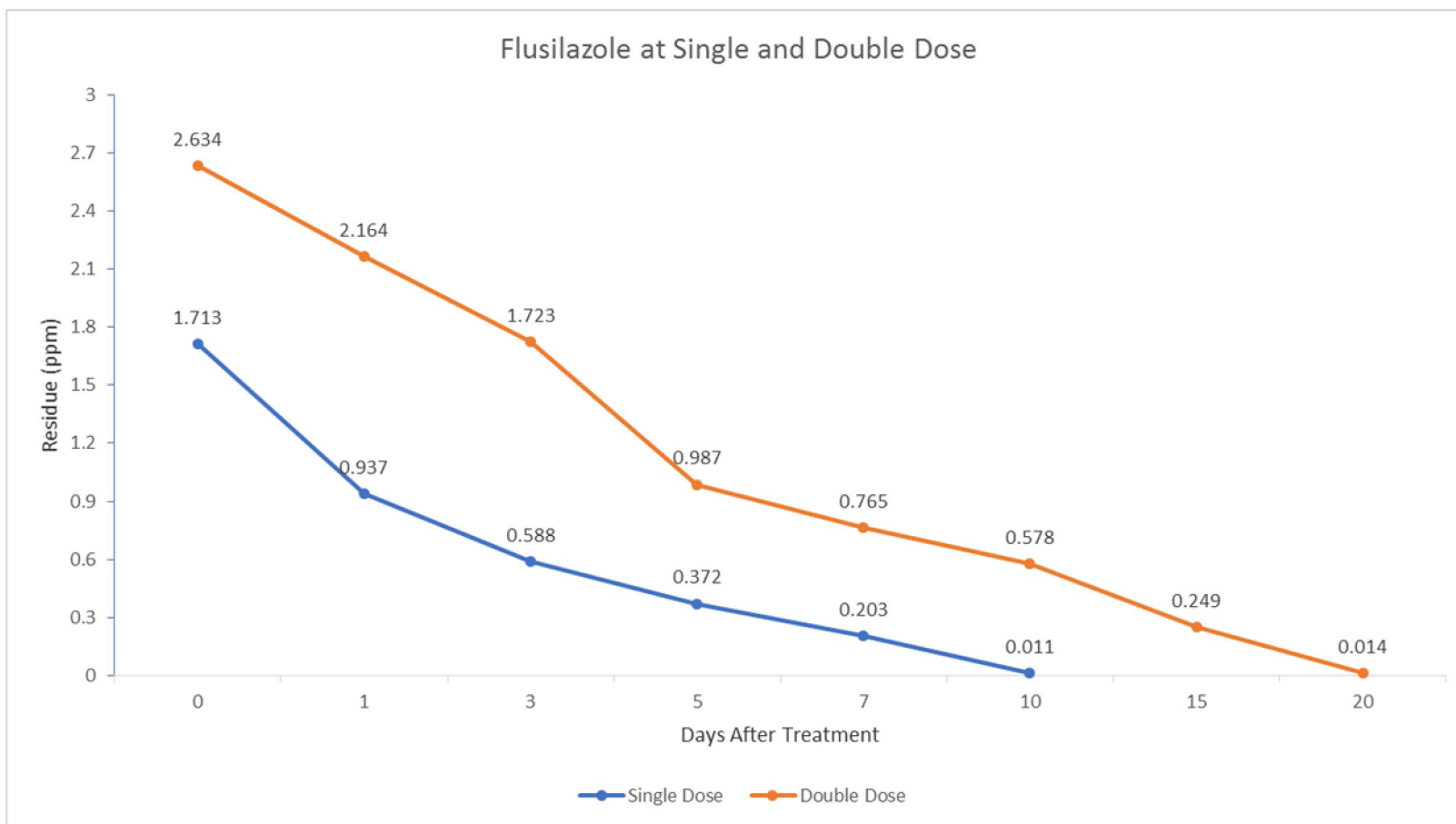


Fig. 4: Average flusilazole 40EC residues in chilli fruit cv. Kashmir Long-1 with days after treatment at the recommended concentration of 0.02 percent and at double the concentration of 0.04 percent

4.2.2 Tebuconazole

The data on tebuconazole (25.9EC) persistence and dissipation at 0.05 percent concentration is presented in Table 10 and depicted in Fig.5.

The initial deposit of tebuconazole on chilli fruit at day zero(1hr) after application was found 1.909±0.004 ppm. The appendix XI-XIII shows chromatograms of Tebuconazole 25.9EC @ 0.05 percent at various intervals after treatment. With the passage of time, the residue decreased gradually and reduced to 1.579±0.008, 1.279±0.006, 0.889±0.004, 0.576±0.005 and 0.224±0.003 ppm in 1, 3, 5, 7 and 10 days, after application respectively. The dissipation percentage of 17.28, 33.00, 53.43, 69.82 and 88.26 was observed in 1, 3, 5, 7 and 10 days, respectively. The pesticide was not detectable after 15 days, indicating that it had either degraded below the detection limit or had completely dissipated in that time. The residues had been found dissipated with a half-life period ($T_{1/2}$) of 3.37 days. The waiting period ($T_{tol.}$) of 4.07 days was worked out on the basis of MRL of 0.4 ppm for tebuconazole on chilli.

Table 10: Dissipation of tebuconazole 25.9 EC in chilli fruit variety Kashmir Long-1 at recommended concentration of 0.05 percent

Days after treatment (X)	Residues (ppm) ± S.D.	Dissipation (%)	$T_{1/2}$	T_{tol}
0	1.909±0.004	0.00	3.37	4.07
1	1.579±0.008	17.28		
3	1.279±0.006	33.00		
5	0.889±0.004	53.43		
7	0.576±0.005	69.82		
10	0.224±0.003	88.26		
15	BDL	BDL		
Y= 1.79 – 0.166X				
R²= 0.983				

The data on tebuconazole (25.9EC) persistence and dissipation at 0.10 percent concentration is presented in Table 11 and depicted in Fig.5.

The initial deposit of tebuconazole on chilli fruit at day zero after application was found 2.666 ± 0.020 ppm. The appendix XIV-XVIII shows chromatograms of Tebuconazole 25.9EC @ 0.10 percent at various intervals after treatment. With the passage of time, the residue decreased gradually and reduced to 1.989 ± 0.008 , 1.795 ± 0.004 , 0.989 ± 0.004 , 0.942 ± 0.004 , 0.689 ± 0.006 , 0.586 ± 0.005 , 0.333 ± 0.002 and 0.256 ± 0.005 ppm in 1, 3, 5, 7, 10, 15, 20 and 25 respectively. The dissipation percentage of 25, 32, 62, 64, 74, 78, 87 and 90 was observed in 1, 3, 5, 7, 10, 15, 20 and 25 days, respectively. The pesticide was not detectable after 30 days, indicating that it had either degraded below the detection limit or had completely dissipated in that time. The residues dissipated with a half-life period ($T_{1/2}$) of 7.76 days. The waiting period ($T_{tol.}$) of 8.13 days was worked out on the basis of MRL of 0.4 ppm for tebuconazole on chilli.

Table 11: Dissipation of tebuconazole 25.9EC in chilli fruit variety Kashmir Long-1 at recommended concentration of 0.10 per cent

Days after treatment (2X)	Residues (ppm) \pm S.D	Dissipation (%)	$T_{1/2}$	T_{tol}
0	2.666 ± 0.020	0.00	7.76	8.13
1	1.989 ± 0.008	25.39		
3	1.795 ± 0.004	32.67		
5	0.989 ± 0.004	62.90		
7	0.942 ± 0.004	64.66		
10	0.689 ± 0.006	74.15		
15	0.586 ± 0.005	78.01		
20	* 0.333 ± 0.002	87.50		
25	0.253 ± 0.005	90.05		
30	BDL	BDL		
$Y=1.923 - 0.082X$				
$R^2=0.752$				

* $Y= 2.0228 - 0.1015X$ and $R^2=0.769$ for calculation of Waiting period (T_{tol})

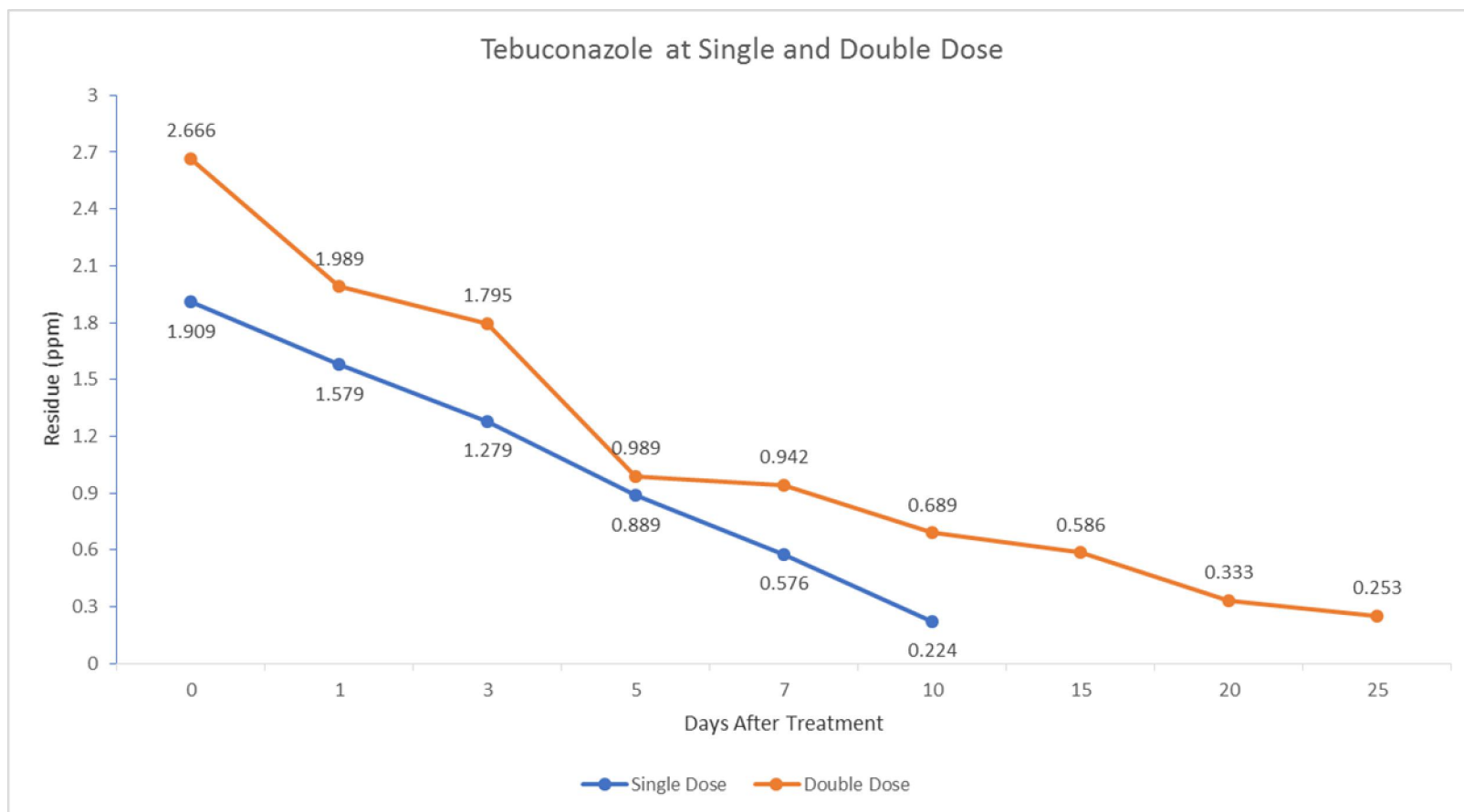


Fig. 5: Average tebuconazole 25.9EC residues in chilli fruit *cv.* Kahmir Long-1 with days after treatment at the recommended concentration of 0.05 percent and at a double concentration of 0.10 percent

4.3 Statistical constant of pesticides

The statistical constants obtained due to the analysis of pesticides persistence data after spray application on chilli crop are given in Table 12.

Table 12: Statistical constants of test pesticides in green chilli fruits

Pesticides	Dose(%)	Statistical Constant			
		Regression equation ($y = a + bx$)	Correlation coefficient (R^2)	Half-life ($T_{1/2}$)	Waiting period (T_{tol})*
Flusilazole	0.02	$Y = 1.273 - 0.147X$	0.809	1.55	15.19
	0.04	$Y = 2.07 - 0.122X$	0.835	3.01	19.83
Tebuconazole	0.05	$Y = 1.79 - 0.166X$	0.983	3.37	4.07
	0.10	$Y = 2.0228 - 0.101X$	0.769	7.76	8.13

* Waiting period calculated at FSSAI MRLs: Flusilazole: 0.01 mg kg^{-1} and Tebuconazole: 0.4 mg kg^{-1} (FSSAI, 2020)

Chapter-5

DISCUSSION

Pesticides used carelessly and indiscriminately on crops result in undesirable residues in food, posing serious health risks. The amount of pesticide residues on crops and their toxicity determines the severity of the threat. Because most pesticides are hazardous by nature, their unrelenting uptake by humans, even at little amounts, can result in a build-up in human tissues, causing serious health issues (Handa *et al.*, 1999). It is not possible to totally eliminate the usage of fungicides due to the food losses caused by fungi. However, the use of fungicides in general and the timing of treatment in particular, may be carefully monitored to ensure that their residues food are kept to a minimum, making it safe for human consumption and the environment. The current study looked into the dissipation of two commonly used fungicides, flusilazole and tebuconazole, on chilli fruits. The current study's investigational findings are briefly summarised as follows:

5.1 Recovery studies

5.1.1 Recovery of flusilazole from fortified chilli fruits

The recovery of flusilazole from fortified chilli fruits was between 77-99.5 percent (Table-5). The work conducted by Jeong *et al.* (2016) provides a conformity to our results who achieved similar trend of recoveries ranging from 75.7-77.6 percent from lettuce which were spiked at 0.2 to 1.0 mg kg⁻¹ fortification level. Similar trend of recovery has been reported by Malhat *et al.* (2015) who recovered mean per cent recoveries more than 80 percent from grapes which were spiked at 0.02, 0.05 and 1.0 µg g⁻¹ fortification level. Chen *et al.* (2002) observed 99.5, 107.7 and 99.4 percent recovery of flusilazole from grapes at 0.005, 0.01 and 0.1 mg kg⁻¹ fortification level, respectively. Kyung *et al.* (2017)

recovered 75.6-87.1 percent flusilazole from fortified tomatoes which were spiked at 0.05 and 0.25 mg kg⁻¹ fortification levels.

5.1.2 Recovery of tebuconazole from fortified chilli fruits

The recovery of tebuconazole from fortified chilli fruits was between 72-104.5% (Table-6). The work conducted by Sahoo *et al.* (2012) provides a conformity to our results who achieved similar trend of recoveries ranging from 86.90-89.33 percent from chilli which were spiked at 0.20 and 0.50 mg kg⁻¹ fortification level. Similar trend of recovery has been reported by Saha *et al.* (2016) who recovered 89.5-91.4 percent tebuconazole from chilli which were spiked at 0.25 and 0.05 mg kg⁻¹ fortification level. Dhanker (2017) observed 91.02-93.84 percent recovery of tebuconazole from chilli at 0.10 and 0.25 mg kg⁻¹ fortification level. Dubey *et al.* (2020) recovered 93.80-100.36 percent tebuconazole from fortified chillies which were spiked at 0.10, 0.50 and 1.00 mg kg⁻¹ fortification levels.

5.2 Initial deposits and persistence of fungicides(s) deposits

Initial deposits of a pesticide depend upon a number of factors like concentration, formulation, weather conditions, substrate characteristics, type of sprayer used, distance between the nozzle and plant surface and properties of carrier etc. Besides these factors, plant type (erect or prostrate), shape of plant parts (broad, narrow or linear) and growth of plant parts (slow or fast) influence the deposit of pesticide on plant surface (Ebling, 1963) whereas the dissipation of pesticides depends upon crop factors like stage of crop, rind of fruit, internal material, weather factors like moisture, temperature and rain. The residue data from test pesticides on chilli fruits indicated that initially there was a rapid loss of pesticide followed by slower rate of dissipation. Keeping most of these factors constant the discussion will be around the deposits and dissipation due to the levels of pesticides.

5.2.1 Flusilazole

5.2.1.1 Initial deposits

The average initial deposits of flusilazole were 1.713 mg kg⁻¹ and 2.634 mg kg⁻¹ on chilli fruits due to application of flusilazole at the rate of 0.02 and 0.04 percent on chilli crop, respectively. Sreeja (2014) reported an initial deposit of 0.61 mg kg⁻¹ at the application rate of 0.1 percent. Malhat *et al.* (2015) observed the initial total residues of flusilazole in mandarin fruit sample were 0.476 mg kg⁻¹ at recommended rate of 0.4 percent and 0.920 mg kg⁻¹ at double the recommended rate of 0.8 percent. Chen *et al.* (2002) observed 0.39 and 0.42 mg kg⁻¹ at the application rate of 150 mg a.i kg⁻¹ from two different sites. Jeong *et al.* (2016) observed 2.01 and 2.31 mg kg⁻¹ average initial deposits of flusilazole on lettuce at the application rate of 1.0 and 2.0 percent, respectively.

5.2.1.2 Persistence of flusilazole in chilli fruits

The data on persistence of flusilazole 40EC at 0.02 and 0.04 percent on chilli fruits is presented in Table 7 and 8, respectively. The result of present findings showed a close proximity to those obtained by Jeong *et al.* (2016) who showed the initial deposits (2.01 mg kg⁻¹) of flusilazole on lettuce dissipated to 89.00 percent at the seventh day at recommended dose of 1ml L⁻¹. Sreeja *et al.* (2014) observed the initial deposit of flusilazole was 0.61 mg kg⁻¹ on cowpea at the application rate of 0.1 percent and dissipated to below MRL (0.02 mg kg⁻¹) on tenth day. Malhat *et al.* (2015) observed that the average initial deposit of flusilazole were 0.476 and 0.920 mg kg⁻¹ at single and double dose, respectively, both dissipated by more than 80 percent in nine days after application. Kyung *et al.* (2017) reported an initial deposit of 0.044 mg kg⁻¹ on tomato which dissipated by 59.13 percent to 0.018 mg kg⁻¹ (below MRL) on seventh day.

5.2.2 Tebuconazole

5.2.2.1 Initial deposit

The average initial deposits of tebuconazole were 1.909 mg kg⁻¹ and 2.666 mg kg⁻¹ on chilli fruits due to application of tebuconazole at the rate of 0.05 and 0.1 percent on chilli crop. Sahoo *et al.* (2012) observed the initial total residues of tebuconazole in chilli fruit sample were 0.950 and 1.880 mg kg⁻¹ at recommended rate (125 g a.i. ha⁻¹) and double the recommended dose (250 g a.i. ha⁻¹), respectively. The result of present findings showed a close proximity to those obtained by Dubey *et al.* (2020) who observed 1.760 and 2.784 mg kg⁻¹ initial deposit of tebuconazole on chilli fruit following its application at the rate of 215 and 430 g a.i. ha⁻¹, respectively. Dhanker (2017) observed that the initial deposit of tebuconazole on chilli fruit sample were 0.21 and 0.44 mg kg⁻¹ at recommended rate of 125 g a.i. ha⁻¹ and double the recommended dosage 250 g a.i. ha⁻¹, respectively. Saha *et al.* (2016) observed the initial deposit of tebuconazole on chilli fruits as 0.569 and 0.620 mg kg⁻¹ following its application rate of 100 and 200 g a.i. ha⁻¹, respectively. Mohapatra (2015) observed the initial deposits of tebuconazole on gherkin fruits as 0.842 and 1.682 mg kg⁻¹ at the application rate of 150 and 300 g a.i. ha⁻¹ of tebuconazole (Nativo 75 WG). Sreeja (2014) reported an initial deposit of 0.61mg kg⁻¹ at the application rate of 0.1 percent.

5.2.2.2 Persistence of tebuconazole on chilli fruits

The data on persistence of tebuconazole 25.9EC at 0.05 and 0.1 percent on chilli fruits is presented in Table 9 and 10, respectively. Dhanker (2017) observed that tebuconazole initial deposits of 0.21 and 0.44 mg kg⁻¹ at the application rate of 125 and 230 g a.i. ha⁻¹, dissipated to 95.23 and 95.45 percent after 7 and 10

days, respectively. Paramasivam *et al.* (2015) observed that tebuconazole initial deposits of 0.67 and 1.29 mg kg⁻¹ at the application rate of 150 and 300 g a.i. ha⁻¹, dissipated to 58.78 and 50.22 percent after 5 days of treatment, respectively. Sreeja *et al.* (2014) reported an initial deposit of tebuconazole on cowpea 0.56 mg kg⁻¹ which dissipated by 96.43 percent in 7 days. Sahoo *et al.* (2016) observed that the average initial deposit of tebuconazole on chilli fruits were 0.95 and 1.88 mg kg⁻¹ at 100 and 200 g a.i. ha⁻¹, dissipated to 87.37 and 95.21 percent after 5 and 7 days, respectively. The result of present findings showed a close proximity to those obtained by Dubey *et al.* (2020) who showed the initial deposit 1.760 and 2.784 mg kg⁻¹ of tebuconazole on chilli fruits at application rate of 215 and 430 g a.i. ha⁻¹, dissipated to 96.42 and 97.44 percent after 7 days and 10 days, respectively

5.3 Statistical constant of pesticides

5.3.1 Half-life values and Waiting Period

The statistical constants obtained due to the analysis of pesticides persistence data after fungicide application on chilli crop are given in Table 11.

Residue half-life of a pesticide is the time taken by initial deposits to decrease its amount by half. Half-life values are influenced by the volatility of the compounds, initial deposits, growth and physiology of plants, weather conditions, oxidation, reduction and hydrolytic biodegradation factors.

The waiting period of pesticides on chilli was calculated on the basis of FSSAI MRLs. FSSAI MRLs for flusilazole and tebuconazole is 0.01 and 0.4 mg kg⁻¹ on chilli fruits, respectively (FSSAI, 2020).

5.3.1.1 Flusilazole

The half-life ($T_{1/2}$) values of flusilazole deposits on chilli fruits at single and double doses were 1.55 and 3.01 days and a waiting period of 15.19 and 19.83 days, respectively. According to the findings of Sharma, D. and Choudhury, P. P. (2018) the waiting period of flusilazole 40EC in chilli is 5 days. Also, according

to the study conducted by Sreeja *et al.* (2014), the half-life value and waiting period of flusilazole on cowpea were 1.5 days and 8.53 days, respectively. Chen *et al.* (2002) observed that half-life of flusilazole on grapes at two different doses varied between 6.3 to 8.4 days and observed a waiting period of 14 days. Jeong *et al.* (2016) reported half-life period for flusilazole as 2.3 and 2.4 days at recommended and double the recommended dosage, respectively in lettuce and a waiting period of 7 days. Malhat *et al.* (2015) reported the half-life period of flusilazole on grapes for both treatments was 4.5 days.

5.3.1.2 Tebuconazole

The half-life ($T_{1/2}$) values of tebuconazole deposits on chilli fruits at single and double doses were 3.37 and 7.76 days and a waiting period of 4.07 and 8.13 days, respectively. The result of present findings showed a close proximity to those obtained by Dubey *et al.* (2020) who has reported that the half-life of tebuconazole on chilli fruits were 1.50 and 1.80 days at single and double doses, respectively and a waiting period of 7.339 days. Sahoo *et al.* (2012) observed that the half-life periods for tebuconazole on chilli fruits were found to be 1.37 and 1.41 days at single and double doses, respectively. Also, Dhanker (2017) reported that the half-life value of tebuconazole on chilli fruits ranged between 1.61-2.12 days at single and double doses. Mohapatra (2015) also suggested 14 days safe Pre-Harvest Interval (PHI) for gherkin fruit consumption after treatment of Nativo 75WG at the standard and double doses, respectively. Paramasivam *et al.* (2015) observed that half-life of tebuconazole on gherkin fruits at two different doses varied between 3.0 to 3.04 days. However, Saha *et al.* (2016) investigated the safety parameters of a fluopyram-tebuconazole combination product in chilli and found that the first deposits were near to MRL at both application rates. Whereas Sreeja *et al.* (2014) reported that the half-life of tebuconazole on cowpea was 3.5 days and a waiting period of zero days.

Chapter-6

SUMMARY AND CONCLUSION

The investigations entitled "**Residue Dynamics of Flusilazole and Tebuconazole on Chilli (*Capsicum annuum* L.) in Kashmir,**" was carried out at Research Centre for Residue and Quality Analysis Laboratory of Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir (SKUAST-K), Shalimar, Srinagar during the year 2020. based upon the data, the results are summarised below:

- Chilli Kashmir Long-1 variety was sprayed with, flusilazole 40 EC at recommended dose (0.02%) and double the recommended dose (0.04%) and tebuconazole 25.9EC at recommended dose (0.05%) and double the recommended dose (0.10%). Fruit samples were taken at 0 (1hr), 1, 3, 5, 7, 10, 15, 20, 25 and 30 days after application. Standard techniques were used to extract and clean the samples of fruit for estimation of residues. Gas Chromatography Mass Spectroscopy (GC-MS) was used to examine residue of flusilazole 40EC and tebuconazole 25.9EC.
- Mean percent recovery of pesticides from fruit samples fortified at three concentrations were 88.96 percent and 87.70 percent for flusilazole and tebuconazole, respectively.
- Flusilazole left an initial deposit of 1.173 ppm at the recommended dose (0.02%), which dissipated by 99 percent in 10 days and was undetectable after that. The half-life duration ($T_{1/2}$) and waiting period ($T_{tol.}$) was 1.55 and 15.19 days, respectively.

- Flusilazole left an initial deposit of 2.634 ppm at double the recommended dose (0.04%) which dissipated by 99.46 percent in 20 days and was non-detectable after that. The half-life period ($T_{1/2}$) and waiting period ($T_{tol.}$) was 3.01 days and 19.83 days were recorded, respectively.
- Tebuconazole left an initial deposit of 1.909 ppm at recommended dose (0.05%) which dissipated by 88 percent in 10 days and was undetectable after that. The half-life period ($T_{1/2}$) and waiting period ($T_{tol.}$) of 3.37 days and 4.07 days were recorded, respectively.
- Tebuconazole left an initial deposit of 2.666 ppm at double the recommended dose (0.10%) which dissipated by 90 percent in 25 days and was undetectable after that. The half-life period ($T_{1/2}$) and waiting period ($T_{tol.}$) of 7.76 days and 8.13 days were recorded, respectively.

CONCLUSIONS

The two fungicides *viz.*, flusilazole and tebuconazole used judiciously at recommended doses and time, residue free fruit safe for consumption can be produced.

- Flusilazole persisted up to 10 and 20 days at single and double dose, respectively. Tebuconazole persisted for 10 and 25 days at single and double dose, respectively in temperate condition of Srinagar.
- Application of flusilazole 40 EC should be given before flowering to allow the residues to dissipate below the MRLs at harvest. The Pre-Harvest Interval (PHI) when sprayed at recommended dose could be 15.19 days between the last application of the formulation and the harvest of chilli, in temperate condition of Srinagar. The results would be valuable for the safe use of flusilazole and to prevent any health problem to consumers.
- When sprayed at a recommended concentration, tebuconazole ensures

consumer safety when applied significantly prior to harvest. When applied at recommended concentration, the pre-harvest gap might be 4.07 days between the last treatment of the formulation and the chilli harvest.

LITERATURE CITED

- Ahmed, N. S. 2005. Residual behaviour of flusilazole and triflumizole fungicides on and in apple fruits under field conditions. *Arab Universities Journal of Agricultural Sciences* **13**(3): 997-1004.
- Anand, A., Gupta, H. B. and Chourasia, H. K. (2020). Management of anthracnose of red chilli caused by *Colletotrichum capsaci*. *Annals of Plant and Soil Research* **22**(4): 390-395.
- Anastassiades, M., Lehotay, S. J., Stajnbaher, D. and Schenck, F. J. 2003. Fast and easy multi residue method employing acetonitrile extraction partitioning and dispersive solid phase extraction for the determination of pesticide residues in produce. *Journal of Association of official Agricultural Chemists International* **86**: 412-131.
- Angioni, A., Aguilera, A., Russo, M., Melis, M., Cabitza, F. and Cabras, P. 2003. Triazole fungicide degradation in peaches in the field and in model systems. *Food additives and contaminants* **20**: 368-374.
- Anonymous, 2019. Area and Production of Chilli. Department of Agriculture. Jammu and Kashmir (UT).
- Badgujar, L. S., Dhoke, K. P., Shiradkar J. M. and Jagdhane, S. 2020. To assess the effectiveness of fungicides against anthracnose disease of chilli *in-vivo*. *International Journal of Chemical Studies* **9**(1): 3574-3577
- Barrett, B. K. and Dixon, G. 1995. Ergosterol biosynthesis inhibition: a target for antifungal agents. *Acta Biochimica Polonica* **42**(4): 465-79.
- Begum, S., Yumlembam, R. A., Marak, T. R. and Nath, P. S. 2015. Integrated management of anthracnose of chilli caused by *Colletotrichum capsaci* in West Bengal condition. *Bioscan* **10**: 1901-1904.

- Blasco, C., Font, G. and Pico, Y. 2006. Evaluation of 10 pesticide residues in oranges and tangerines from Valencia (Spain). *Food Control* **17**: 841–846.
- Boobis, A. R., Ossendorp, B. C., Banasiak, U., Hamey, P. Y., Sebestyen, I. and Moretto, A. 2008. Cumulative risk assessment of pesticide residues in food. *Toxicology Letters* **180**: 137–150.
- Chau, N. D. G., Son, L. L. and Hop, N. V. 2020. Dissipation of the pesticides fipronil, cypermethrin and tebuconazole in vegetables: A case study in Thua Thien-Hue province, Central Vietnam. *Journal of Pesticide Science* **45**(4): 245-252.
- Chen, L., Chen, J. M. and Xia, F. L. 2002. Residue analysis method of flusilazole in grape and soil by HPLC. *Journal of Agro-Environmental Protection* **21**: 150-152.
- Dhanker, P. 2017. Persistence of tebuconazole in chilli and soil. M. Sc. Thesis. Department of Biochemistry, Haryana Agricultural University, Hisar-125004. Haryana, India.
- Dong, B. and Hu, J. 2014. Dissipation and residue determination of fluopyram and tebuconazole Residues in watermelon and soil by GC-MS. *International Journal of Environmental Analytical Chemistry* **94**(5): 493-505.
- Dubey, J. K., Patyal, S. K., Katna, S., Shandil, D., Devi, N., Singh, G. and Singh, G. 2020. Persistence and dissipation kinetics of tebuconazole in apple, tomato, chilli and onion crops of Himachal Pradesh, India. *Environmental Science and Pollution Research* **27**: 11290-11302
- Ebling, W. 1963. Dissipation, degradation, persistence and effectiveness of pesticides. *Residue Reviews* **3**: 35-163.

- FAO/WHO. (2000). Pesticide residues in food-1999 evaluations. Part II-toxicological. Joint FAO/WHO Meeting on Pesticide Residues. World Health Organization, Geneva
- FSSAI, 2020. www. fssai. gov. in. (19/08/2020).
- Garland, S. M., Menary, R. C. and Davies, N. W. 1999. Dissipation of propiconazole and tebuconazole in peppermint crops (*Mentha piperita* (Labiatae)) and their residues in distilled oils. *Journal Agricultural and Food Chemistry* **47**(1): 294-298.
- Golakiya, B. B., Bhimani, M. D. and Akbari, L. F. 2018. Efficacy of different fungicides for the management of chickpea wilt (*Fusarium oxysporum* f. sp. *ciceri*). *International Journal of Chemical Studies* **6**(2): 199-205.
- Hafiz, P. A. 2016. Efficacy of bioagents and fungitoxicants against *Colletotrichum capsaci* (Syd.) Butler & Bibsy causing Anthracnose of chilli. M. Sc. Thesis. Division of Plant Pathology, SKUAST-K, Srinagar-190025. Jammu and Kashmir, India.
- Hallenbeck, W. H and Cunningham, K. M. 1985. *Pesticides and Human Health* pp 06-20. In: Springer-Verlag, Berlin.
- Handa, S. K., Agnihotri, N. P. and Kulshrestha, G. 1999. Pesticide residues. Significance, Management and Analysis, Research Periodicals and Book Publishing Home, Texas, USA.
- Hoskin, W. M. 1961. Mathematical treatment of the rate of loss of pesticides residues. *Plant Protection Bulletin* FAO **9**(9): 163-168.
- Huatao, J., Xiangwei, Y., Cheng, S., Guangyu, Z., Xiuguo, W. and Yiqiang, L. 2018. Degradable residues characterization and possible MRL setting for

albendazole, flusilazole and imazalil in tobacco. *CORESTA Congress*, Kunming, China.

Jain, S., Singh, A. and Kaur, Y. 2017. Relative efficacy of triazole, dithiocarbamate and chloronitrile fungicides against fruit rot of chilli. *Plant disease Research* **31**(2): 142-145.

Jat, S. L. 2015. Detection of *Colletotrichum capsaci* (Syd.) Butler and Bibsy causing Die-back of chilli and its management. M. Sc. Thesis. Department of Plant Pathology, S. K. N. College of Agriculture, Jobner-303329. Rajasthan, India

Jeong, S. I., Rahman., Musfiqur, M., Aty, E. A., Kim, M., Woo, S., Humayun, K., Waziha, L., Lee, T., Jung, J. Y., Choi, D., Shin, H. J., Im, C. H., Hong, J. G., Shim, M. S. and Han, J. 2016. Simultaneous detection of fluquinconazole and flusilazole in lettuce using gas chromatography with a nitrogen phosphorus detector: decline patterns at two different locations. *Biomedical Chromatography* **30**(6): 946–952.

Jianlu, Q. U., Haibin, W. U., Kun, F., Yugang, S. U. N., Jun, L. I. and Jihan, T. A. O. 2012. Biological characteristics of *Colletotrichum gloeosporioides* and inhibitory effects of several fungicides. *Chinese Journal of Pesticide Science* **14**(5): 503-509.

Jyot, L. G., Arora, K. P., Sahoo, S. K., Singh, B. and Bartu, R. S. 2010. Persistence of trifloxystrobin and tebuconazole on Grape leaves, Grape berries and soil. *Bulletin of Environmental Contamination and Toxicology* **84**: 305-310.

Kaur, S., Takkar, R. and Bhardwaj, U. 2012. Dissipation kinetics of trifloxystrobin and tebuconazole on wheat leaves and their harvest time

residues in Wheat grains and soil. *Bulletin of Environmental Contamination and Toxicology* **89**: 606–610.

Kavadia, V. S. and Lal, R. 1967. Residues of malathion on Indian rapeseed. *Indian Journal of Agricultural Sciences* **38**: 708-713.

Kolkat, G. S., Davidson, R. H. and Bris, C. L. 1961. The effect of controlled temperature and humidity on the residual type of certain pesticides. *Journal of Economic Entomology* **54**: 1186-1190.

Kumar, S. and Jakhar, S. S. 2019. Effect of seed treatments and container on chilli seed viability. *Journal of Experimental Agriculture International* **36**(2): 1-10.

Kumbhar, C. T. and More, S. M. (2013). Efficacy of triazole fungicides in controlling fruit rot of chilli. *International Journal of Plant Protection* **6**(2): 257-261.

Kundu, C., Goon, A. and Bhattacharyya, A. 2011. Harvest residue study of fungicide tebuconazole EC formulation in Groundnut and Paddy. *Journal of Environmental Protection* **2**: 424-428.

Kyung, K. S., Noh, H. H., Lee, J. Y., Park, H. K., Jeong, H. R., Jin, M. J., Jeong¹, L. H. S. and Jeong², L. H. S. 2017. Residual characteristics of fluquinconazole and flusilazole in tomatoes during greenhouse cultivation and processing. *The Korean Society of Pesticide Science* **21**: 84-89.

Lewis, K. A., Tzilivakis, J., Warner, D. and Green, A. 2016. An international database for pesticide risk assessments and management. *Human and Ecological Risk Assessment: An International Journal* **22**(4): 1050-1064.

Li, W. and Leng, Y. F. 2004. Residue dynamics of flusilazole in cucumber and soil. *Chinese Journal of Pesticide Science* **6**: 87–89.

- Liu X, Wang X, Xu J, Dong F, Song W and Zheng Y. 2011. Determination of tebuconazole, trifloxystrobin and its metabolite in fruits and vegetable by a Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) method using gas chromatography with a nitrogen – phosphorous detector and ion trap mass spectrometry. *Biomedical Chromatography* **25**: 1081-1090.
- Mai, S., Ninga, E., Mukaj, M., Liti, A. and Cara, M. 2018. Dissipation kinetics of tebuconazole residues in Grape. *European Journal of Engineering Research and Science* **3**(10): 28-30.
- Malhat, F., Loutfy, N. M. and Ahmed, M. T. 2015. Validation of QuEChERS-based method for determination of flusilazole residues in grape by high-performance liquid chromatography with photodiode array detector. *Toxicological & Environmental Chemistry* <http://dx.doi.org/10.1080/02772248.2015.1092736>.
- Manjunath, M., Saha, S., Kumar, A., Rai, B. A. and Singh, B. 2015. Comparative efficacy of different fungicides in the control of chilli anthracnose. *Indian Journal of Plant Protection* **43**(2): 214-216.
- Mehta, I. 2017. Chillies-The Prime Spice-A History. *Journal of Humanities and Social Science* **22**(7): 32-36.
- Mohapatra S. 2015. Comparison of the residue persistence of trifloxystrobin (25%)+tebuconazole (50%) on gherkin and soil at two locations. *Environmental Monitoring and Assessment* **187**: 769.
- Mohapatra, S. and kumar, R. A. 2014. Persistence of trifloxystrobin and tebuconazole in banana Karnataka India. *International Journal of Environmental Analytical Chemistry* **94**(5):506-518.

- Mohapatra, S., Deepa, M. and Jagdish, G. K. 2011. Residue dynamics of tebuconazole and quinalphos in immature Onion bulb with leaves, mature Onion bulb and soil. *Bulletin of Environmental Contamination and Toxicology* **87**: 703-707.
- Muzzafar, M. 2017. Etiology and management of wilt complex disease of chilli. M. Sc. Thesis. Department of Plant Pathology, Sher-e-Kashmir University of Agricultural Sciences and Technology Kashmir, India.
- NHB, 2019. Area and Production of Chilli. www.nhb.gov.in
- Pandey, K. K. and Gupta, R. C. 2015. Management of anthracnose (*Colletotrichum capsaci*) in chilli (*Capsicum annum* L.) through fungicides, bioagents and hand picking methods. *Journal of Spices and Aromatic Crops* **24**(2): 141-144.
- Paramasivam, M., Selvi, C. and Deepa, M. 2015. Simultaneous determination of tebuconazole, trifloxystrobin and its metabolite trifloxystrobin acid residues in gherkin under field conditions. *Journal of Separation Science* **38**: 958 – 964.
- Patil, C. S., Vemuri, S., Deore, B. V., Saindane, Y. S., Kavitha, K. and Anitha, V. 2018. Dissipation behaviour of fluopyram and tebuconazole on Pomegranate. *Advances in Nutrition and Food Sciences* pp. 113.
- Patyal, S. K., Sharma I. D., Chandel, R. S. and Dubey, J. K. 2013. Dissipation kinetics of trifloxystrobin and tebuconazole on apple (*Malus domestica*) and soil a multi-location study from north western Himalayan region. *Chemosphere* **92**: 949-954.

- Prabhavathi, Y., Kishore, N. T. K. and Seema, 2013. Analysis of supply chain of spices in India: A case study of red chillies. *International Journal of Scientific and Research Publications* **3**(9): 1-4.
- Rajabaskar, D., Kumar, R. S., Ragupathy, A. and Sridhar, K. 2004. Persistence and dissipation of propiconazole in chillies. *Pestology* **28**: 141-144.
- Saha, A. 2017. Dissipation and Safety Evaluation of Tebuconazole Residues in Peanut-Field Ecosystem. *Proceedings of the National Academy of Sciences, India Section B*, **87**(3): 753-760.
- Saha, S., Manjusha, R., Jadhav, T. P., Shabeer, A., Banerjee, K., Sharma, K. B., Loganathan, M. and Rai, A. B. 2016. Safety assessment and bioefficacy of Fluopyram 20%+Tebuconazole 20% 40SC in chilli, *Capsicum annum* L. *Proceedings of the National Academy of Sciences, India Section B: Biological Science* **86**(2): 359-366.
- Sahoo, S. K., Jyot, G., Battu, R. S. and Singh, B. 2012. Dissipation kinetics of trifloxystrobin and tebuconazole on chilli and soil. *Bulletin of Environmental Contamination and Toxicology* **88**(3): 368-371.
- Sarkar, S., Nandi, A., Dash, S. K., Senapati, N., Pandey, G., Das, S. and Patnaik, A. 2016. Bio-efficacy of different fungicides along with bio-agents against chilli Anthracnose (*Colletotrichum capsici*) disease under field condition. *Journal of Mycopathological Research* **54**(1): 85-87.
- Sharma, D. and Choudhary, P. P. 2018. Pesticide use and their residue management in vegetable. NIPHM, Technical Bulletin No. 74.
- Shuang, Y., Dong, Q., Qiong, W., Xing, Li, G., Li Jun, H. and Shuren, J. 2011. Residue and dissipation dynamics of flusilazole in apple and soil. *Bulletin of Environmental Contamination and Toxicology* **86**: 319-322.

- Singh, G. and Singh, B. 2014. Residue Dynamics and Risk Assessment of Trifloxystrobin and Tebuconazole on Tomato (*Lycopersicon esculentum* Mill.). *American Journal of Environmental Protection* **2**(3): 59-63.
- Sreeja, S. J., Girija V. K. and Beevi, S. N. 2014. A potential and safe new generation fungicide for the management of anthracnose of Cowpea (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdcourt). *Pesticide Research Journal* **27**(2): 191-198.
- Srivastava, B. P., Kavadia, V. S. and Sharma, G. K. 1969. Effect of temperature and humidity on residual toxicity of some pesticides. *Indian Journal of Entomology* **31**: 127-131.
- Teotia, T. D. S. and Dahm, P. A. 1950. The effect of temperature, humidity and weathering on residual toxicities of five organic pesticides. *Journal of Economic Entomology* **43**: 864-876.
- Tomlin C. D. S. 1994. The Pesticide Manual: Incorporating The Agrochemicals Handbook (ed)-10th ed. Crop Protection Publications, United Kingdom. 1341p.
- Velayutham, L. K. and Damodaran, K. 2015. Growth Rate of Chilli Production in Guntur District of Andhra Pradesh. *International Journal of Research in Humanities and Social Studies* **2**(11): 1-5.
- Wang, C., Qiu, L., Zhao, H., Wang, K. and Zhang, H. 2013. Dissipation dynamic and residue distribution of flusilazole in mandarin. *Environmental Monitoring and Assessment* **185**(11): 9169-9176.
- Wang, Z., Cang, T., Qi, P., Zhao, X., Xu, H., Wang, X., Zhang, H. and Wang, X. 2015. Dissipation of four fungicides on greenhouse strawberries and an assessment of their risks. *Food Control* **55**: 215-220.

- Wani, A. A., Dar, A. A., Jan, I., Sofi, K. A., Sofi, J. A. and Dar, I. H. 2019. Dissipation, risk assessment, half-life period and method validation of carbendazim and triazophos in green pea by high-performance liquid chromatography. *Journal of Separation Science Plus* **2**: 284-290.
- Wu, Y. T., Zhang, Y. H., Zhang, M., Liu, F., Wan, Y. C., Huang, Z., Ye, L., Zhou, Q., Shi, Y. and Lu, B. 2014. Selective and simultaneous determination of trace bisphenol A and tebuconazole in vegetable and juice samples by membrane-based molecularly imprinted solid-phase extraction and HPLC. *Food Chemistry Journal* **164**: 527-535.
- Yadav, I. C., Devi, N. L., Syed, J. H., Cheng, Z., Li, J., Zhang, G. and Jones, K. C. (2015). Current status of persistent organic pesticides residues in air, water and soil and their possible effect on neighbouring countries: A comprehensive review of India. *Science of the Total Environment* **511**: 123-137.

Appendix I

Meteorological data from the day of pesticide application up to final sampling for the year 2020

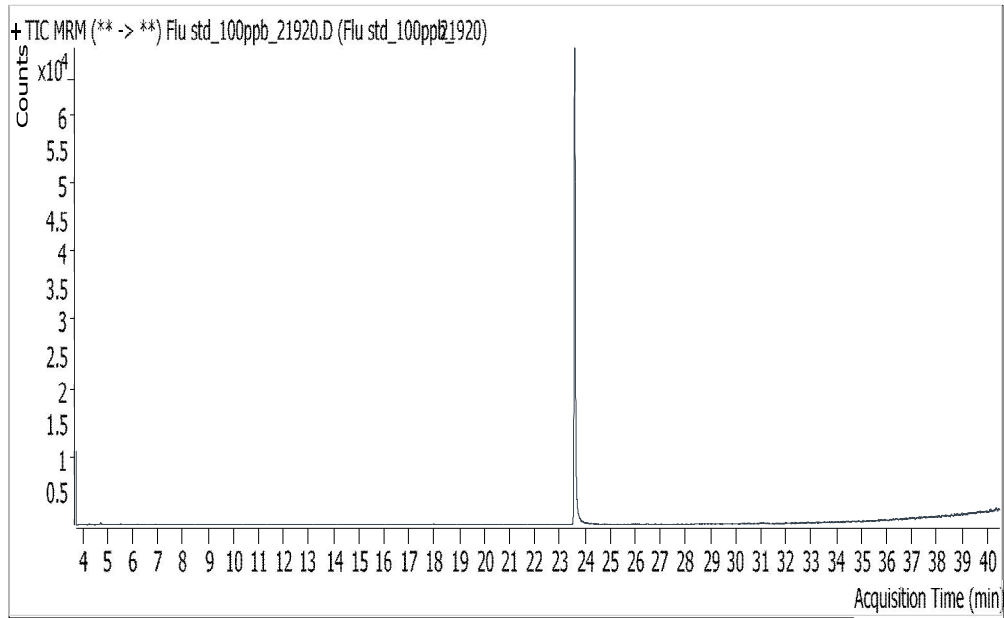
Date	Max temp (°C)	Min temp (°C)	Rain (mm)	RH Max. (%)	RH Min. (%)	Evap. (mm)
01-09-2020	28.5	15.5	6	91	69	3
02-09-2020	30.5	15.5	0	91	57	3.5
03-09-2020	30	15.5	0	91	70	4.3
04-09-2020	19.5	16	17.4	91	94	4
05-09-2020	27	16	0.6	91	58	2
06-09-2020	27.5	15.5	27	94	64	4
07-09-2020	26.5	14	19	90	54	4.4
08-09-2020	29	12	0	74	48	4.4
09-09-2020	30	12	0	85	44	4.4
10-09-2020	30	11	0	70	47	5
11-09-2020	31	11.5	0	81	50	5.2
12-09-2020	30.5	11.5	0	74	48	5.3
13-09-2020	31	10.5	0	81	36	5.3
14-09-2020	30.5	10.5	0	91	32	4.3
15-09-2020	31	12	0	82	35	5.3
16-09-2020	31	11	0	74	30	4.7
17-09-2020	31	10	0	69	34	6
18-09-2020	31.5	10	0	66	42	5
19-09-2020	30.5	8	0	87	39	3.8
20-09-2020	30.2	7.5	0	57	29	4.2
21-09-2020	30.5	7.5	0	69	29	3.5
22-09-2020	31	8.5	0	65	46	4
23-09-2020	31	9	0	61	32	3.5
24-09-2020	30	8	0	65	39	3.4
25-09-2020	29	8	0	77	54	3.6
26-09-2020	25	8.5	0	65	49	3.4
27-09-2020	24	7	0	83	61	3.1
28-09-2020	26.5	7	0	80	58	3.6
29-09-2020	27	6	0	84	36	3.8
30-09-2020	27	5	0	80	33	3.7
01-10-2020	27	5.5	0	73	39	3
02-10-2020	27.5	5	0	87	33	3.6
03-10-2020	28	4.8	0	78	35	3.6

Contd...

Appendix I: contd...

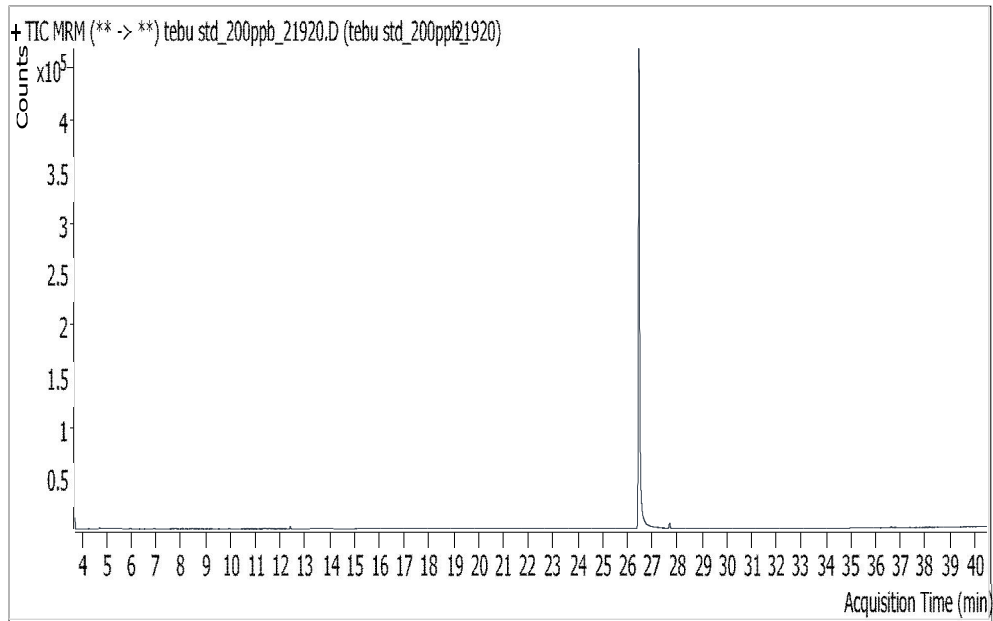
Date	Max temp (oC)	Min temp (oC)	Rain (mm)	RH Max. (%)	RH Min. (%)	Evap. (mm)
04-10-2020	28	4.5	0	88	39	3.4
05-10-2020	28	4.5	0	78	39	3.3
06-10-2020	29	3.5	0	73	26	3.5
07-10-2020	28	4	0	84	41	3.4
08-10-2020	27.5	3.5	0	87	39	2.9
09-10-2020	28	3.6	0	79	39	3.2
10-10-2020	27	3.5	0	77	33	3.2
11-10-2020	27	2.5	0	68	26	3.3
12-10-2020	27	3	0	77	26	2.5
13-10-2020	26.5	3	0	78	25	3.2
14-10-2020	26	4.5	0	88	38	3.1
15-10-2020	27	4	0	84	38	3.1
16-10-2020	26.5	3.5	0	74	32	3.1
17-10-2020	26	4	0	78	39	3.3
18-10-2020	26	2.8	0	84	26	2.7
19-10-2020	25.5	2.5	0	84	32	2.4
20-10-2020	25.5	1.4	0	84	34	2.7
21-10-2020	24.5	2.1	0	78	29	2.6
22-10-2020	24.5	2	0	75	36	2.4
23-10-2020	24	1.6	0	78	31	2.4
24-10-2020	22.5	2	0	74	42	2.4
25-10-2020	18.5	1	0	81	53	2.6
26-10-2020	20	-0.5	0	65	44	2.5
27-10-2020	21.5	-1.5	0	68	37	3.5
28-10-2020	21.5	-1	0	65	38	3
29-10-2020	20.5	-1	0	62	40	2.5
30-10-2020	21	-2	0	62	46	3.2
31-10-2020	20	0	0	58	51	3

Appendix II



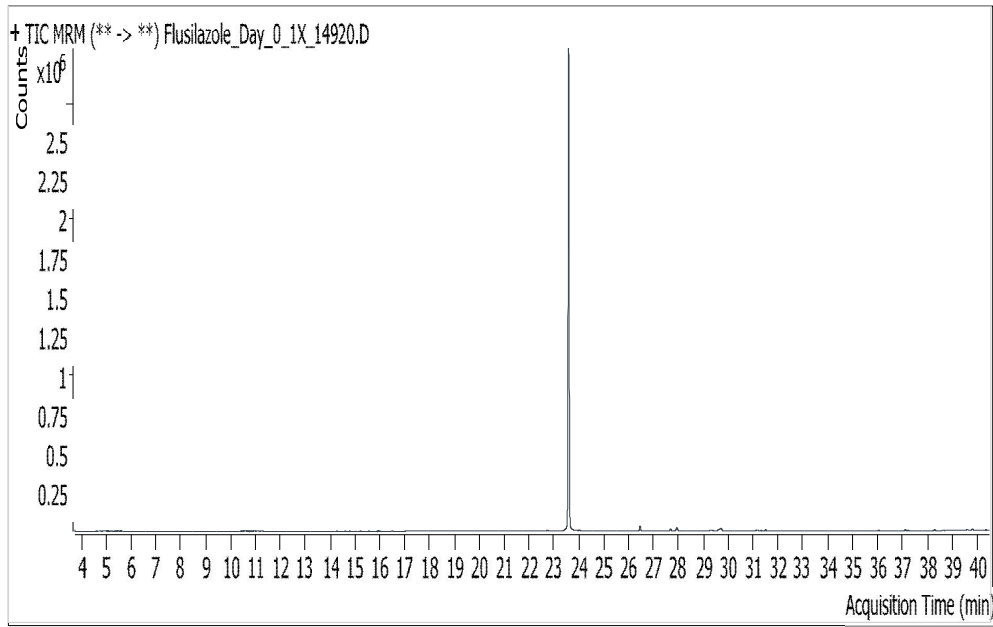
Chromatogram of Flusilazole Standard 0.1 ppm (100 ppb)

Appendix III



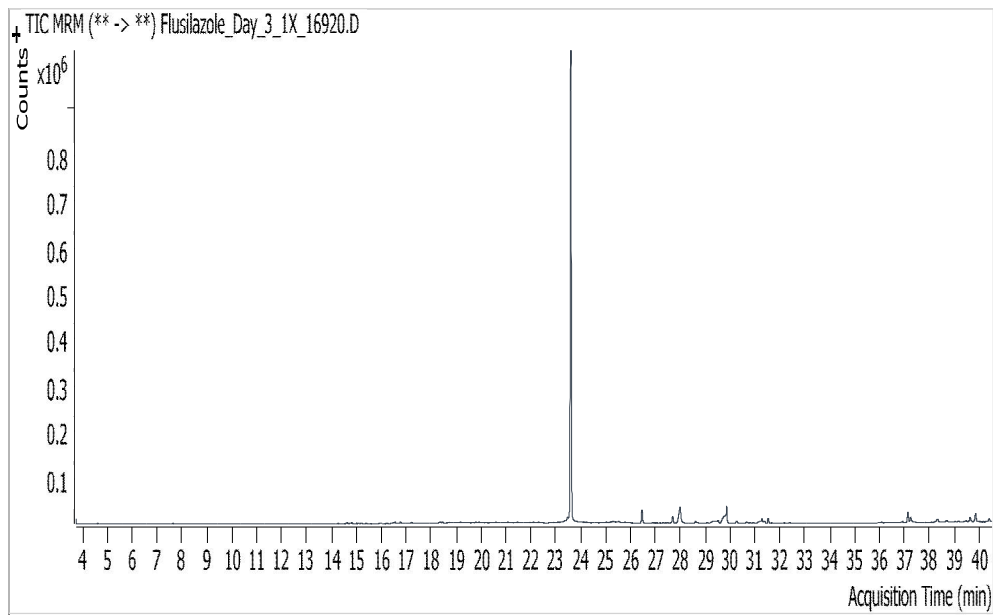
Chromatogram of Tebuconazole Standard 0.2 ppm (200 ppb)

Appendix IV



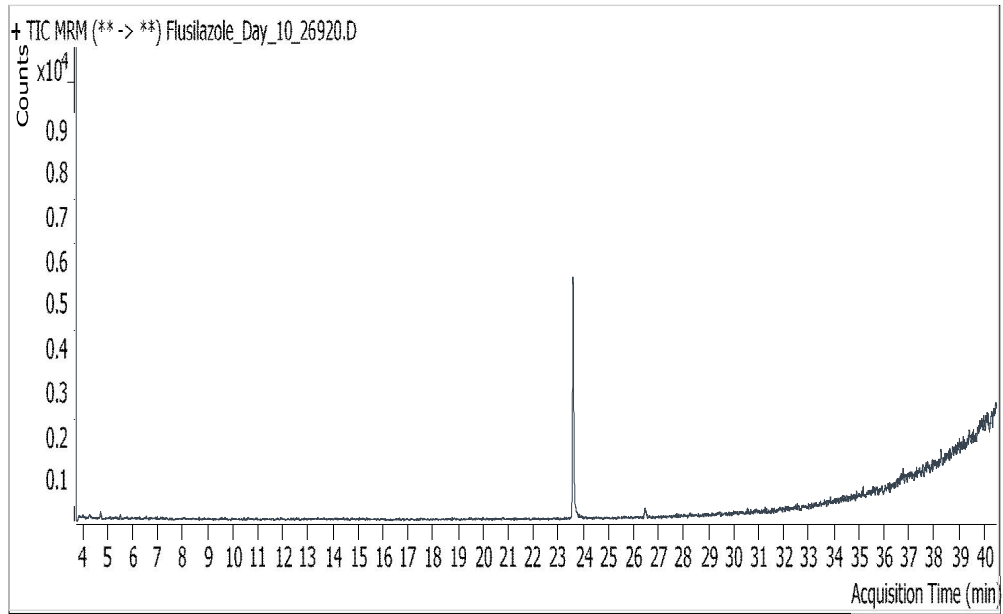
Chromatogram of Flusilazole @ 0.02 % at Day 0 on Chilli

Appendix V



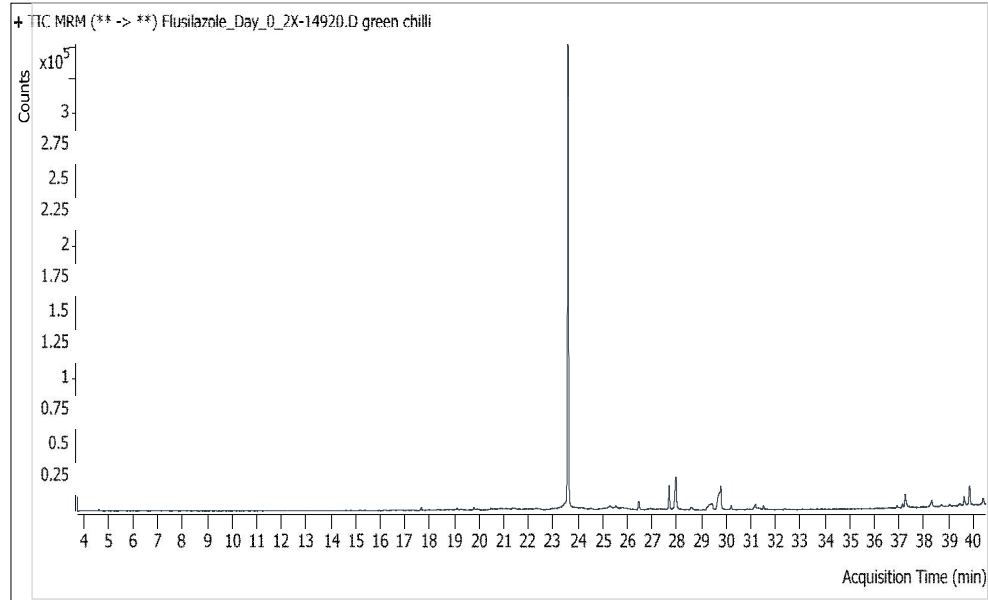
Chromatogram of Flusilazole @ 0.02 % at Day 3 on Chilli

Appendix VI



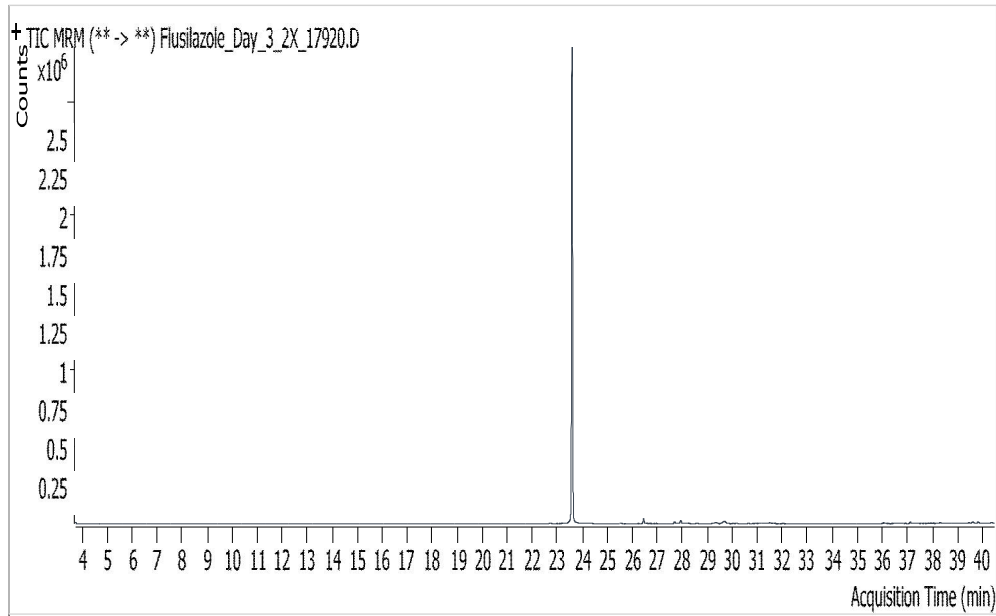
Chromatogram of Flusilazole @ 0.02 % at Day 10 on Chilli

Appendix VII



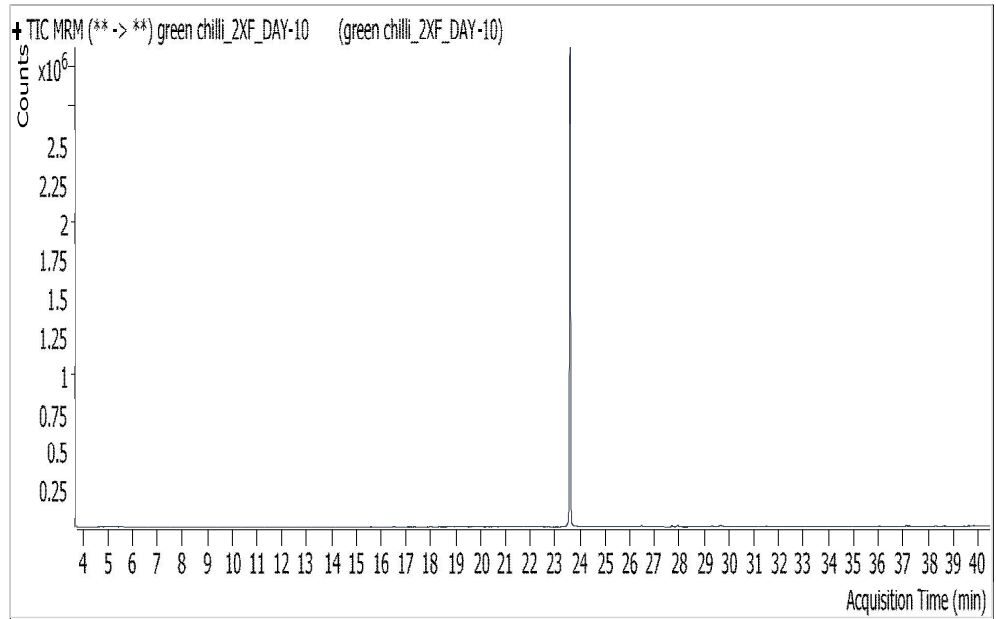
Chromatogram of Flusilazole @ 0.04 % at Day 0 on Chilli

Appendix VIII



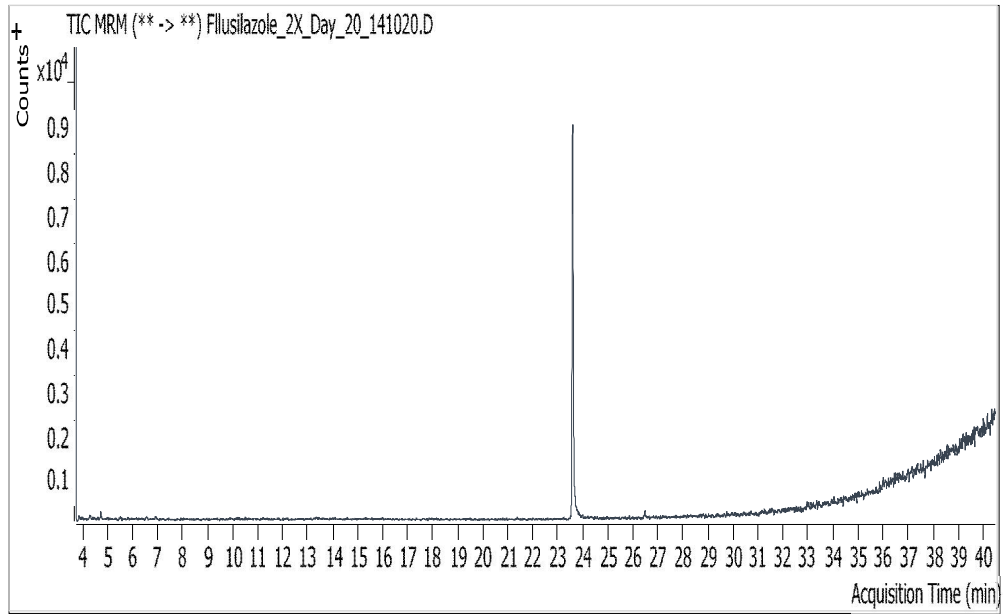
Chromatogram of Flusilazole @ 0.04 % at Day 3 on Chilli

Appendix IX



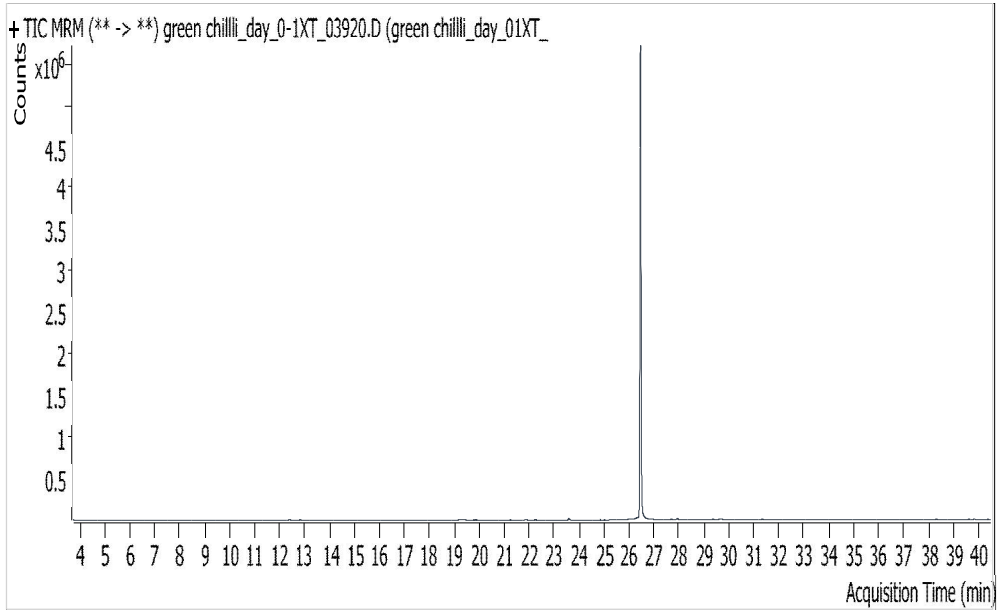
Chromatogram of Flusilazole @ 0.04 % at Day 10 on Chilli

Appendix X



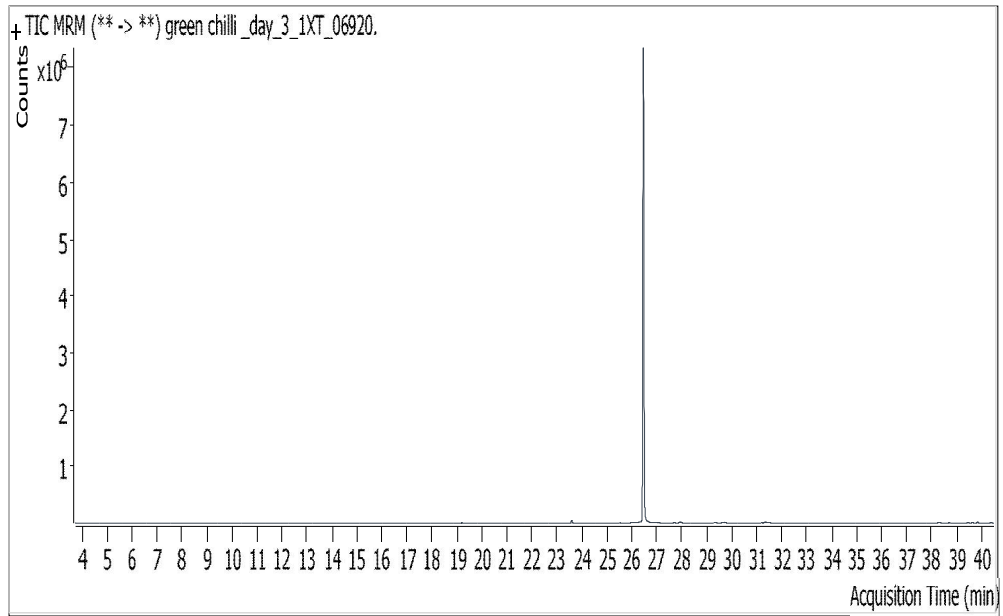
Chromatogram of Flusilazole @ 0.04 % at Day 20 on Chilli

Appendix XI



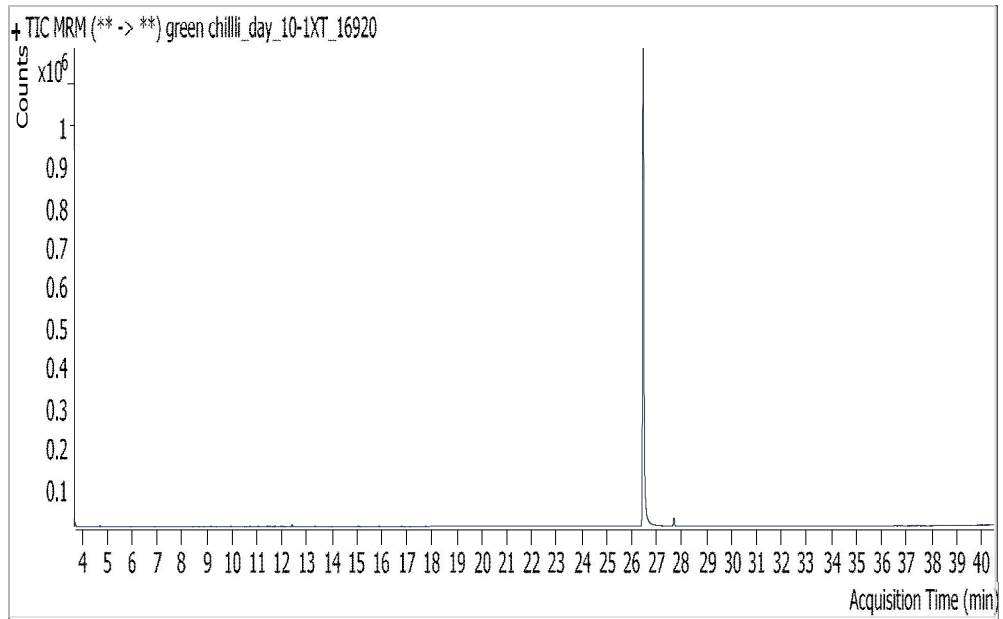
Chromatogram of tebuconazole @ 0.05 % at Day 0 on Chilli

Appendix XII



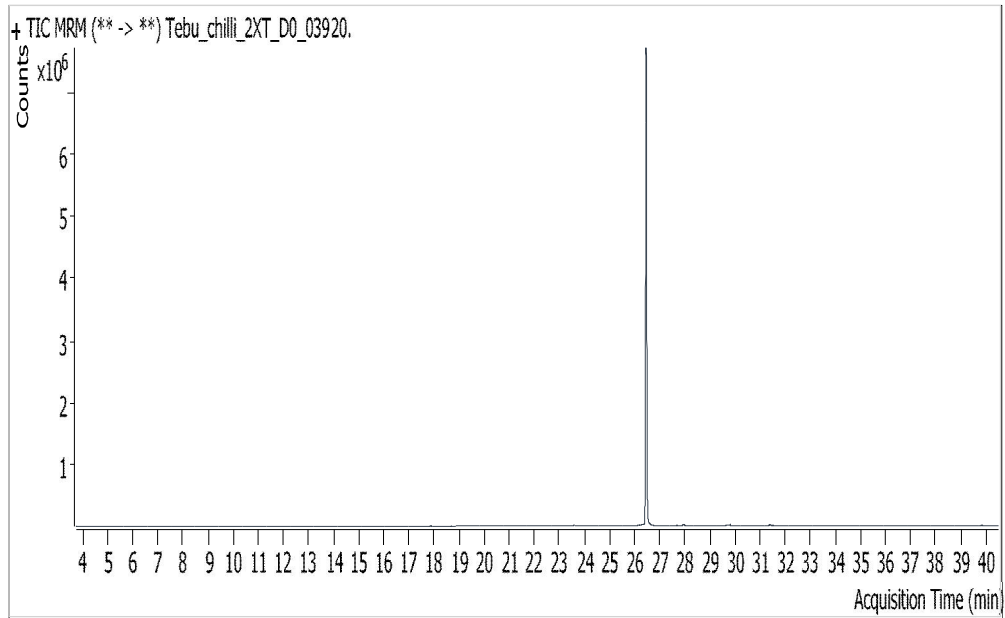
Chromatogram of tebuconazole @ 0.05 % at Day 3 on Chilli

Appendix XIII



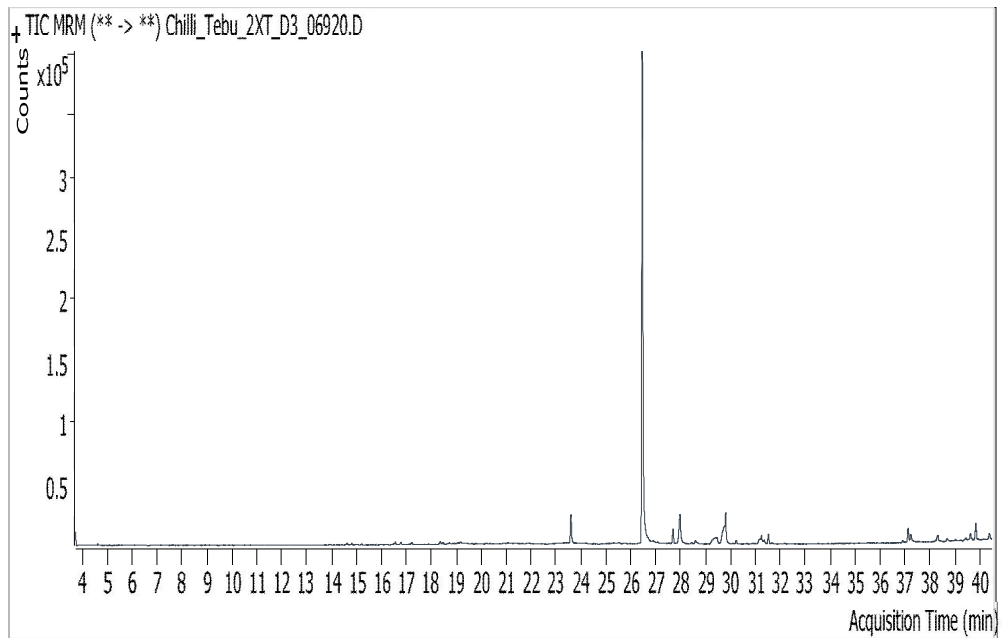
Chromatogram of tebuconazole @ 0.05% at Day 10 on Chilli

Appendix XIV



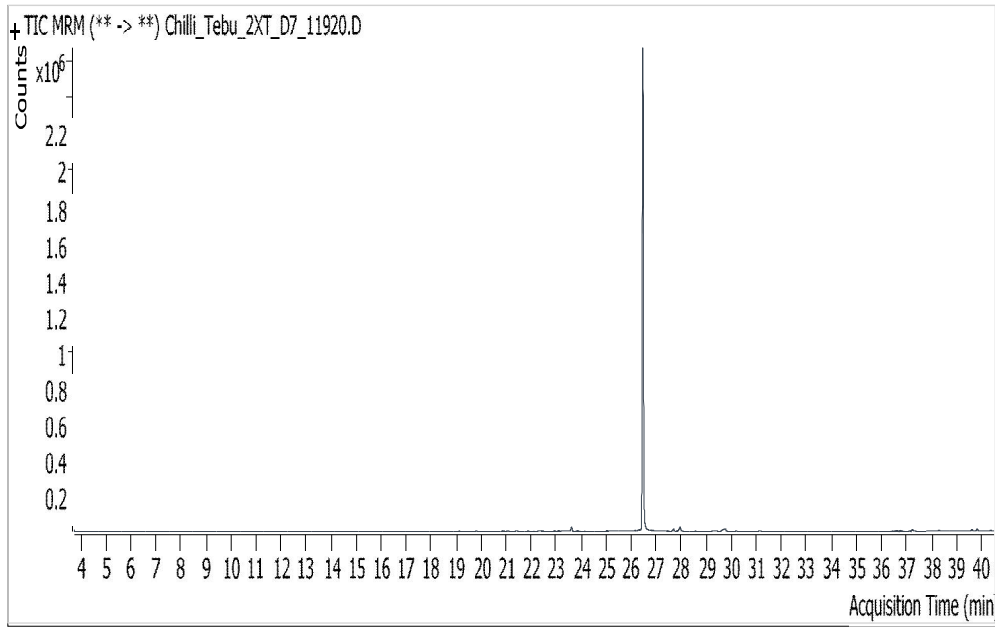
Chromatogram of tebuconazole @ 0.10% at Day 0 on Chilli

Appendix XV



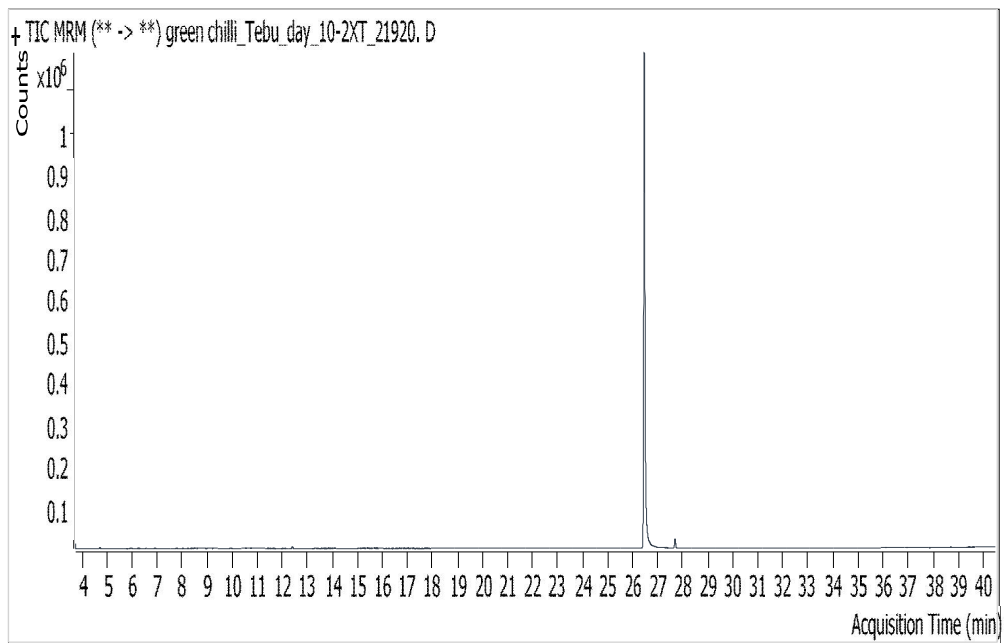
Chromatogram of tebuconazole @ 0.10% at Day 3 on Chilli

Appendix XVI



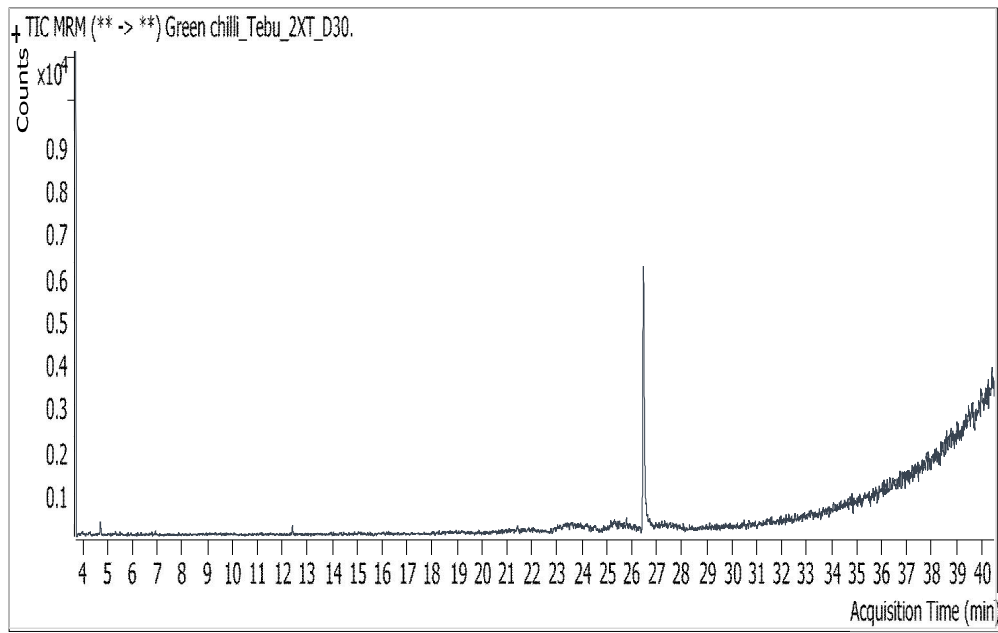
Chromatogram of tebuconazole @ 0.10% at Day 7 on Chilli

Appendix XVII



Chromatogram of tebuconazole @ 0.10% at Day 15 on Chilli

Appendix XVIII



Chromatogram of tebuconazole @ 0.10% at Day 30 on Chilli

Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
Faculty of Horticulture, Division of Plant Pathology

CERTIFICATE

Certified that all the corrections/amendments as suggested by External Examiner **Dr. P. K. Chakarabarty (Member ASRB, New Delhi)** during Viva-Voce examination held on **09-01-2022** have been incorporated in the manuscript entitled “**Residue Dynamics of Flusilazole and Tebuconazole on Chilli (*Capsicum annuum* L.) in Kashmir**” submitted by **Mr. Vinod Kumar (Regd. No. MSH-2019-279)**.

(Dr. Ashraf Alam Wani)
Chairman
Advisory Committee