DISSIPATION STUDIES OF BOSCALID (A CARBOXAMIDE FUNGICIDE) IN SOILS OF DIFFERENT TEXTURES

Thesis

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By

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(Adarsh Butola)
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Pantnagar
September, 2022

CERTIFICATE–I

This is to certify that the thesis entitled **“DISSIPATION STUDIES OF BOSCALID (A CARBOXAMIDE FUNGICIDE) IN SOILS OF DIFFERENT TEXTURES”,** submitted in partial fulfillment of the requirements for the degree of **Master of Science in Agriculture** with major in **Agricultural Chemicals** of the College of Post Graduate Studies, G. B. Pant University of Agriculture & Technology, Pantnagar, is a record of bonafide research carried out by **Mr. Adarsh Butola,** Id. No. **56994** under my supervision and no part of the thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been acknowledged.

Anjana Srivastava

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CERTIFICATE–II

We, the undersigned, members of Advisory Committee of **Mr. Adarsh Butola,** Id. No. **56994,** a candidate for the degree of **Master of Science in Agriculture** with major in **Agricultural Chemicals,** agree that the thesis entitled **"DISSIPATION STUDIES OF BOSCALID (A CARBOXAMIDE FUNGICIDE) IN SOILS OF DIFFERENT TEXTURES"** may be submitted in partial fulfillment of the requirements for the degree.

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Member

Pachauri
(S.P. Pachauri)
Member

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LIST OF ABBREVIATIONS

g - gram
mg - milligram
mol - mole
ml - millilitres
min - minute
nm - nanometres
˚C - degree Celsius
aq. - aqueous
cm - centimetre
mg/l - milligram per litre
hr/hrs - hours
mg/g - milligram per gram
g/kg - gram per kilogram
g/ml - gram per millilitre
mm - millimetres
WHO - World Health Organization
i.e. - in other words
K - Kelvin
mg/l - milligram per litre
λ_{max} - Wavelength at maximum absorbance
rpm - Revolutions per minute
pH - Power of hydrogen ion concentration
UV-VIS - Ultraviolet-Visible
R^2 - Coefficient of determination
e.g. - For example,

et. al.	-	Co-workers
EPA	-	Environmental Protection Agency
STMR	-	Supervised trial median residue
PHI	-	Pre harvest interval
MRL	-	Maximum residue limit
GAP	-	Good agricultural practices
Kg	-	Kilogram
R&D	-	Research and development
SDHI	-	Succinate dehydrogenase inhibitor
OC	-	Organic carbon
ACN	-	Acetonitrile
Log	-	Logarithm
CAS	-	Chemical abstracts service
EC	-	Emulsifiable concentrate
QoI	-	Quinone outside inhibitor
v/v	-	Volume per volume
a.i.	-	Active ingredient
hpa	-	Hectopascal
spp.	-	species
lbs	-	Pound
µg	-	microgram
ml	-	Mililitre
RQ	-	Risk quotient
$EC_{50}/DT_{50}/t_{1/2}$	-	Half maximal effective concentration
WD	-	Water dispersible granules
SC	-	Suspension concentrate
EC	-	Emulsifiable concentrate

LOD - Limit of detection

LOQ - Limit of quantification

QuEChER'S - Quick, effective, cheap, easy, rugged, safe

HPLC - High performance liquid chromatography

HPLC/DAD - High performance liquid chromatography with diode arry detection

FAO - Food and agricultural organization

BDL - Below detection limit

GC-ITMS - Gas chromatography/ion trap mass spectrometry

EU - European union

ln - Natural logarithm

LC/MS - Liquid chromatography-mass spectrometry

K - Degradation constant

SD - Standard deviation

USDA - United states department of agriculture

Introduction

Chapter 1 INTRODUCTION

Pesticides are substances that are meant to control any pests, which are harmful to crops and can cause economic damage to mankind by preventing, destroying, and mitigating any pest ranging from insects, rodents and weeds to microorganisms. Pesticides include herbicides, insecticides, nematicides, avicides, rodenticides, bactericides, and fungicides. Around the world, an estimated 2.5-3 billion kilograms of pesticides are applied annually **(Alavanja, 2009; Pimental and Burgess, 2014).** Pesticides are an effective way to enhance quality and quantity, and maintain food security in the world. Among the world's most pesticide consuming countries China tops the table, USA is second and Argentina is at third position, which is increasing rapidly over the year and by the end of 2030, global pesticide usage is estimated to be increased up to 3.5 million tonnes. Pesticide manufacturing in India started in 1952, with DDT (Dichlorodiphenyltrichloroethane) and HCH (Hexachlorocyclohexane) and today it produces approx. 90,000 metric tonnes of pesticide and ranks twelfth in pesticide production in the world **(Sharma, 2019)**.

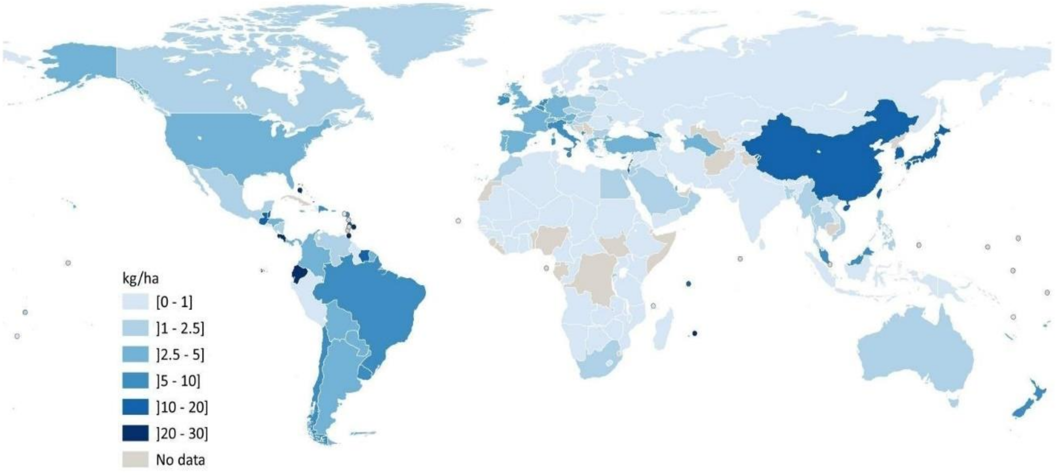


Figure 1.1 Pesticide use per cropland area, 2018 (FAOSTAT pesticide use and pesticide indicators, 2020)

Most of the pesticides have a pre-harvest interval (PHI), a minimum window of time that is required between the final application of the pesticide and the date of harvest but some produced fungicides have not been given any such PHI. The absence of PHI in some instances makes fungicides important targets for post-harvest techniques that can be capable of degrading fungicide residues **(Skanes, 2021).**

However, there is a maximum residue limit (MRLs) which has to be adhered to, for such pesticides in food commodities. The MRL is the pesticide residue level in a specific food after it has been produced under Good Agricultural Practices (GAP). Pesticide residues do not exceed the MRL if crops are cultivated appropriately and all pesticide treatments are made in accordance with GAP (i.e. at the correct time and according to label guidelines). The MRL is calculated by a small-scale farm research in which the pesticide under test is sprayed on a specific crop, the appropriate withdrawal period is permitted (i.e. the time required between pesticide application and harvest), before the crop is harvested, and residues are determined. If the residue level exceeds the MRL in accordance with GAP guidelines then it is not authorised to be sold, imported and exported **(Shaw, 2001).** MRL is different for different crop commodities and is set by different countries according to the application rate. For example, MRL for boscalid in cucumber is (5.0 mg/kg, China) and potato is (2 mg/kg, European union) Tomato (3 mg/kg, European union). The tomato and cucumber crops come under dark and light class of vegetables respectively whereas potato comes under tuber and their MRL's are used to calculate the corresponding national estimated daily intake (NEDI) for their respective class. For multiple fruits, STMR (supervised trials median residue) are used to calculate the NEDI of boscalid in fruits.

Fungicides which are used to inhibit or kill fungal growth in crops and are categorized into contact, translaminar and systemic types. Fungal infections were responsible for 7–24 percent of yield losses in commodity crops such as potatoes worldwide as reported in 2001–2003, even with the application of chemical-based crop protection methods **(Oercke, 2006).**

During 1940s diseases control is mainly through Inorganic chemicals and the key areas in which these chemicals are used were Horticulture and Vegetable production. As damaging effects of diseases become more obvious by use of chemical control new areas of chemical classes were introduced which aim for better effectiveness than the previous chemical class. This leads to patent busting strategies in order to monetize developing fungicide market. R & D costs have increased dramatically in recent years and are dominated by providing all the additional data needed for registration, not the discovery process itself. These cost increases occurred when the market shows little growth or is somewhat down. As a result, the industry has to constantly strive to reduce costs, usually through mergers and acquisitions. As a result, many crop disease problems are not addressed by the industry and special measures have to be taken to make appropriate disease control available in these smaller markets. Integrated control using chemicals, biocontrols, and biotechnology approaches is expected to begin to dominate, but plant disease control continues to be needed as fungicides are important for such control **(Russel, 2005).**

Development of first fungicides took place around 17th century on the basis of observations that soaking of grain and then followed by liming is effective for controlling bunt of wheat. The most important discovery was of Bordeaux mixture done by PMA Millardet of France in 1882 where he noticed that bluish-white mixture of copper sulfate and lime is effective in controlling downy mildew of grapes **(Morton, 2008).** The use of synthetic fungicide for post-harvest decay management had been used for several decades, but 1986 report of US National Academic of Sciences concluded that fungicides residues pose health risk to consumer as well as environment and therefore is a need for safe alternative for synthetically prepared fungicides **(Martinez, 2012).**

Boscalid is a pyridine carboxamide obtained by formal condensation of the carboxy group of 2-chloronicotinic acid with the amino group of 4’-chlorobiphenyl-2-amine **(PubChem CID, 213013).** It is expected to accumulate moderately in fish tissue, with more accumulation occurring in non-edible tissue than edible tissue, but once cleansed out from the tissue, the tissue is no longer exposed to the compound. Boscalid is moderately toxic to aquatic species and does not have an acute or chronic exposure effect. It also poses no risk to plants and bird species and in mammals too it does not pose any acute effect but on long term exposure it leads to weight loss in the second generation **(USEPA, 2003).** The carboxamide fungicides include carboxin, benodanil, furametpyr, penthiopyrad, flutolanil, mepronil, fenburam, and thifluzamide which are single-site inhibitors of the succinate ubiquinone reductase or succinate dehydrogenase inhibitor (SDHI) complex in the respiratory chain **(Avenot, 2007).**

Boscalid has been proposed for use as a seed treatment on rapeseed (*Brassica napus*) including canola. It is transported both translaminarily and acropetally. It is very safe for the plants and covers a wide range of disease spectrum. Boscalid controls a number of fungal pathogens in aerable and speciality crops including ornamentals. Following terrestrial application, it has a moderate potential to reach aquatic ecosystems, including surface and groundwater. Boscalid is one of the fastest-growing fungicide in terms of new compounds produced. It has been launched into the fungicide market for controlling turfgrass and other general agricultural pests **(Stammler, 2008).**

Several studies on the environmental fate of boscalid have been conducted. **Reilly *et al.* (2012)** found boscalid to be the most common pesticide in ground and surface waters of selected regions in the United States. **Smalling and Orlando (2011)** discovered high levels of boscalid, up to 36 mg L^{-1}, in water samples from 12 California coastal watersheds. **Smalling *et al.* (2013)** reported the presence of boscalid in water, fish, and sand crab samples and 89 % of sediment samples. Furthermore, boscalid was found in 12 of the 24 surface water samples taken from Rhineland-Palatinate (Germany) streams in 2008 and 2009, with water samples of two rivers containing the fungicide > 0.1 mg L^{-1} pesticide; a threshold limit of the pesticide in drinking water **(Karlsson *et al.*, 2016).**

Persistence / dissipation studies are conducted to estimate the dissipation rate of parent compounds under actual usage conditions, provide information on the formation and degradation of major metabolites, provide their leachate behavior and accumulation of their residues in soil **(Barefoot, 2003).** The degradation rate of these compounds is influenced on the basis of their structure. The pesticides with high water solubility and low absorptivity which support the microbial growth are responsible for faster dissipation rates. These pesticides are structurally similar to natural substances occurring naturally. Therefore, these natural substances are used as energy source and easily get degraded by most microbial organisms as soon as they enter into the environment. In the case of structurally different compounds such as xenobiotic compounds, which are synthesized by man, do not contain suitable degradation genes and as a result bacteria etc. are not be able to evolve enzymes capable of degrading these compounds **(Arbeli, 2007).**

Soil is a biologically active and porous medium developed in the uppermost layers of the earth's crust. It acts as a reservoir for water and nutrients, and as a medium for filtration and decomposition of hazardous wastes. It also helps to circulate carbon and other elements through global ecosystems. Value of half-life of pesticides in soil is determined under various conditions, as it is an indicator of pesticides persistence in soil rather than degradation rates. Pesticides with half-life less than 30 days are considered non persistent and that with half-life above 100 days are considered persistent **(Buyuksonmez, 1999).** In aerobic soil boscalid metabolizes to form an intermediate which is then transformed into CO_2 and soil bound residues. Majority degradation of this compound is due to its conversion to bound residues. It’s metabolites are 2-chloronicotinic acid (M510F47), 2-hydroxy-N-(4’-chlorobiphenyl-2-yl)-nicotinamide (M510F49), and an unknown (M510F50). Boscalid is hydrolytically and photolytically stable on soil and in water. It is not transformed to any significant extent in both aerobic and anaerobic aquatic system, but it is rapidly transferred (dissipation half-lives of < 2 weeks) to sediment phase from water phase by getting sorbed to sediment **(USEPA, 2003).**

As boscalid is a novel fungicide, there is very little information about its dissipation behaviour in agricultural crops and soil. Since application of boscalid on agricultural crops, can lead to its falls on soil too from where it can leach to ground water the persistence / dissipation studies of boscalid from soil are of utmost importance. Hence the present studies have been undertaken to evaluate the persistence / dissipation pattern of this pesticide from two different soils with the objectives mentioned below:

OBJECTIVES

- To determine λ_{max} of boscalid fungicide [2-chloro-N-(4'-chlorobiphenyl-2-yl) nicotinamide].
- To determine soil properties of two different soils.

- To standardize the extraction method for analysis of boscalid from soil and determine it through the chromatographic method.
- To study the dissipation pattern of boscalid from two soils at two different concentrations during different time intervals (0, 1, 3, 5, 7, 14, 21, 28, 35, 42 and 60 days).

Review of Literature

Chapter 2 REVIEW OF LITERATURE

Modern agriculture is fully dependent on chemical pesticides and fertilizers during the last century for supplying of nutrients as well as controlling of pest and diseases. Today we have reached a stage where we have to manage the derogatory effects of these chemicals on the environment as well as on human health along with meeting the demands of increased food production around the globe. Pesticides tend to remain longer time in soil as compared to plants and animals as they are actively growing living system in comparison to soil which is a static system. Soil is a rich diversity of micro flora and fauna which effect its properties. Apart from plant growth, soil also performs processes such as exchange of gases, flow of energy, nutrients and water and detoxification of pollutants. Thus, soil health is necessary for sustainable agriculture and to maintain soil biodiversity such as microbial diversity which is most diverse and favourable habitat for a variety of microorganisms including bacteria, fungi, protozoa, algae and virus **(Prashar, 2016).** Degradation rate of pesticide is influenced by soil physicochemical properties (such as pH and organic carbon (OC) content, biological properties (microbial activity and distribution), and environmental conditions that control soil temperature and water content. Both the paths and rate of decomposition also depend on the properties of the chemical. Fluctuations in degradation rates are expected and many studies provide evidences of variations in pesticide degradation rates between fields **(Kah, 2007).** Plants play an important role in producing secondary metabolites that play as a defence barrier for plants for controlling of other animal and microbial predators. These metabolites are classified on the basis of their chemical point of view such as alkaloid and phenolic compounds on biosynthetic origin and terpenoids, phenylpropanoids and polyketides etc. Many of these metabolites are used for defence against animal predators and some of them are toxic to plants but get inactivated as glycosides in intercellular spaces. Some fungi and bacteria produce some antifungal properties containing substances like strobilurins, myxothiazols, oudemansins that suppress other organism competing for nutrients in the same environment **(Martinez, 2012).** Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the Joint FAO/WHO Meeting on Pesticide Residues (JMPR), regional scientific committees such as the European Union's, and national regulatory agencies all use the safety factor approach to determine acceptable or tolerable intakes of substances with toxicity thresholds. The acceptable daily intake (ADI) is a commonly used phrase to indicate safe intake levels; additional terminology includes the reference dose (Rf D) and tolerated intakes, which are expressed on a daily (TDI) or weekly basis **(Herrman, 1999).** The ADI of boscalid and pyraclostrobin is 0.04 and 0.03 mg/kg of body weight respectively **(Chen, 2019).** One of the major contributing fungicides in the global market are Strobilurins that contribute about 23-25% of global fungicide sales. They are generally obtained from fungus Strobilurus tenacellus. They belong to class of QoIs (Quinone outside Inhibitors) that inhibit the respiratory complex III. Now there are synthetic strobilurins like azoxystrobin, pyraclostrobin, picoxystrobin and trifloxystrobin available in the market by big giants of pesticide market. These synthetic compounds are more effective than natural compounds in terms of volatility and increased resistance to UV breakdown and share the same β-methoxyacrylate moiety **(Tang, 2017).** Pathogens can develop resistance to newly introduced fungicides within a few years after exposure, depending on the pathogen's genome and mode of fungicidal action (single or multiple pathways). Within two years of enrolment, field and laboratory studies with boscalid fungicide were detected with a strain of Alternaria alternata (the cause of Alternaria blight in pistachios) resistant to boscalid **(Avenot and Michailides, 2007).**

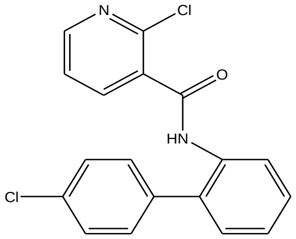
2.1 Carboxamide Fungicides

These are the class of compounds which inhibit complex II, a SDH enzyme (succinate ubiquinone oxidoreductase) and an essential component of respiratory chain. It plays a role in respiration by oxidation of succinate to fumarate in mitochondrial Krebs cycle. SDH inhibitors are classified into two main categories according to their binding site, succinate binding (e.g., malonate) and ubiquinone binding (e.g., carboxamide). The first compound of this class was carboxin, marketed in 1966-67, a narrow spectrum of herbicide generally used for seed treatment to control smut. Boscalid is the first truly broad-spectrum fungicide of this class launched in 2003. The compounds in this group are very diverse but they show a common feature which is the presence of amide bond **(Sierotzki, 2013).** Carboxamide fungicides include boscalid, penflufen, pydiflumetofen, penthiopyrad, fluxapyrosad, benzovindiflupyr, bixafen. Carboxamide fungicide have more greening effect as compared to other SDHI fungicides group which include benzamide and amide group **(Bayer, 2021).**

2.2 Boscalid (SDHI Inhibitor)

Boscalid is a pyridine carboxamide made by combining the carboxy group of 2-chloronicotinic acid with the amino group of 4'-chlorobiphenyl-2-amine in a formal condensation reaction. Hence it is a biphenyl derivative, a pyridine carboxamide, a monochlorobenzene derivative, and an anilide fungicide. It is made up of nicotinic acid **(PubChem CID 213013)** and is effective against a variety of fungal infections, including Botrytis spp., Alternaria spp. etc. It can be used on a variety of crops, including fruits, vegetables, and ornamentals. It functions as an inhibitor of EC 1.3.5.1 [succinate dehydrogenase (quinone)], a pollutant in the environment, a xenobiotic, and an antifungal agrochemical.

Table 2.1 Physicochemical properties of boscalid (USEPA, 2003; PubChem CID 213013)

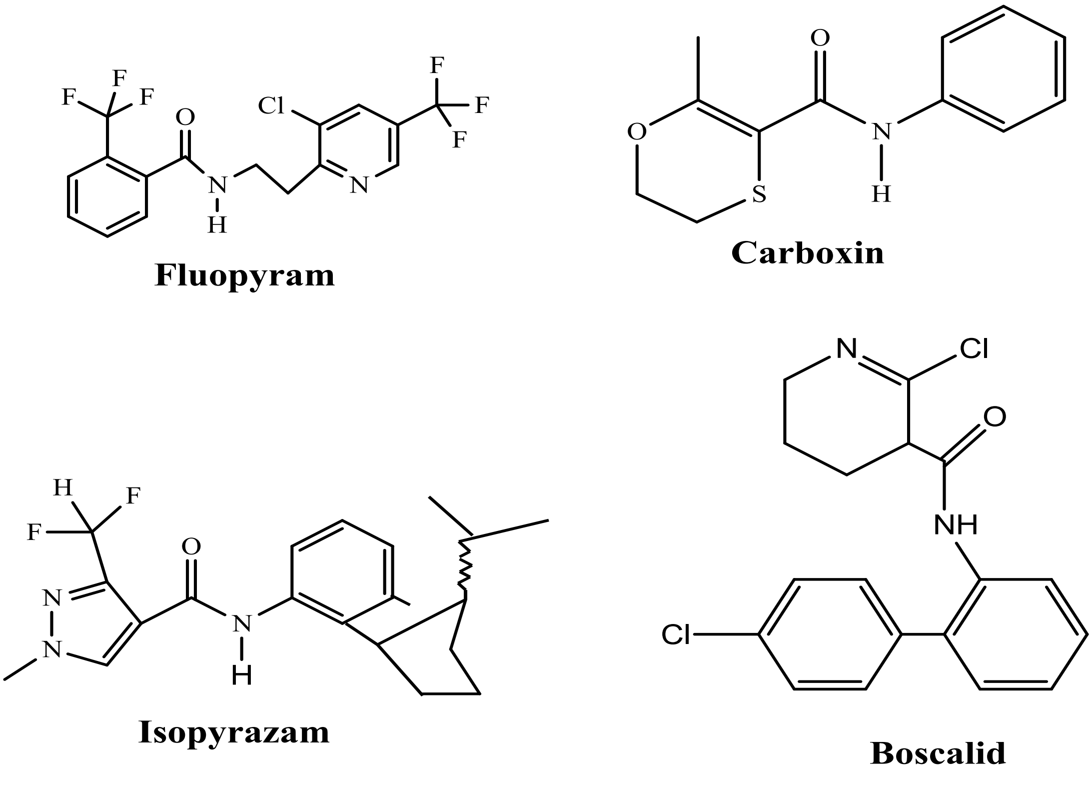
Common name	:	Boscalid
Chemical name	:	3-pyridinecarboxamide,2-chloro-N-(4’chloro [1,1’biphenyl]-2-yl)
CAS Registry Number	:	188425-85-6
Trade name	:	Emerald, endura, and pristine
Year of initial registration	:	2003
Molecular weight	:	343.2 g/mol
Physical form	:	Solid
Colour	:	White powder (TGAI); White crystalline (PAI)
Odour	:	TGAI- faint smoky; PAI- odorless
Melting point	:	143.4-143.6EC (TGAI); 142.8-143.8EC (PAI)
Density	:	1.394 g/cm^3 (TGAI); 1.381 g/cm^3 (PAI)
Octanol/water partition coefficient (K_{ow})	:	Log P o/w=2.96; P o/w = 915 @ 21^{o}C
Vapour pressure at 25^{o}C	:	7x10^{-9} hPa (PAI at 20^{o}C) 2x10^{-8} hPa (PAI at 25^{o}C)
Chemical structure of Boscalid	:	

2.3 Structure Activity Relationship of Carboxamide Fungicide with Chemical Structure of Boscalid

The chemical structure of carboxamide can be divided into three parts**:**

1) The acid core which is different from its ring structure oxathiin (carboxin), pyrazole (isopyrazam), pyridine (boscalid), substituted benzene (fluopyram).
2) Linker which consist up of benzene ring or a 2-carbon aliphatic spacer in fluopyram.
3) Bulky hydrophobic group absent in carboxin **(Scalliet, 2012).**

Chemical structures of some carboxamide fungicide have been depicted in **Figure 2.1**



Fluopyram

Carboxin

Isopyrazam

Boscalid

Figure 2.1 Chemical structures of carboxamide fungicides

General structure required for activity of carboxamide fungicides and related compounds are shown in **Figure 2.2.** These compounds have a general structure depicted in **Figure 2.2** where R = CH_3 and $R^/$ = H or R and $R^/$ form a ring system and unsubstituted aniline ring or having one or more electron donating group. As a consequence, the anilide must be obtained from α, β unsaturated acids, and the β carbon atom must possess a methyl group. Moreover, the double bond may form part of planar, aromatic system (e.g. benzene, furan, oxazole, thiazole) or a non-planar system (e.g., dihydro-oxathin, dihydrofuran, dihydropyran). Implicitly, the p-methyl group of such a system is cis relative to the carboxamide group. If the double bond is part of an open chain system, the þ -methyl group must be cis with respect to the amide functional group. Finally, the nitrogen of the carboxamide group must have a free proton and can replace the aniline ring. In that case, the electron donating group appears to give more activity than the electron withdrawing group **(White, 1975).**

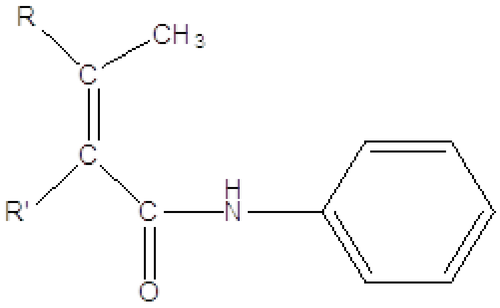


Figure 2.2 Basic structure of boscalid

2.4 Mode of action of boscalid

Boscalid is a systematic fungicide having protective and curative action. It inhibits germ tube elongation, spore germination, sporulation and mycelial growth. It gets translocated acropetally within the plant. Boscalid inhibits the enzyme succinate ubiquinone reductase which is also known as complex II. This enzyme is a component of inner mitochondrial complex which consist of 4 nucleus encoded subunits (SDH A,B,C,D). SDH C and D anchor the complex in membrane while the A and B are present in mitochondrial matrix where they catalyze to oxidation of succinate to fumarate as part of tricarboxylic acid (TCA) cycle. The electrons released are transported to electron transport chain via co-substrate ubiquinol. Complex II plays an important role in fungal metabolism as it transfers the electron for energy production and also forms an essential junction where components of TCA cycle can be diverted to formation of building blocks for amino acid and lipids. Their mode of action is similar to that of strobilurins, but have no risk of cross resistance, as they have different site of action in electron transport chain. Pathogens which have become resistance to other classes of fungicide may be controlled by boscalid **(Kulka, 1995).** The metabolic pathway of boscalid in soil has been shown in **Figure 2.3.**

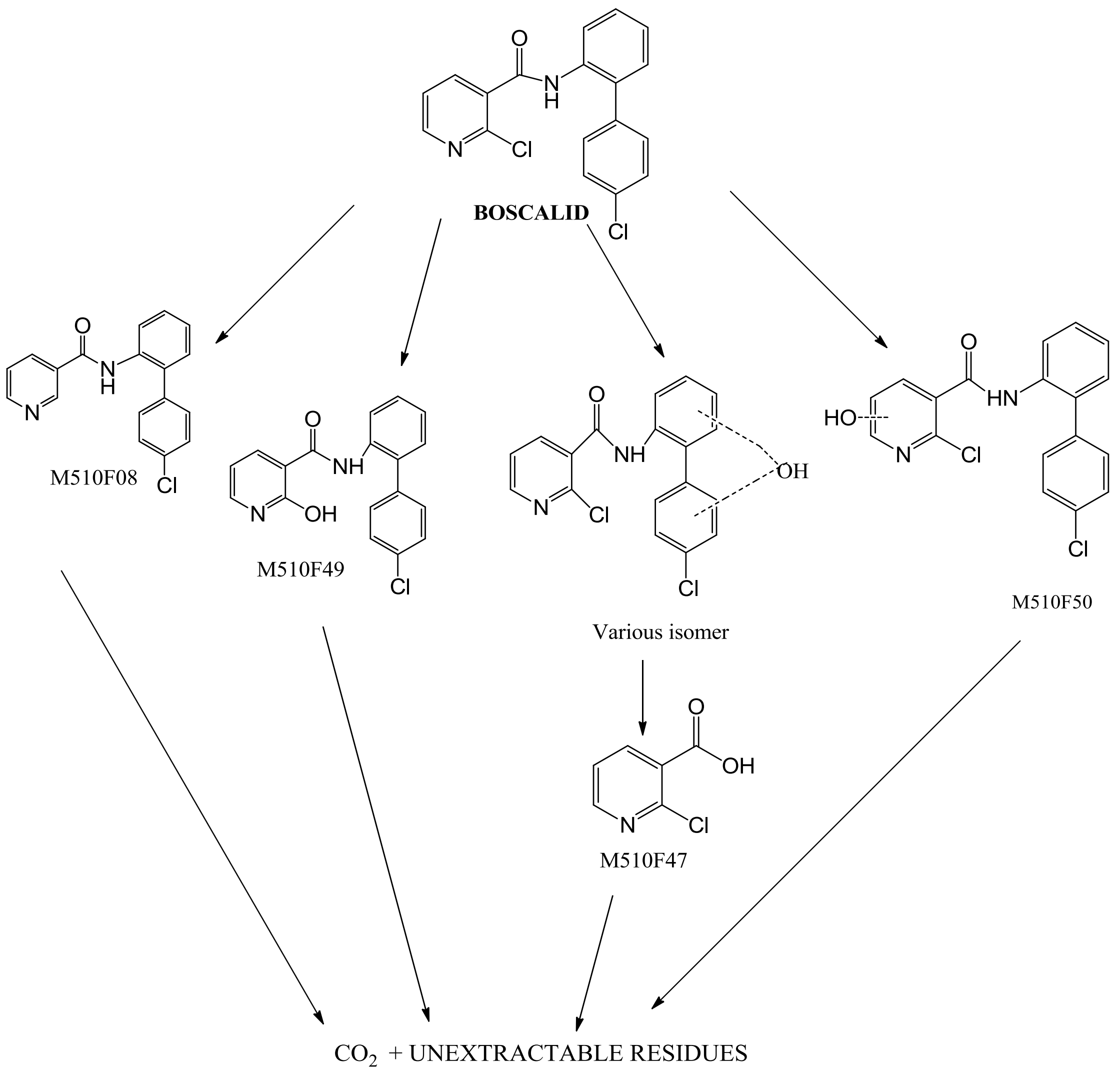


Figure 2.3 Metabolic pathway of boscalid

IUPAC NAME OF METABOLITES FORMED BY DEGRADATION OF BOSCALID IN SOIL

M510F08: N-(4'-chlorobiphenyl-2-yl)nicotinamide

M510F49: N-(4'-chlorobiphenyl-2-yl)-2-hydroxynicotinamide

M510F47: 2-chloronicotinic acid

M510F50: 2-chloro-N-(4'-chlorobiphenyl-2-yl)-hydroxynicotinamide

Aerobic soil metabolism of [^{14}C] boscalid in sandy loam soil showed that boscalid degraded slowly and identified metabolites were minor part of residue and degraded almost at same rate. Pyridine labelled test compound mineralized more quickly to $^{14}CO_2$ than diphenyl labelled. No volatiles were found except $^{14}CO_2$. Non-extractable diphenyl labelled boscalid begins to decline within 266 days and that of soil treated with pyridine labelled continued to increase during whole period of incubation. In case of anaerobic soil metabolism, the experiment was conducted in two soil types that showed that boscalid degraded slowly and identified metabolites were minor part of residue except M510F47, which is formed in maximum amount (6.7%) during study using pyridine labelled boscalid. The non-extractable ^{14}C in soil treated with diphenyl labelled and pyridine labelled continued to increase during whole incubation period **(FAO, 2006).**

2.5 TOXIC EFFECTS OF BOSCALID ON DIFFERENT FLORA AND FAUNA

Qian *et al.* (2018) exposed the freshwater algae (chlorella vulgaris) to boscalid to see if the compound has any inhibitory effect on metabolism of algae. He found that when algae were exposed to different concentrations of boscalid (0, 0.8, 1.6, 2.4 and 3.2 mg/L) for 96 hours to see effects on photosynthetic activity, antioxidant enzyme system and endogenous substances, there was significant inhibition of growth of algae and decrease in content of chlorophyll content and carotenoids by 1.6mg/L boscalid. Chlorella vulgaris Reactive Oxygen Species (ROS) and oxidative damage can be induced by boscalid, consistent with significant changes in ROS levels and various antioxidant enzyme activities. This shows that boscalid could affect the microalgae by disorganizing the normal process of photosynthesis, oxidative damage and energy metabolism. He also investigated chronic toxic effects of boscalid on adult zebrafish (an aquatic organism) for 28 days. Result demonstrated that boscalid damages the liver and kidney of the fish. Boscalid effect caused increase in hexokinase, a content of glycogen, glucose-6-phosphatase and insulin in liver and decrease in blood glucose content and succinate dehydrogenase activity. Boscalid reduced the activity of triacyl glycerides (TG) and cholesterol (TC), fatty acid synthase (FAS), and acetyl coenzyme A carboxylase (ACC) in the liver. Therefore, the expression of genes associated with carbohydrate and lipid metabolism in the liver and intestine was affected by boscalid. The results suggest that boscalid may affect carbohydrate metabolism in adult zebrafish through regulation of gluconeogenesis and glycolysis at 0.1 mg / L. In addition, 0.01 mg / L boscalid can cause increased beta-oxidation and decreased lipid synthesis. In conclusion, their study confirmed that carbohydrate and lipid metabolism is a potential biological pathway that mediates boscalid-induced developmental effects.

Vu *et al.* (2016) investigated effects of boscalid on marine amphipods allorchestes compressa for 6 weeks under laboratory conditions. The amphipod was exposed to boscalid with concentration having 1, 10 and 40 μg active ingredient a.i./L. with 5 replications per treatment. Growth rate and energy reserves were also affected by boscalid and are concentration dependent. Lipid content and reproduction was most affected by it and lipid content was reduced by 53.8% at highest concentration. At 40 μg a.i.L^{-1} antennae deformity was also seen in the amphipod. These effects seen in the amphipod were in environment relevant concentration. The decline and eradication of boscalid can affect environment in adverse way because Allochestes compressa play an important role in trophic transfer and nutrient recycling. The results suggest that it may not pose acute effect to aquatic ecosystem shortly but may pose affect in the long run.

Boscalid risk to aquatic organic does not seems very toxic as it readily gets sorbed to the sediment. Based on estimated exposure levels, the proposed use of boscalid is unlikely to pose an acute or chronic ecological risk to freshwater fish, invertebrates, or estuary / saltwater fish, at multiple application risk as high as 6 application of 0.350 pounds a.i./acre. However, in the case of estuary / marine invertebrates, the acute risk level of vulnerable species is exceeded. Boscalid is not expected to affect aquatic animals that live primarily in the open ocean. Benthic fauna is more likely to encounter boscalid because the chemicals are persistent and tend to sorb to sediments. Boscalid is practically nontoxic to bird species in acute and subacute levels but on chronic exposure of boscalid to strawberry that was incorporated with 0.350 lbs a.i./acre for 7-day reapplication interval results in chronic risk level of concern for short grass consuming birds (RQ=1.08). In case of rats it in nontoxic on acute exposure but in long term for 2 generation it leads to decreased body weight. Ecological impact studies of various terrestrial and semi-aquatic plants have shown less than 25% adverse effects compared to pooled controls at all test endpoints. As a result, EC 25 was above 0.55 lb a.i./acre. Therefore, RQ values for terrestrial and semi-aquatic plants have not been calculated and it is believed that there is no risk to the plants using boscalid below 0.55 lbs/acre **(USEPA, 2003).**

2.6 EFFECT OF BOSCALID ON ACTIVITIES OF SOIL ENZYMES AND SOIL RESPIRATION

Effects of boscalid was investigated on four soil enzymes using flourimetric microplate enzyme assay for 60 days that are directly involved in P-cycling and C-cycling. At 100-200 mg/kg, boscalid inhibited phosphatase activity whereas β-D-glucosidase activity which stimulated in the first 7 days inhibited at 35 to 60th day of incubation, while at 100-200 mg/kg boscalid inhibit phenol oxidase and peroxidase activity in first 7 days, and possessed a stimulating and no inhibitory effect on latter incubation period. Boscalid at 10-100 mg/kg also inhibited respiration activity in soil. These results indicated inhibitory effect of boscalid on soil phosphatase, β-D-glucosidase, and soil respiration, and some positive effects on phenol oxidase and peroxidase in a long term **(Xiong, 2014).** Since boscalid is a complex II inhibitor which is present in both eukaryotic and prokaryotic organism it may affect the non-target soil bacteria present in soil which is useful for soil fertility **(Yang, 2011)**

2.7 EFFECT OF BOSCALID IN CONTROLLING FUNGAL DISEASES

Study was conducted to test the effect of new fungicides like boscalid, fenhexamid, fluazinam and fludioxonil in contrast to vinclozolin, with its effects on growth of Sarracenia minor and Sclerotinia sclerotium in agar plate as well as effect on lettuce drop in the field. Fludioxonil and fluazinam at 0.01μg/ml reduced the growth of Sarracenia minor to 82-84% and 1-16% by boscalid, fenhexamid and vinclozolin. At same concentration, Sclerotinia sclerotium growth was reduced to 78% by fluazinam and 0-12% by boscalid, fludioxonil, fenhexamid and vinclozolin. At 0.1 μg / mL, all chemicals except vinclozolin suppressed Sarracenia minor mycelium growth by 70-98%, but growth of Sclerotinia sclerotium was suppressed by 95-99% by fludioxonyl and fluazinam that was significantly less (40-47%) than boscalid and fenhexamid, but not from vinclozolin. At a rate of 1μg/ml, all fungicides supressed growth of Sarracenia minor and Sclerotinia sclerotiorum by 87-100% and 77-100% respectively. Boscalid and fluazinam provide more diseases control in comparison to fenhexamid, fludioxonil and vinclozolin in lettuce plots infested with Sarracenia minor. In the presence of S. sclerotiorum, the use of fluazinum, fludioxonyl, and vinclozolin resulted in the highest degree of disease suppression, but the least potent compound was fenhexamid. Boscalid and fluazinam was more effective against lettuce drop caused by Sarracenia minor than disease caused by Sclerotinia sclerotiorum **(Matheron, 2004)**

Isolates of botrytis cinerea having five different mutations at three different codons of SDHB which is one of four protein subunits forming succinate dehydrogenase (P225F, N230I, H272L/R/Y) were tested for sensitivity to eight SDHI (succinate dehydrogenase inhibitor) fungicide. In vitro, P225F mutants were resistant to all SDHIs examined. In the same way, isolates with the H272L mutation were highly resistant to boscalid but had low to moderate resistance to other SDHIs. Boscalid, fluopyram, and fluxapyroxad resistance was moderate in the N230I mutants, whereas isopyrazam, bixafen, fenfuram, benodanil, and carboxin resistance was low. The H272R mutants had moderate resistance to boscalid and low resistance to isopyrazam, fenfuram, and carboxin but they were still susceptible to fluopyram, bixafen, fluxapyroxad, and benodanil. Similar to the H272Y, the H272Y demonstrated moderate resistance to boscalid and extremely poor resistance to isopyrazam, bixafen, fenfuram, and carboxin but increased sensitivity to benodanil and fluopyram. In detached fruit experiments, boscalid gave moderate to high control of H272R/Y and N230I mutants, but little control of H272L and P225F mutants. Fluopyram, on the other hand, controlled H272R/Y mutants and had moderate control over H272L, N230I, and P225F mutants. This suggested that sensitivity to SDHI fungicide vary greatly depending upon the point mutation in SDHB subunit **(Veloukas, 2013).**

The hydrophilic flavoprotein (SdhA), the iron-sulphur protein (SdhB), and the two lipophilic transmembrane C- and D-subunits make up SDH (SdhC and SdhD). Mutations that result in amino acid alterations in the SdhB, SdhC, or SdhD subunits of SDH provide laboratory resistance to SDHI fungicides. According to studies.

Furthermore, field isolates resistant to boscalid have mutations in the SdhB, SdhC, or SdhD subunits of A. alternata, resulting in amino acid changes **(Matheron, 2004).** Laboratory tests were performed to assess the risk of developing resistance to boscalid. Boscalid-resistant mutants were obtained by UV treatment at lower frequencies and with smaller resistance factors than the pyrimethanyl-resistant mutants. All boscalid-resistant mutants were also much more sensitive to Qo inhibitors (quinone outside inhibitor group of fungicides used in agriculture) than wild-type parents, and with less in vitro sporulation and pathogenicity to eggplant leaves. The results showed that resistance to boscalid was less likely to develop than resistance to pyrimethanil. However, B cinerea is a high-risk disease and appropriate resistance precautions need to be taken. A synergistic effect of the activities of boscalid and kresoxim-methyl was observed **(Zhang, 2007).**

In Shandong, the main application methods for controlling Botrytis include fruit dipping and spraying. According to their findings, the incidence of B. cinerea resistance to boscalid in the sprayed area increased significantly and rapidly during the five years. Boscalid showed higher inhibitory activity against B. cinerea isolate at the fruit dipping site than the spray application area. Greenhouse studies showed that dipping in 30 mg / liter or spraying 300 mg / liter boscalid was effective in controlling grey mold in the fruit dipping areas. However, neither soaking the fruits nor spraying boscalid succeeded in controlling the grey mold in the spray application area. Fungicide application strategies appear to have a significant impact on fungicide resistance in all of the above phenomena, and application of fruit dips can slow the development of boscalid resistance. (**Cui, 2021)**

Kim *et al.* (2016) tested the effectivenss of six classes of fungicides for controlling Botrytis cinerea pathogen which is a necreotrophic pathogen and causes severe problem in post-harvest of strawberries. Out of the tested fungicides, fludioxonil was most effective in controlling the pathogen (EC_{50}<0.1 μg/ml), whereas pyrimethanil was least effective (EC_{50}=50 μg/ml), for controlling mycelial growth of B.cinerea. fenpyrzamine, Pyrimethanil also showed relatively low effectiveness in controlling germination and conidia production of *B. cinerea*.

2.8 FIELD CONTROL EFFICIENCY OF BOSCALID FUNGICIDE

Chen MF (2007) reported that EC 50 values for boscalid, were found to be 1.0692 μg mL^{-1}. The control efficiency of a 50 percent boscalid WDG (600-times dilute solution) against rice sheath blight on the 14th day after the second spraying was 63.53 percent, which was significantly lower than the control fungicides 20 percent Jinggangmycin A WP (1500-times dilute) and 5 percent Hexaconazole SC (600-times dilute solution).

Smith (2008) compared fluazinam and boscalid in greenhouse studies, as well as a pre-and post-inoculation fungicide or no fungicide applications to reduce S. minor infections. When fungicide was applied before inoculation or up to 2 days after inoculation, there was a significant reduction in successful infections in the greenhouse, but not when it was applied 4 days after inoculation. From 2004 to 2006, field tests were done to compare the efficacy of fluazinam and boscalid, employing alternate sequences of those fungicides or no fungicide for each of three sprays every season. In the field, fungicide applications made before the highest incremental rise in disease incidence offered the best disease control or yield improvement. In both field and greenhouse studies, boscalid outperformed fluazinam by a little margin.

Myresiotis (2008) utilized fifty-five Botrytis cinerea isolates to establish the pathogen's baseline sensitivity to two novel fungicides: boscalid, which inhibits the electron transport chain enzyme succinate dehydrogenase, and pyraclostrobin, which hinders electron transport between cytochrome b and cytochrome c1. Sensitivity to boscalid was determined by inhibiting both mycelial growth and spore germination, whereas pyraclostrobin sensitivity was determined solely by inhibiting spore germination. The sensitivity distribution for both fungicides was a unimodal curve, with mean EC$_{50}$ values of 0.033 g ml^{-1} for pyraclostrobin and 2.09 and 2.14 g ml^{-1} for boscalid based on inhibition of mycelial growth and spore germination, respectively. There was no evidence of a cross-sensitivity association between the two fungicides ($r = 0.09$). Furthermore, there was no cross-resistance between these two fungicides and the botryticides: cyprodinil, pyrimethanil, fenhexamid, fludioxonil, and iprodione. The control efficacy of the two fungicides was also tested against two isolates with anilinopyrimidine resistance and two isolates with benzimidazole resistance, as well as two isolates with wild-type sensitivity. Pyraclostrobin and boscalid were both effective in controlling all six isolates, regardless of their susceptibility to benzimidazoles and anilinopyrimidines. Benzimidazole-resistant isolates were not well controlled by carbendazim, while anilinopyrimidine-resistant isolates were not adequately controlled by cyprodinil.

2.9 FACTORS AFFECTING CHEMICAL DEGRADATION OF PESTICIDE IN SOIL (Bansal, 2012).

1. **Organic matter and clay content:** These two play a major role in chemical degradation of pesticide as they provide large surface area for hydrolytic degradation.
2. **pH:** This generally depends on nature of pesticides i.e. some pesticides are acid hydrolyzed while some other are base hydrolyzed. At slightly alkaline conditions bacteria have better degradable ability for pesticides.
3. **Temperature:** As temperature increases the molecules move and react faster causing hydrolytic process to occur faster.
4. **Nature of substituents:** Some substitutes of pesticides are easily replaced by substrate by hydrolytic reaction since the product form is very stable in water. The substitute that pull the electron density away from substrate encourage hydrolysis.
5. **Effluent irrigation:** Effluent irrigation increases the chemical degradation of pesticides by changing the pH of soil solution and increasing dissolved organic matter content.

2.10 DISSIPATION STUDIES OF BOSCALID

The processes of diffusion, adsorption, hydrolysis, and biodegradation are important for controlling the behavior of pesticides. Through these studies, the contribution of suspended solids and dissolved organic matter to these processes has become more acceptable. In case of laboratory conditions of water sediment system, the factors affecting results are water sediment ratio, sediment phases, and depth of water **(Katagi, 2006).**

Jackson (2003) applied boscalid at two field locations and the time required for 50% of the initial compound concentration to dissipate (half-life) ranged from 27 to 200 days. Field soil studies indicated that the DT50 for the compound was about 108 days. In order to determine how true field dissipation-time kinetic calculations were, a 60-day DT50 (some masked data), 150-day DT50 and 196-day rate constant ($t_{1/2}$) kinetic result were entered into the Pesticide Root Zone Model (PRZM-3) in a California site. A 27-day DT50, and a 200-day $t_{1/2}$ kinetic result were also entered into the model on a Florida site. Outcome indicated that lowest DT50 in PRZM-3 provided the best fit to actual field data. This study suggests that PRZM-3 can be used successfully to address uncertainty of compound behaviour between a laboratory and the actual behaviour observed in the field.

USEPA (2003) conducted dissipation studies of boscalid on bare and cropped land. The DT_{50} for dissipation of the parent compound from the surface solid for boscalid applied to bare ground plots (U.S. and Canada) ranged from 27 to 372 days, were typically greater than 100 days, and frequently exceeded 200 days. For the bare ground plots, the residual carryover ranged from 11.9 to 52.3% of the total treatment. Boscalid treated cropped plots (turf, peach, almond) had DT50 values for parent compound dissipation from the surface soil that ranged from 44 to 360 days and were typically greater than 100 days. For the cropped plots, the residue carryover as a proportion of the entire application ranged from 6.2-20.1 percent.

Lee *et al.* (2008) estimated the dissipation pattern of boscalid in cucumber under greenhouse conditions to establish pre-harvest residue limit (PHRL) and biological half-life. The initial concentration of boscalid in cucumber at the standard application rate was 7.29 mg kg^{-1} and after 15 days with a half-life of 1.9 days it decreased to 0.04 mg kg^{-1}, while the initial concentration was 14.69 mg kg^{-1} (double application rate) it decreased to 0.11 mg kg^{-1} after the same period with a half-life of 2.0 days. Due to fast increase in weight of cucumber during cultivation there was decrease of boscalid residue. The final residue levels of boscalid were predicted based upon dissipation curve and guideline on safe use when it was applied for control of powdery mildew and grey mould.

Chen and Zhang (2010) carried out an experiment to look into the dissipation of boscalid in strawberries and soils. Two doses of boscalid (50 percent water granule) were used (349.5 and 525.0 g a.i./ha). At 0, 1, 2, 3, 5, 7, 14, and 21 days after treatment of boscalid, soil and strawberry samples were taken. The results demonstrated that boscalid dissipation followed first-order kinetics in strawberries, with half-lives of 4.9 and 6.4 days and 6.1 and 8.0 days in soil from the Jinan and Beijing trial locations, respectively. After three days of treatment, boscalid residues in strawberries were below the EU maximum residue level (5 mg/kg). According to this study, boscalid is safe to use on strawberries in the prescribed dosage.

Angioni *et al.* (2011) studied fate of residues of three pesticides in grapes and its transfer during the process of wine making. The residue levels were far below MRLs for grapes according to EU (European union) which accounts for 0.81, 0.43 and 4.23 mg/kg for iprovalicarb, indoxacarb, and boscalid at harvest time respectively. The residue level in samples treated with boscalid showed residue problem when repeated treatment was performed. The pesticides were completely transferred from grapes to must (freshly crushed fruit juice that contain skins, seeds and stems of fruit), but in wine it showed very low or negligible value due to its absorbance by lees (mix of dead yeast, grapes, skins, seed stems and tartrates) and pomace (solid remain of grapes). The GC-ITMS method worked well with reasonable recovery rates in the 75-115% range and quantification limits (LOQs) and quantification limits (LODs) well below the MRL.

Table 2.2 MRL of boscalid for different commodities (FAO, 2006)

Commodity	MRL (mg/kg)
Apple	**2**
Banana	**0.6**
Coffee	**0.05**
Grapes	**5**
Pomegranate	**2**
Berries and other small fruits	**10**
Almond hulls	**15**
Pistachio	**1**
Tree nuts	**0.05**
Stone fruit	**3**
Dried grapes	**10**

Li *et al.* (2015) studied degradation of carboxamide fungicide fluxoapyroxad in which he observed that its degradation is more in soil with higher organic matter and soil with neutral pH as compared to acidic pH as microbial biomass is reduced under acidic pH. He also conducted dissipation studies on sewage water system in contrast to reservoir water system and found out that dissipation was more in sewage water sample as compared to reservoir due to presence of high organic matter and oxygen. Its half lives in reservoir water and sewage water were 82.3 and 75.2 days, respectively. In water sediment system only 20% loss was recorded, which was due to high affinity of fluxapyroxad with sediments. It can be due to its presence in non-ionic form under environmental conditions (pka=12.58) and its lipophilicity (log k_{ow-} =3.08). The biphasic model (Hoerl eq) produced a better fit, yielding high correlation coefficient values ($\geq$ 0.9932) and low residue standard error (RSE$\leq$ 6.05). This model is widely used to describe dissipation of pesticides from water. The degradation half-lives of fluxapyroxamad is 157.6 days indicating that it is a persistent pesticide.

Jankowska *et al.* (2016) treated two varieties of tomato with six fungicides azoxystrobin, boscalid, chlorothalonil, cyprodinil, fludioxonil, and pyraclostrobin under greenhouse conditions to estimate dissipation rate kinetics and behaviour of selected pesticides after processing factor at single and double dose. Risk assessment known as hazard quotient was also performed. The processing studies showed that it had a significant effect in removal of selected fungicides. The processing factor was generally less than 1 and did not depend on variety. Initial deposits concentration of fungicide on tomatoes and concentration after processing posed no risk to human health. The average initial residue of six fungicide for two varieties were in the range of 0.158 - 1.076 and 0.217 - 1.143 mg/kg respectively. At the end of the experiment concentration decreased to 0.090 – 0.541 and 0.121 – 0.568 mg/kg which showed that 99% of initial concentration dissipated over 21 days of experiment. The dissipation rate is initially faster and then becomes slower that shows nonlinear trend of first order kinetic model. The persistence of the fungicides was in the following order: cyprodinil > fludioxonil > pyraclostrobin > boscalid > azoxystrobin > chlorothalonil.

Vallee *et al.* (2016) estimated dissipation rates of three fungicides and three herbicides on four substrates (two soils, sediment and straw). Over 120 days the influence of water content was determined in sequence of three steps (flooded-unsaturated-flooded). The dissipation rates were higher in isoproturon and MCPA (2-methyl-4-chlorophenoxyaceticacid) which varied between 61% to 100% of applied quantities whereas boscalid and tebuconazole showed lowest dissipation rates which were 1.4 to 43.9% of applied quantities during 120 days of incubation. During unsaturated conditions soil and straw had lowest DT50 values and in flooded condition sediment had lowest DT50 value. This showed that most favourable dissipation conditions of soil and straw were observable when drainage ceases. This result suggests that the application of pesticides to man-made wetlands contributes to the long-term effectiveness of these buffer zones in reducing water pollution.

Karlsson *et al.* (2016) applied boscalid to an agricultural location in western Germany in 2010-11, and it was extracted three years later using rapid solvent extraction (ASE). The amount of boscalid in the plough horizon ranged from 0.12 to 0.53 g kg^{-1}, with a field mean of 0.20 - 0.09 g kg^{-1}. These levels were significantly lower than those calculated using literature DT50 values, which predicted a concentration of 16.89 mg/kg based on a 345-day DT50 value. As a result, the field boscalid concentration measured produced just 1.2 percent of the expected value. Furthermore, a short-term incubation experiment with 14 C-labelled boscalid yielded DT50 values of 297-337 days, as opposed to the 345 days reported in the literature. However, when compared to the 104 - 224 days, this DT50 value was still significantly higher than that which was calculated based on field experiment. The result indicated that boscalid dissipation was much faster at agricultural sites with sandy soil types as expected from laboratory incubation experiments.

Taha *et al.* (2016) performed studies on photocatalytic degradation of boscalid under sunlight using N-TiO_2 doped catalyst having higher photocatalytic activity as compared to undoped TiO_2. Persulfate ions increase the photolytic activity of photocatalyst and act as scavenger for elecrtron hole recombination. Persulfate alone is responsible for better degradation of boscalid than TiO_2 and persulfate system under experimental conditions. At 80^0C persulfate totally degrade the 3000 μg/L boscalid and its degradation products in less than 1hour. On the other hand, with the presence of methyl alcohol boscalid degradation forms complex and makes it easier for identification than when boscalid degradation was carried out by persulfate at 80^0C. These identified products were then confirmed by negative MRM (Multi Reaction Monitoring) Method. After that transition that confirm each structure was selected on the basis of structure of best transition for boscalid optimization via negative ESI MS (Electron Spray Ionization). This showed that boscalid degradation occurs efficiently with persulfate with thermal activation with sunlight or by a thermal activation it can be used in poor countries with high pesticide contamination levels.

Mukherjee *et al.* (2016) investigated the dissipation of three contrasting (14) C- labeled pesticides (bentazone, boscalid, and pyrimethanil) in laboratory incubation experiments using sandy soil, biochar produced from pine wood chips, and /or digestate obtained from anaerobic digestion process using maize silage, chicken manure, beef, and pig urine as feedstock. The result indicated that the addition of digestate increased pesticide mineralization, whereby mineralization was not proportional to digestate loads in a mixture, indicating a saturation effect in the turnover rate of pesticides. Mixing biochar into soil generally reduced total mineralization and led to larger sorption / sequestration of pesticides, resulting in the faster decrease of an extractable fraction. Also, the addition of biochar to soil/digestate mixtures reduced mineralization compared to digestate alone mixture but mineralization rates were still higher than for biochar / soil alone. In consequence, the addition of biochar to the soil generally decreased pesticide dissipation times and larger amounts of biochar led to high amounts of non-extractable residues of the pesticide in the substrates. Among the mixtures tested, a mixture of digestate (5%) and biochar (5%) gave optimal results concerning mineralization and simultaneous sorption for all three pesticides.

Jo *et al.* (2017) examined dissipation patterns of boscalid and pyraclostrobin in jujube and their pre-harvest residue limits (PHRLs) based on their dissipation patterns and biological half-lives. Pesticides were sprayed at a normal dose on jujube in two distinct fields. The jujube fruit used in the study were harvested at 0 (2 hours), 1, 3, 5, 7, 10, and 14 days following pesticide treatment. HPLC/DAD equipment was used to measure the residues of both herbicides. As a result, for boscalid and pyraclostrobin, the method limit of quantification (MLOQ) was 0.02 mg/kg for boscalid and pyraclostrobin. With less than 5% CV (coefficient of variation), boscalid recovery ranged from 101.8 to 109.3 percent, and pyraclostrobin recovery ranged from 104.2 to 115.4 percent. An average initial deposit at field 1 and field 2 samples were observed 0.40 and 0.48mg/kg for boscalid and 0.76 and 0.57mg/kg for pyraclostrobin. In field 1, boscalid and pyraclostrobin had biological half-lives of 11.0 and 6.1 days, and 13.2 days and 12.7 days in field 2 respectively.

Chen *et al.* (2019) estimated boscalid and pyraclostrobin in soil and grapes using rapid resolution liquid chromatography triple quadrupole tandem mass spectrometry with QuEChERS technique using multiwalled carbon nanotubes, where both fungicides follow first-order kinetics, with a linear correlation coefficient of 0.995-0.998 at 95% confidence. The highest levels of boscalid and pyraclostrobin in grape, was 3.99 mg/kg and 0.792 mg/kg with a pre-harvest interval of 14 days and doses of 380–570 mg a.i. kg^{-1} in three or four applications respectively. As recommended by the Chinese Ministry of Agriculture, these are below the maximum residue limits for boscalid and pyraclostrobin in grapes, Risk quotients (RQs) were evaluated by comparing national estimated daily intake with acceptable daily intake. The results yielded Risk quotient for boscalid and pyraclostrobin in grape to 54% and 31.7%, respectively, which suggested a low health risk to consumers.

He *et al.* (2020) investigated dissipation dynamics and residue level of boscalid in soil and cucumber using tandem mass spectrometry (LC/MS) to specify the reason of spraying doses and to found out the safe interval period of suspension concentrate (SC). Field trials were conducted and outcome of the result was that the half-life in cucmber was 2.67-9.90 days which followed the first order kinetic model while in case of soil dissipation kinetics it does not follows first order kinetics and half-life was more than 17 days which shows that degradation of boscalid in soil proceed slowly.

Materials and Methods

Chapter 3 MATERIALS AND METHODS

This chapter contains details regarding material and methodology used during the experimental part of the study. The main subheadings presented in the chapter are as follows:

1. Experimental site
2. Methodology
3. Lambda max (λ_{max}) of boscalid
4. Residue analysis with HPLC
5. Calibration curve of boscalid
6. Recovery experiment and determination of LOD and LOQ
7. Dissipation studies

3.1 EXPERIMENTAL SITE

Lab experiments were conducted at the Agro-chemical laboratory, Department of Chemistry, college of basic science and humanities and soil properties were determined in department of soil science, college of agriculture, G. B. Pant University of Agriculture and Technology, Pantnagar, District-Udham Singh Nagar (Uttarakhand) from June to august.

3.2 EXPERIMENTAL MATERIALS

Analytical grade [2-chloro-N-(4'-chlorobiphenyl-2-yl)nicotinamide] boscalid was bought from sigma-Aldrich

3.2.1 Chemicals

1. Acetonitrile (CH_3CN)
2. Sodium chloride (NaCl)
3. Anhydrous Magnesium Sulphate ($MgSO_4$)
4. Distilled water

5. Primary secondary amine reagent
6. Methanol
7. $K_2Cr_2O_7$ solution
8. Conc. Sulphuric acid (H_2SO_4)
9. Orthophosphoric acid
10. Sodium fluoride
11. Diphenylamine (DPA)
12. Ferrous ammonium sulphate (FAS)
13. H_2O_2 (Hydrogen peroxide)
14. Calgon solution (sodium hexametaphosphate)

3.2.2 Glasswares

1. Beakers
2. Funnels
3. Burettes
4. Measuring cylinder
5. Laboratory bottles
6. Volumetric flasks
7. Filter papers
8. Filtration assembly
9. Centrifuge tubes
10. Stopper
11. Conical flasks
12. Micropipettes with tips

3.2.3 Instruments

1. Electrical analytical balance (Citizen)
2. Centrifuge (Remi)
3. Deep freezer (Remi)
4. Vortex (Biogen)
5. Sieve
6. Sonicator (Spectralab)
7. Quartz water distillation unit
8. Sieve
9. Conductivity meter (Model systronics 306)
10. pH meter (Model systronic 361)
11. Mechanical shaker
12. UV-VIS Spectrophotometer
13. Ultimate 3000 HPLC-Dionex model 322 coupled with a UV detector automatic pumping system, rhenodyne injector with a loop of 20ul, gradient controller, UV-VIS detector of variable wavelength, and Chromeleon software client (version-6.8SP4)

3.2.4 COLLECTION OF SOIL SAMPLE

The soils for carrying out dissipation experiments were collected from Crop research centre, Pantnagar. The soil samples were dried in shade for 2 weeks, crushed and passed through sieve of 2 mm, they were then placed in two plastic tubs for carrying out the dissipation studies on boscalid.

3.3 EXPERIMENTAL METHODOLOGIES

3.3.1 Physiochemical properties of soil

The processed soil samples were analyzed for their fundamental properties by applying methods listed in **Table 3.1.**

Table 3.1 Methods used for determining soil properties

Properties	**Methods**
Soil pH	Combined glass electrode pH meter (1:2 soil water suspension)
Electrical conductivity	Conductivity meter (1:2 soil water suspension)
Organic carbon	Modified Walkely and Black methods as described by **Jackson (1958)**
Soil Texture	Bouyoucos Hydrometer Method **(Bouyoucos, 1962)**

3.3.1.1 Soil pH

The pH of an aqueous solution was calculated using hydrogen ion activity. Using the glass electrode on a microprocessor-based pH metre, soil pH was determined at a 1:2 soil-water suspension ratio after 30 minutes of equilibrium **(Jackson, 1958).** A 50 mL beaker containing 10g of soil was then filled with 20 mL of distilled water. A glass electrode pH metre was then used to measure the pH after the mixture had been periodically agitated for 30 minutes.

3.3.1.2 Electrical conductivity of soil

Soil electrical conductivity was measured in 1:2 soil aqueous solution at 25^0C using a microprocessor based digital conductivity meter and is expressed in dS/m^{-1} **(Bower and Wilcox, 1965).** 10gm soil was taken in a 50 ml beaker to which 20 ml distilled water was added while stirring the contents with a glass electrode which was then kept for overnight without disturbance. The supernatant was separated and its electrical conductivity was determined using a conductivity meter

3.3.1.3 Organic carbon

The organic carbon content of soils was assessed using Walkley and Black methods as described by **Jackson (1958).** In this method, 1g of processed soil sample was taken in a 500 mL conical flask that had been sieved using a 0.5 mm sieve. Then, slowly add 20 mL of concentrated H_2SO_4 and 10 mL of 1N $K_2Cr_2O_7$. Following a gentle shaking for combining the ingredients, the flask was permitted to stand for 30 minutes so that allow the soil organic carbon get oxidised. The flask's contents were then diluted out using 200 mL of distilled water. The next step involved the addition of 30 drops of diphenylamine indicator to 10 mL of 0.2 g of NaF (sodium fluoride) and 10 mL of 85% H_3PO_4 to the mixture in conical flask. Back titration of unreacted chromic acid with 0.5 N ammonium ferrous sulphate was carried out till the colour changed from dull green to turbid blue and then, at the very end, back to a brilliant green. This marked the end point and the same procedure was carried out without using soil as a blank. Soil organic carbon was then determined using the following formula:

$$Soil\ Organic\ carbon\ (\%) = \frac{Me.o_x - Me._{Red}}{weight\ of\ soil\ sample\ x\ 0.76} x 0.003 x 100$$

Where,

$Me._{ox}$ = Volume of potassium dichromate x Normality of potassium dichromate.

$Me._{red}$ = Volume of ferrous ammonium sulfate x Normality of ferrous ammonium sulfate

0.003 = Milli equivalent weight of carbon.

100 = Percent conversion factor.

0.76 = Fraction of organic carbon that was oxidized to carbon dioxide.

3.3.1.4 Texture determination

The soil texture was determined by relative proportions of sand, silt, clay in soil sample using bouyoucos hydrometer method **(Bouyoucos, 1962).** In this experiment, 50 gm soil was taken and poured in a 1 litre measuring cylinder, followed by mixing with Calgon reagent (sodium hexametaphosphate) so that soils do not form aggregates. The soil sample was shaken with Calgon reagent for 10 minutes and after addding 400 ml of distilled water it and left unshaken overnight. After that a plunger was inserted and carefully mixed for 30 seconds until a suspension is obtained. After 40 seconds of adequate suspension mixing, a hydrometer was inserted to determine percentage of (silt+clay). Temperature should be noted before taking hydrometer reading.After 2 hours we can again take reading from hydrometer and the amount of clay was determined in suspension.The soil sample was texturally classified using USDA equilateral triangle as shown in **Figure 3.1.**

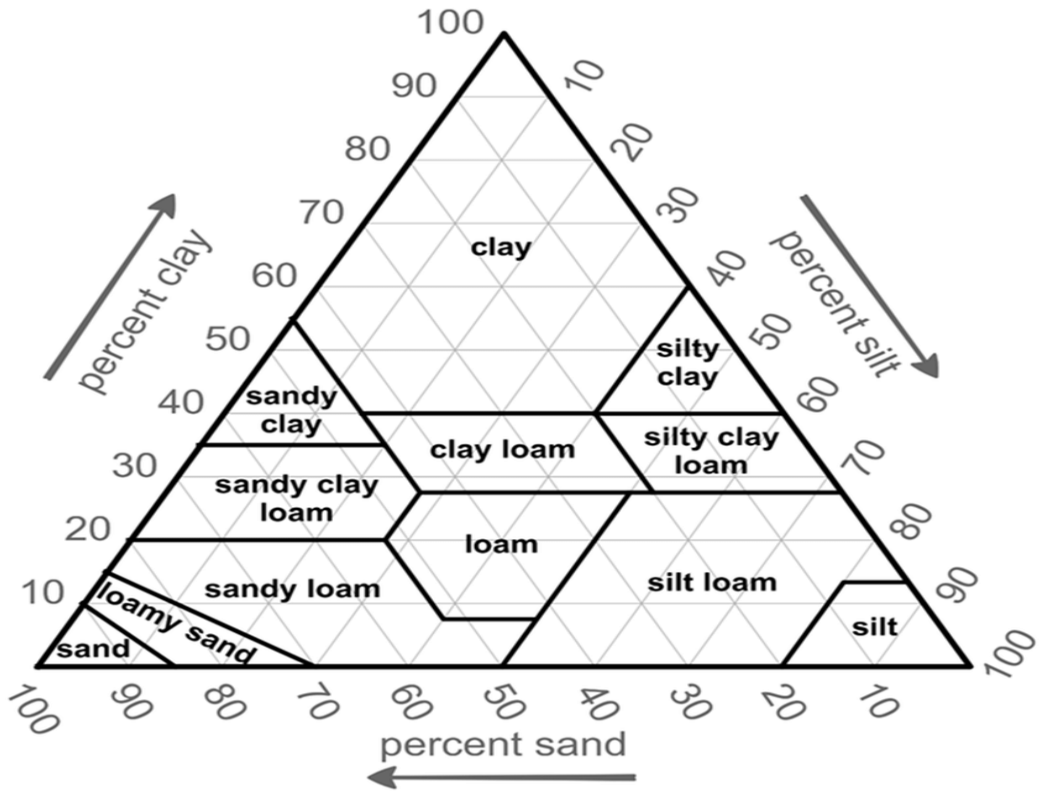


Figure 3.1 USDA textural triangle

The percentage of sand, silt and clay was calculated as follows:

For first 40 sec-

(Silt + clay) % $= \frac{(S-B)\pm CF}{weight\ of\ soil\ sample} x100$

After two hours-

Clay%$= \frac{(S-B)\pm CF}{Weight\ of\ soil\ sample} x100$

CF= (Suspension temperature – 20^0C) x 0.2

Silt%= (silt + clay) - clay

Sand%= 100% - (silt% + clay%)

Where,

S= Sample reading

B= Blank reading

CF= Correction factor

“+ve’’ if temperature of solution < 20^{o}C

“-ve’’ if temperature of solution > 20^{o}C

3.4 DETERMINATION OF λ_{max}

A 2mg mL^{-1} solution of boscalid was prepared for finding out the λ_{max} by UV-VIS spectrophotometer using acetonitrile as reference. This concentration was scanned between UV ranges of 200-400 nm. The maximum absorbance taken as λ_{max} was observed at 245nm which was depicted in **Figure 3.2.**

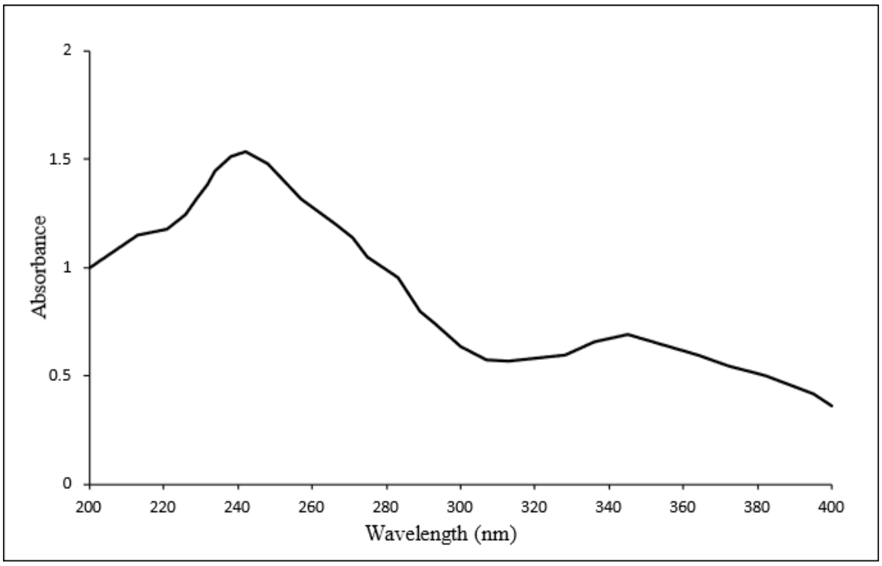


Figure 3.2 Graph between Absorbance and Wavelength

3.5 HPLC CONDITION OF BOSCALID ANALYSIS

The HPLC conditions utilized to determine boscalid in soil were:

a) Mode: Isocratic

b) Column: C18

c) Mobile phase: 60:40 (ACN: Water containing 0.1% orthophosphoric acid (v/v))

d) Flow rate of mobile phase: 1ml min^{-1}

e) Detector: UV at 245 nm

f) Run time: 11 minutes

The retention time (Rt) of boscalid was determined to be 8.9 minutes under the aforementioned chromatographic conditions.

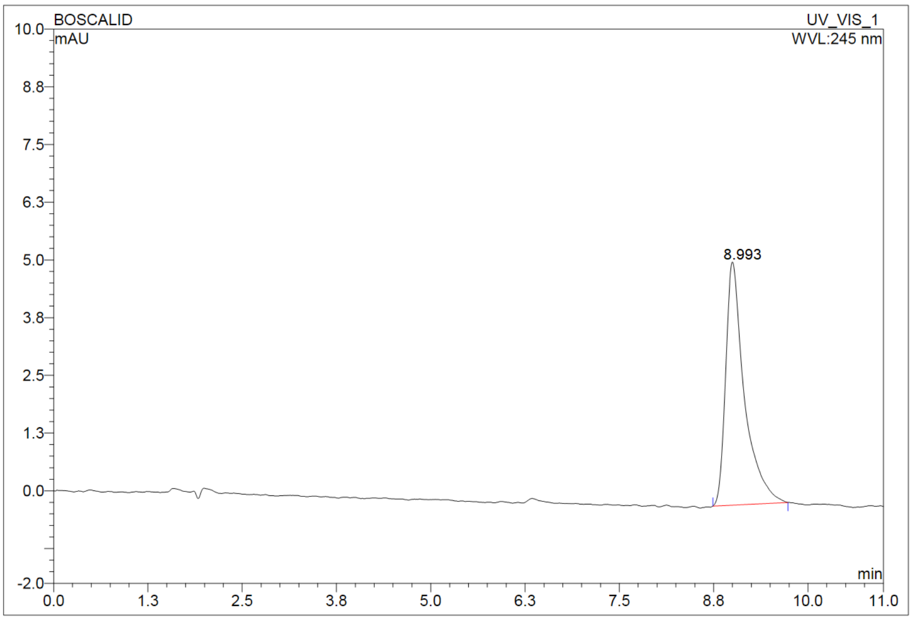


Figure 3.3 Standard Chromatogram

3.6 CALIBRATION CURVE OF BOSCALID

The calibration curve was plotted to determine the concentration of boscalid at various time intervals. Analytical grade boscalid was dissolved in acetonitrile (ACN) in a 100 mL volumetric flask to prepare a standard stock solution of 100 µg mL^{-1} up to the mark. The solution was further diluted to 10 µg ml^{-1} and then serial dilutions of various concentrations of 0.2, 0.5, 1, 2, 3, 4, and 5 µg mL^{-1} were prepared with HPLC grade acetonitrile. For analysis, 20 µl aliquots were injected into the HPLC system each time. The peak area of each concentration was recorded and a calibration curve was plotted between the concentration on x-axis and peak area on y-axis. Detector response of boscalid showed a good linearity within the concentration range as presented in **Figure 3.4.**

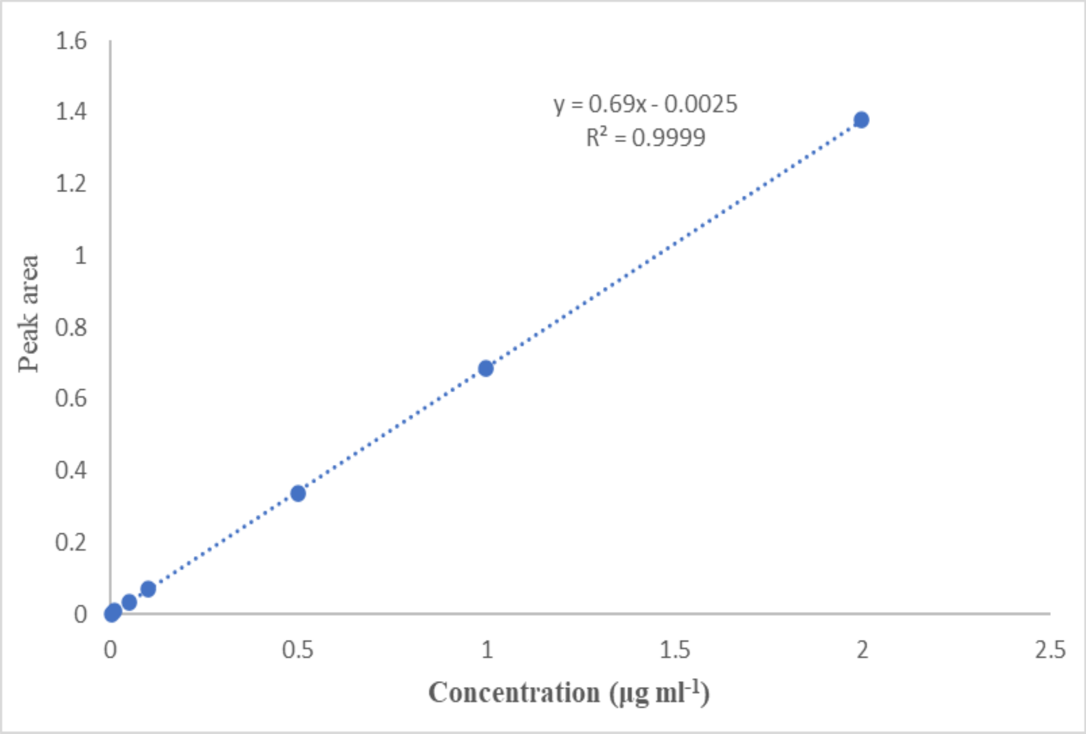


Figure 3.4 Calibration curve of boscalid at different concentrations

3.7 RECOVERY EXPERIMENTS

Recovery experiments were conducted to establish the reliability of the analytical method and to determine the efficiency of the steps used to extract and analyze boscalid in the soil. Fifty-gram soil samples were supplemented with 0.05, 0.5, and 1.0 µg g-1 analytical grade boscalid standards. The sample was drawn out after one hour of fortification and analyzed. The recovery rate was calculated as:

$$\textbf{Recovery\%}=\frac{concentration\ of\ pesticides\ in\ fortified\ samples}{concentration\ of\ analytical\ standard\ of\ pesticide}\,x100$$

3.8 LOD AND LOQ

The limit of detection (LOD) and limit of quantification (LOQ) values of boscalid were determined on the basis of signal to noise ratio before proceeding to analysis of dissipation experiment. The LOD and LOQ value for boscalid were .004 and .012µg mL^{-1} as shown in **Figure 3.5 and Figure 3.6.**

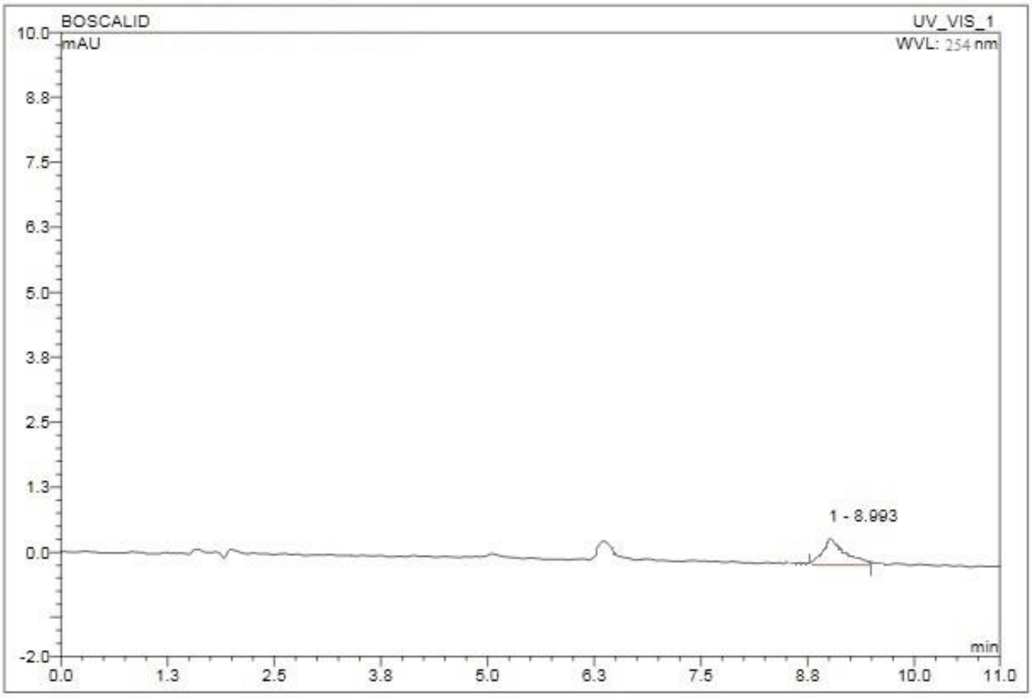


Figure 3.5 Chromatogram depicting LOD (0.004 µg mL^{-1}) of boscalid

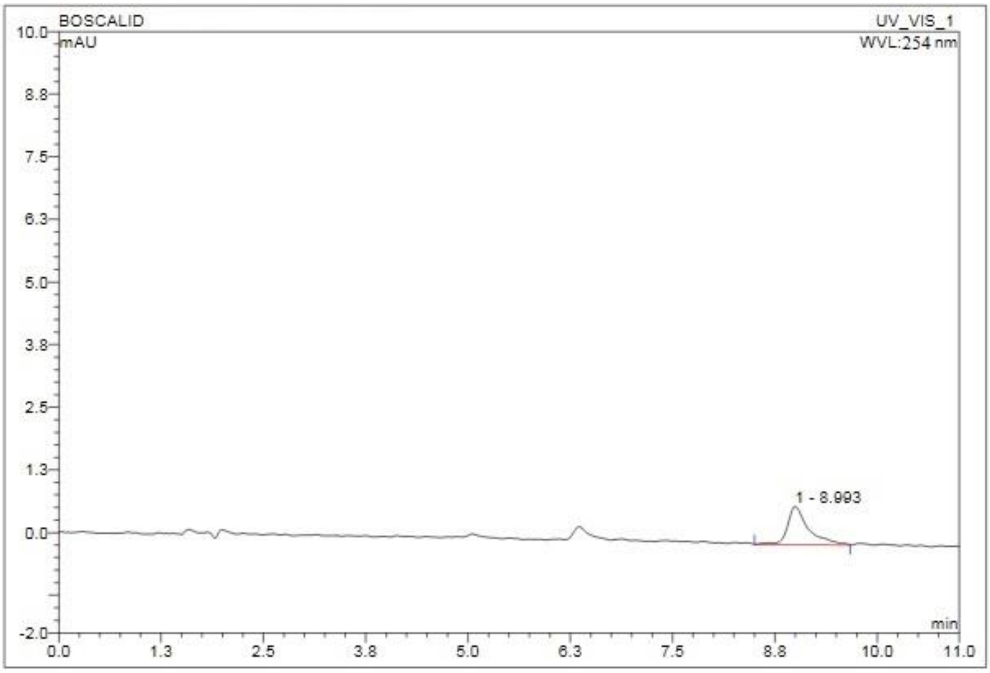


Figure 3.6 Chromatogram depicting LOQ (0.012 µg mL^{-1}) of boscalid

3.9 DISSIPATION STUDIES

Dissipation studies of boscalid was carried out in two different texture soils. Both soils were dried out and passed through 2mm sieve. Two replicates of both the soils were collected in two plastic tubs and inoculated with 1 and 2 µg g^{-1} of boscalid stock solution. The day we inoculated the fungicides is considered as zeroth day where the recovered fungicide is considered as 100% and we can calculate the dissipation percentage of other days by taking zero day as 100%. The dissipation studies were carried out on 0, 1, 3, 5, 7, 14, 21, 28, 35, 42 and 60 days and analysis was done using HPLC-UV after extraction with QuEChERS method.

3.10 METEOROLOGICAL DATA DURING DISSIPATION STUDIES

The weekly meteorological data depicting maximum and minimum temperatures, relative humidity, rainfall and sunshine hours during experimental period has been presented in **Table 3.2.**

Table 3.2 Weekly Meteorological data during experimental period

Date	Temperature (ºC)		Relative Humidity		Rainfall (mm)	Sunshine (Hrs)
	Max.	Min.	Max.	Min.		
26 June- 02 June	33.85	27.42	89.28	65.71	5.5	9.8
03 July- 09 July	35.57	28.85	89.42	60.85	6.77	9.3
10 July- 16 July	37.42	29.00	84.00	50.14	6.04	9.5
17 July- 23 July	34.00	27.71	90.85	66.57	5.61	9.0
24 July- 30 July	31.71	26.42	96.57	73	4.4	8.7
31July-06 Aug	31.57	26.28	95	75	3.1	8.6
07-13 Aug	34.71	27.85	86.14	59.42	6.3	8.2
14-20 Aug	33.57	27.28	90.71	61.57	2.7	8.5
21-27 Aug	34.28	27.57	90.28	59.28	1.2	8.4

3.11 METHODOLOGY FOR DISSIPATION STUDIES

The details of experiment are as given below:

a) Both types of soils are placed in 4 plastic tubs in equal amount and fortified with 1 and 2 μg g^{-1} of boscalid fungicide respectively

b) The dissipation study was carried out by drawing the soil samples at different time intervals i.e. 0 (2hrs), 1, 3, 5, 7, 14, 21, 28, 35, 42 and 60 days after boscalid application.

c) The soil samples were taken by spatula and extraction of boscalid was done.

d) The method of extraction from soil was optimized. The recovery study was also performed at three different concentrations for validation of analysis method.

e) When the extraction process of each day was over, the residue of boscalid were quantified by HPLC (High Performance Liquid Chromatography).

f) The limit of detection (LOD) and limit of quantification (LOQ) of the instrument on the basis of signal to moise ratio were also determined.

3.12 EXTRACTION METHOD

The extraction of boscalid from soils was done by QuEChERS method which is a streamlined and effective extraction and cleanup approach for an analytical chemist to examine residue in the matrix **(Anastassiades *et al*., 2015).**

Five grams of soil from each tub was taken in 50 mL centrifuge tubes and to it 5 mL of distilled water, and acetonitrile was added to it. The contents were vortexed for 2 minutes and allowed to stand. After this anhydrous $MgSo_4$ (magnesium sulfate) and sodium chloride (NaCl) in the ratio of 2:1 was added to it. The mixture was again vortexed for 2 minutes and then centrifuged for 5 minutes at 4000-5000 rpm. After phase separation, the upper layer (organic layer) was decanted in a separate centrifuge tube containing 150 mg $MgSO_4$ and 50 mg PSA for clean-up, and once again this mixture was centrifuged for 5 min at 5000 rpm. The contents were filtered through a 0.45 μm PTFE (Polytetrafluoroethylene) disc filter prior to HPLC analysis. The quantification of boscalid in the soil was done by injecting 20μl of extract in Dionex Ultimate 3000 HPLC system and peak area was recorded.

3.13 STATISTICAL ANALYSIS

Statistical procedures for determining regression (R^2), standard deviation (SD), relative standard deviation, correlation etc. were applied on the data obtained.

Results and Discussion

Chapter 4 RESULTS AND DISCUSSION

In this chapter the outcome of physicochemical properties and dissipation studies of boscalid fungicide in two soils at two different concentrations (1 and 2μg g^{-1}) have been presented and discussed.

Table 4.1 General physicochemical properties of two soils

Physicochemical properties of soils	
Soil pH	
Soil sample A	7.34
Soil sample B	7.39
Electrical conductivity (dSm^{-1})	
Soil sample A	0.122
Soil sample B	0.364
Organic carbon (g Kg^{-1})	
Soil sample A	16.80
Soil sample B	14.24
Textural analysis	
Soil sample A	
Clay	9.2%
Silt	12%
Sand	78.8%
Soil texture	Sandy loam
Soil sample B	
Clay	25.2%
Silt	22%
Sand	52.8%
Soil texture	Sandy clayey loam

4.1 PHYSICOCHEMICAL PROPERTIES OF SOIL

The data in **Table 4.1** depicts pH, electrical conductivity, organic carbon, and texture of two soils (A and B) taken for studies. The pH of the soil samples of A and B are nearly the same which shows that both the soil samples are slightly alkaline in nature. The electrical conductivity (EC) of soil sample A is lower than that of the soil sample B but the soil organic carbon of soil A is slightly higher that of B. The soil texture analysis revealed that soil sample A has a sandy loam whereas soil sample B has sandy clayey loam texture.

4.2 RECOVERY OF BOSCALID FROM TWO SOIL SAMPLES

Recovery studies were performed to establish the reliability of the analytical method used for extraction of boscalid fungicide from soil. The recovery of boscalid from two soils has been depicted in **Table 4.2.** As evident from the Table the average recoveries of boscalid in soils at two different concentration (0.5 and 1µg g^{-1}) ranged between 80.59 to 98.97% and can hence be considered satisfactory. However, as evident from the table, the recovery of boscalid was higher in sandy loam soil at both the fortification levels.

Table 4.2 Recovery of boscalid from fortified soil samples at two different concentrations

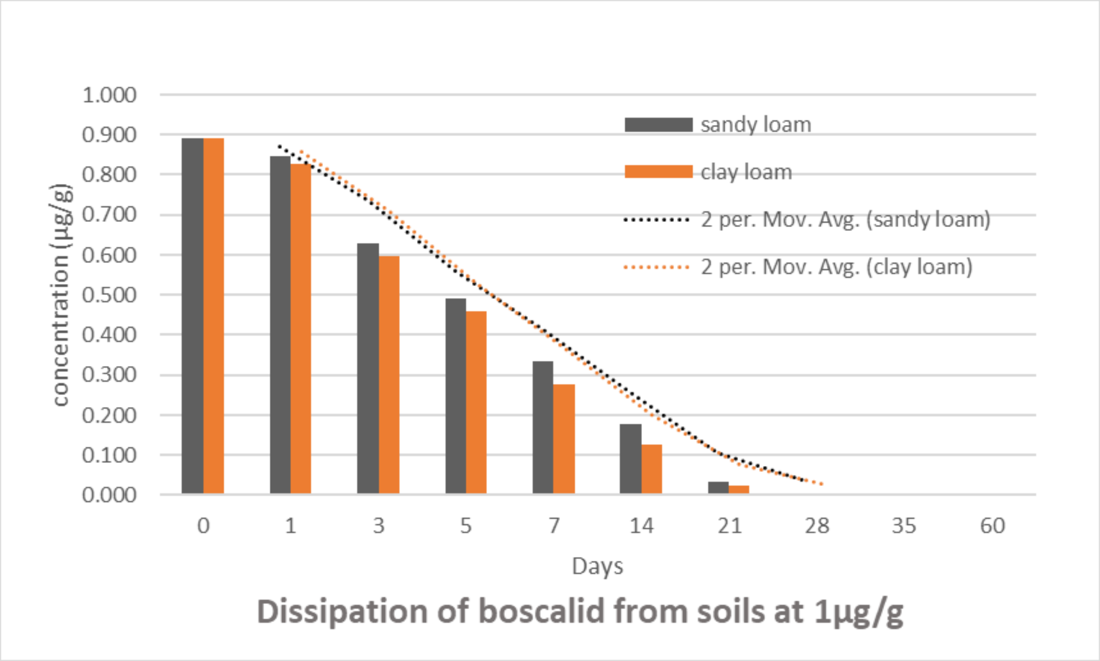
Soil samples	Mean ± S.D.			
	0.5µg g^{1}	1µg g^{-1}	0.5µg g^{-1}	1µg g^{-1}
Soil sample A (sandy loam)	82.08% 86.87% 81.49%	93.57% 95.77% 98.97%	83.48±2.9	96.1±2.7
Soil sample B (sandy clayey loam)	80.59% 80.59% 86.56%	90.51% 91.97% 91.97%	82.58±3.4	91.4±0.8

4.3 Calibration curve LOD and LOQ

A linearity check was done at seven different concentration levels, ranging from 0.005 to 2μg mL^{-1} of boscalid using HPLC. The detector showed a good linearity in the above concentration range as depicted in **Figure 3.4.** The LOD and LOQ were also determined on the basis of signal to noise ratio and have been shown in **Figure 3.5 and 3.6** (Materials and method section). The LOD and LOQ values were found to be 0.004 and 0.012 μg g^{-1} respectively.

4.4 DISSIPATION STUDIES OF BOSCALID IN TWO DIFFERENT SOILS

Boscalid dissipation investigations were carried out under the lab conditions in both the soil samples A and B at 1 and 2μg g^{-1} concentration levels in triplicate. The dissipation study was performed during different time intervals from 0 (2h) to 60 days **(Figure 4.1).** Through this study the boscalid residue concentration persisting at different time intervals and percent boscalid dissipation values were determined. **Table 4.3** depicts the average persistence and dissipation of boscalid on different days. The amount of boscalid residue recovered after the 0th day (2h) extraction process was taken as 100 % persistent value and the amount of boscalid residues at different intervals (1 to 60 d) were calculated relative to this value. From the data obtained in **Table 4.3** it can be clearly observed that the boscalid degraded uniformly with time with no rapid and slow dissipation rate. At lower level of fortification (**1μg g^{-1}**) boscalid could be detected up to 21 days only but at higher fortification level i.e.2μg g^{-1}, it persisted till 28 days. However, boscalid was below detection limit (BDL) at both the fortification rates thereafter i.e on 35, 42 and 60 days.



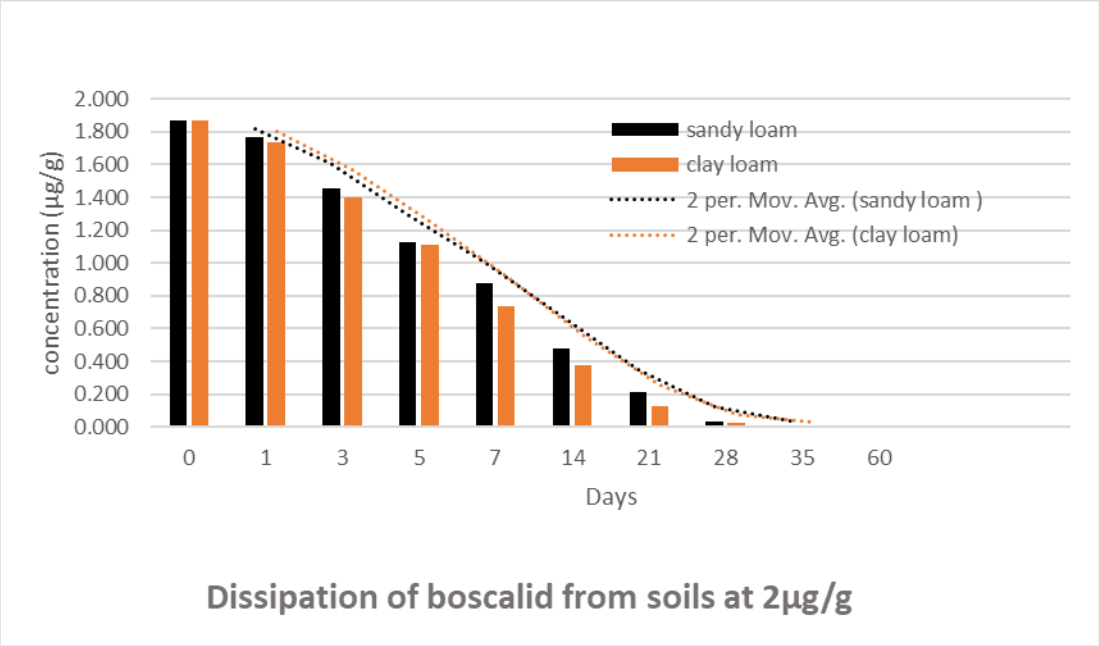


Figure 4.1 Dissipation rates of boscalid in soils at 1 and 2 µg g^{-1} concentrations

Table 4.3 Persistence of boscalid in two different soils at two concentrations

Days	**Soil sample A**		**Soil sample B**	
	1μg g^{-1}	**2μg g^{-1}**	**1μg g^{-1}**	**2μg g^{-1}**
0	0.892±0.003 (0.00)	1.870±0.004 (0.00)	0.891±0.004 (0.00)	1.870±0.010 (0.00)
1	0.846±0.006 (5.21)	1.769±0.001 (5.40)	0.826±0.007 (7.29)	1.739±0.011 (7.01)
3	0.628±0.007 (29.65)	1.452±0.024 (22.33)	0.598±0.006 (32.89)	1.402±0.014 (25.01)
5	0.491±0.003 (44.96)	1.130±0.006 (39.56)	0.461±0.008 (48.27)	1.110±0.008 (40.63)
7	0.336±0.004 (62.38)	0.875±0.003 (53.19)	0.276±0.010 (69.05)	0.735±0.008 (60.68)
14	0.177±0.001 (80.20)	0.479±0.006 (74.41)	0.127±0.007 (85.78)	0.379±0.014 (79.75)
21	0.035±0.006 (96.12)	0.213±0.004 (88.62)	0.025±0.011 (97.23)	0.129±0.011 (93.12)
28	BDL	0.033±0.0014 (98.24)	BDL	0.034±0.008 (98.18)
35	BDL	BDL	BDL	BDL
42	BDL	BDL	BDL	BDL
60	BDL	BDL	BDL	BDL

Values in parenthesis show percent dissipation of boscalid; BDL < 0.012 μg g^{-1}

As evident from the **Table 4.3** the dissipation of boscalid was faster for sandy loam (Soil sample A) in comparison to the sandy clayey loam soil (Soil sample B). Some important parameters which play an important role in pesticide persistence and degradation in soil are

(i) physicochemical and biological properties of the soil which include texture especially clay content, organic matter, pH etc. and the presence of fauna and microbial population of soil

(ii) chemical characteristics of the pesticide itself and

(iii)the climate like wind, rainfall, temperature etc.

Through our study **(Table 4.3),** it is evident that boscalid does not persist for long duration in the soils of this region as more than 90% of the pesticide gets dissipated within 35 days at both the levels of fortification.

The chromatograms depicting boscalid persistence in sandy loam and sandy clayey loam soils at two fortification rates i.e. 1.0 and 2.0 µg g^{-1} along with control are depicted in **Figure 4.2 to 4.37.**

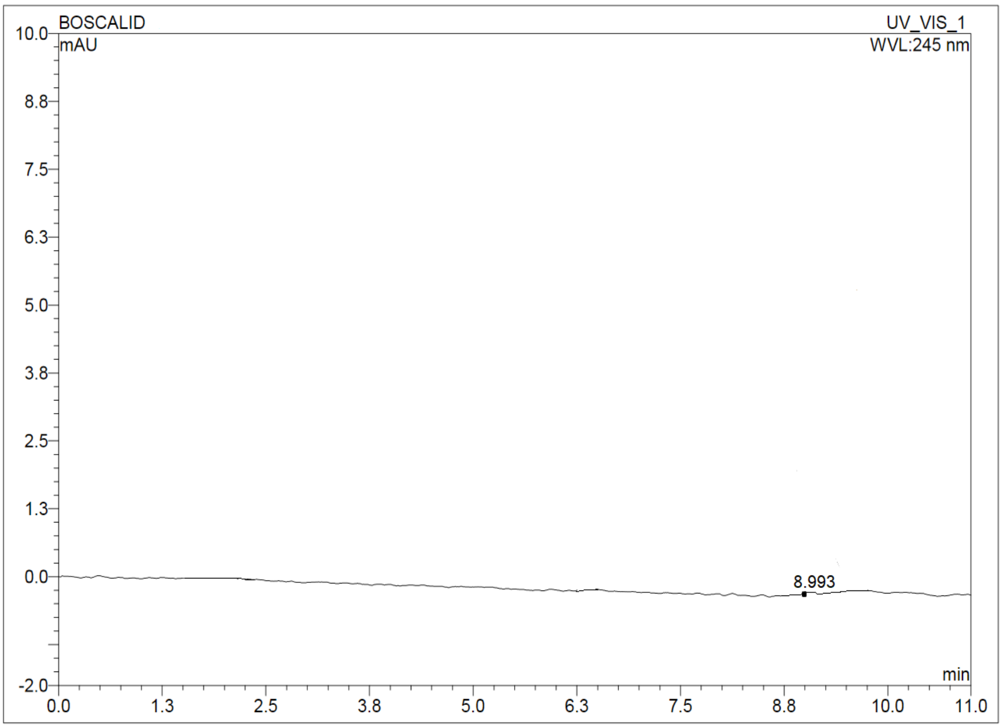


Figure 4.2 Control of soil sample A

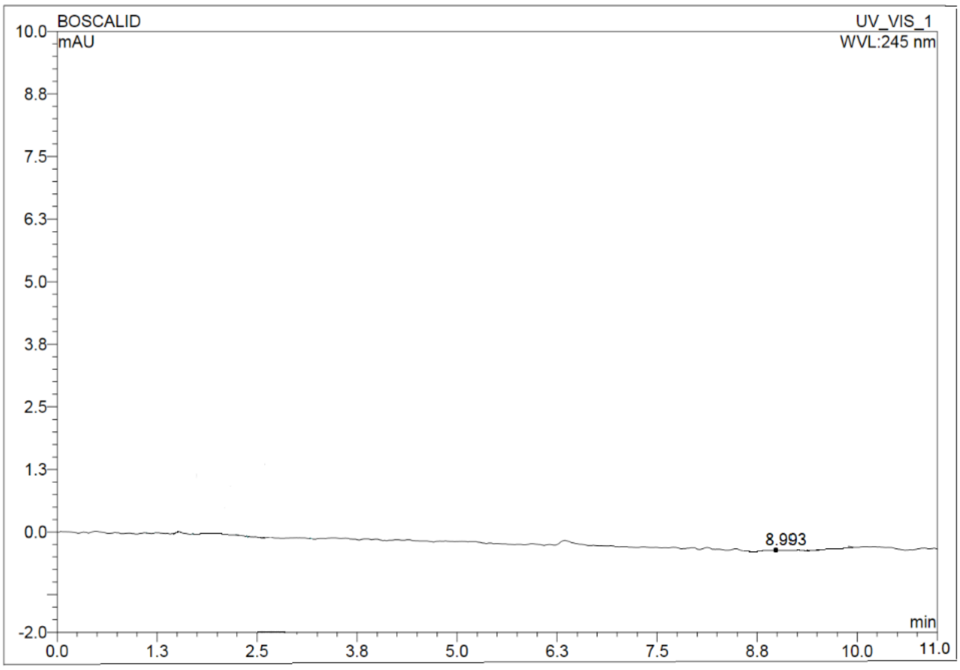


Figure 4.3 Control of soil sample B

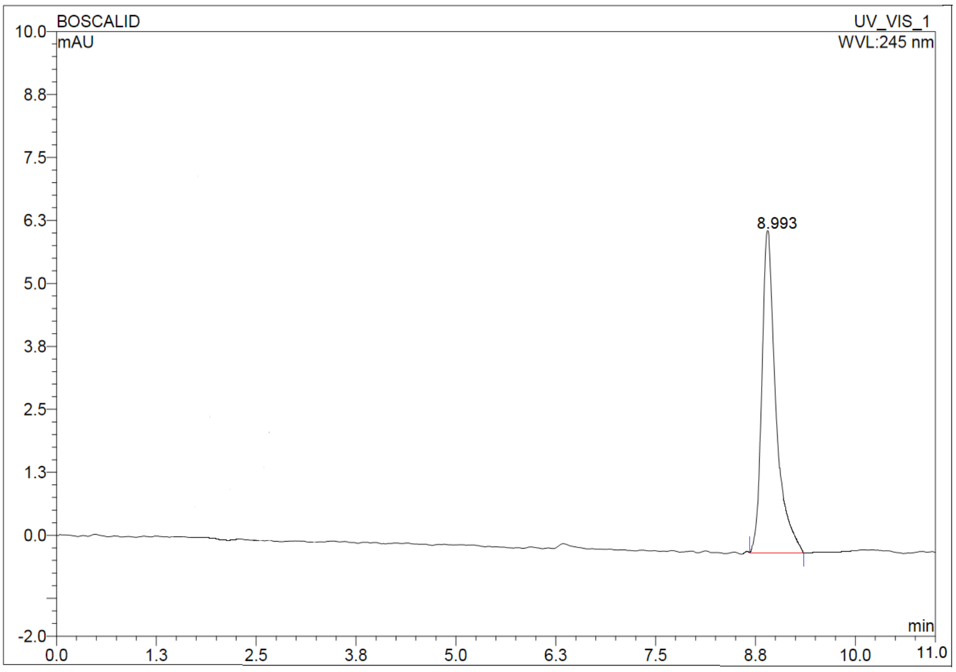


Figure 4.4 Persistence of boscalid in soil sample A @1µg g^{-1} (0^{th} day)

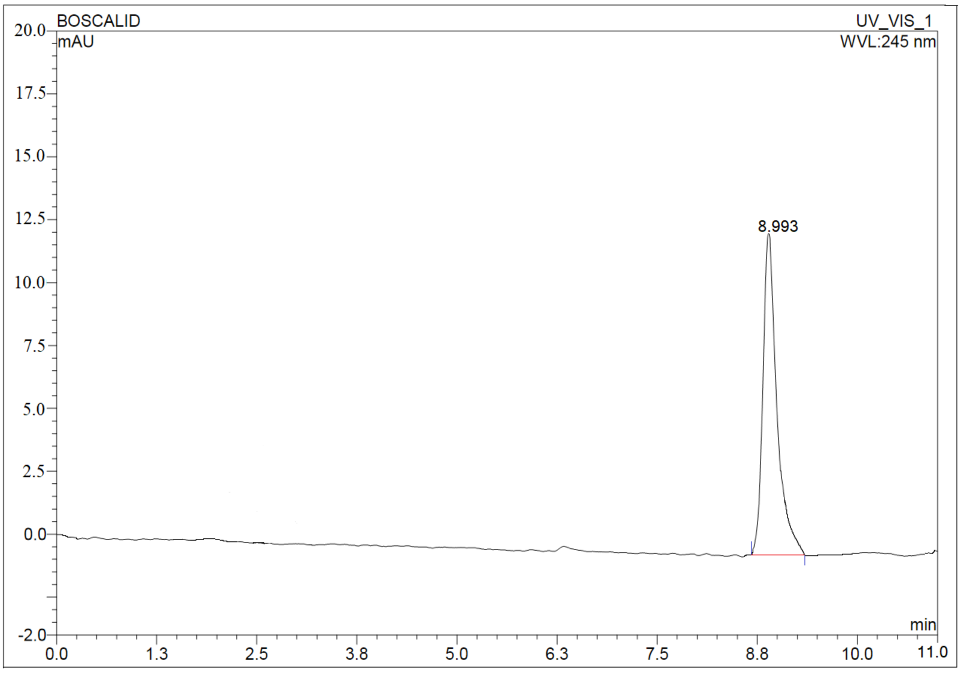


Figure 4.5 Persistence of boscalid in soil sample A @ 2µg g^{-1} (0th day)

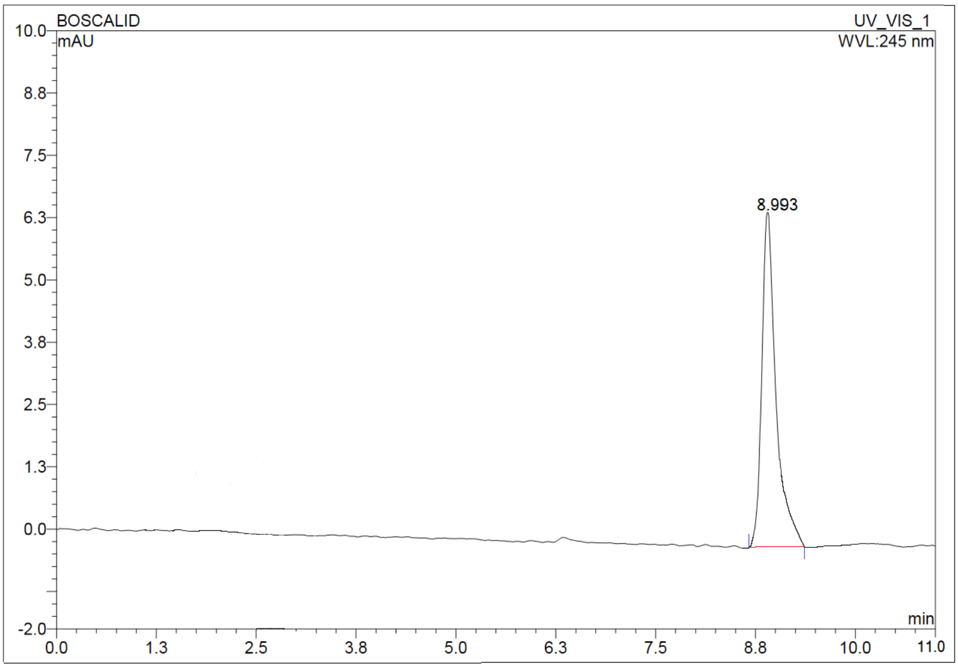


Figure 4.6 Persistence of boscalid in soil sample B @ 1µg g^{-1} (0th day)

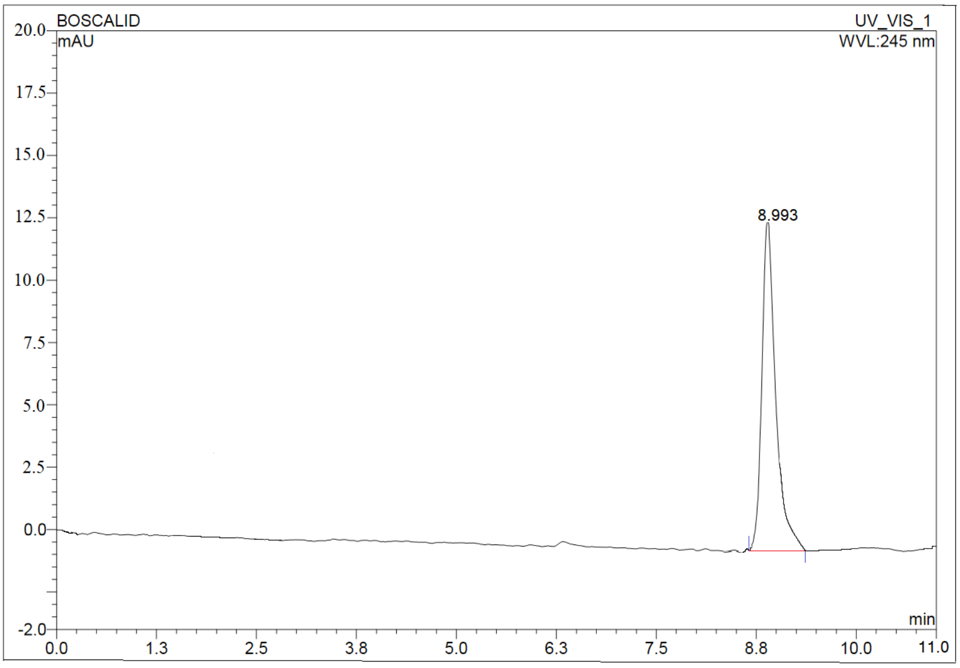


Figure 4.7 Persistence of boscalid in soil sample B @ 2µg g^{-1} (0th day)

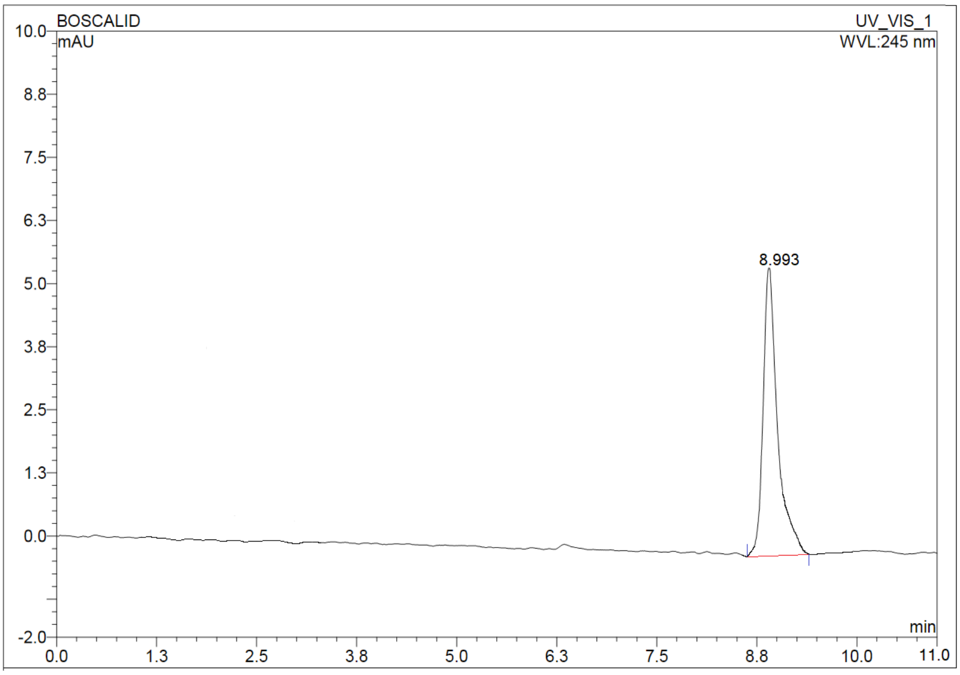


Figure 4.8 Persistence of boscalid in soil sample A @ 1µg g^{-1} (1thday)

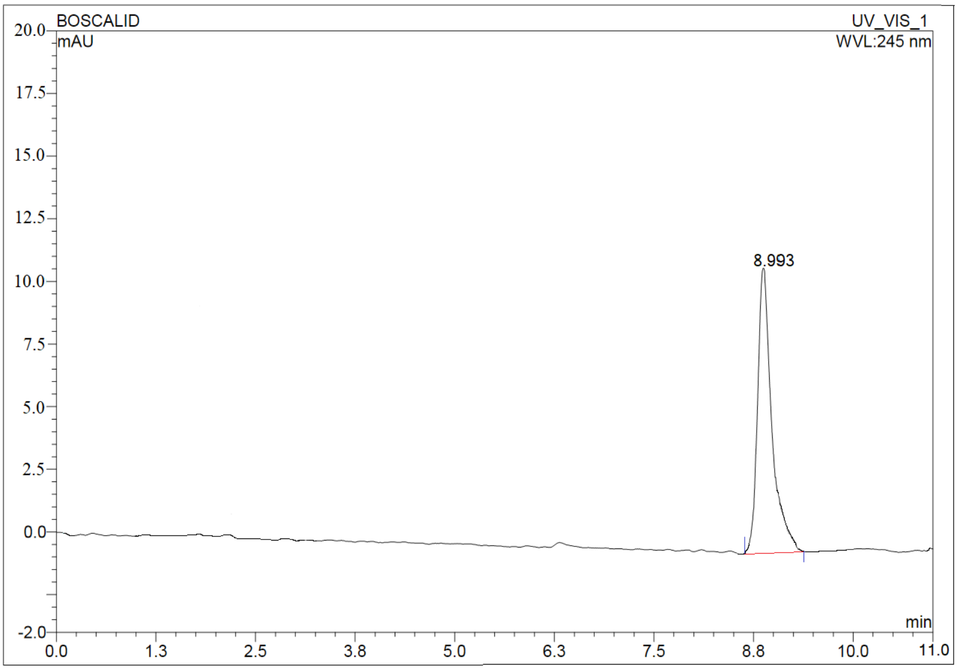


Figure 4.9 Persistence of boscalid in soil sample A @2μg g^{-1} (1st day)

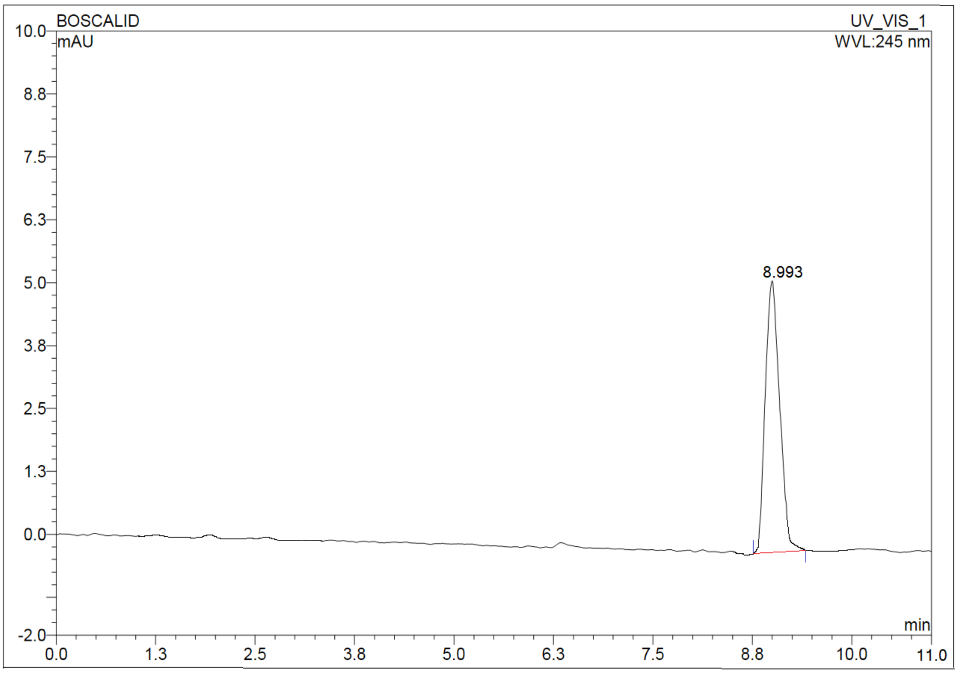


Figure 4.10 Persistence of boscalid in soil sample B @ 1μg g^{-1} (1st day)

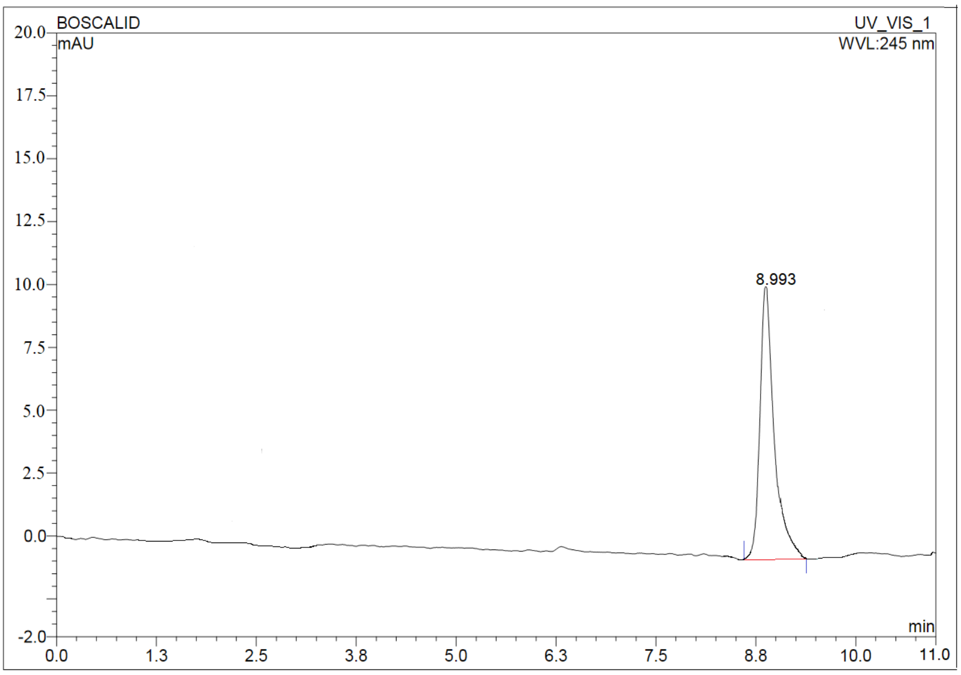


Figure 4.11 Persistence of boscalid in soil sample B @ 2µg g^{-1} (1st day)

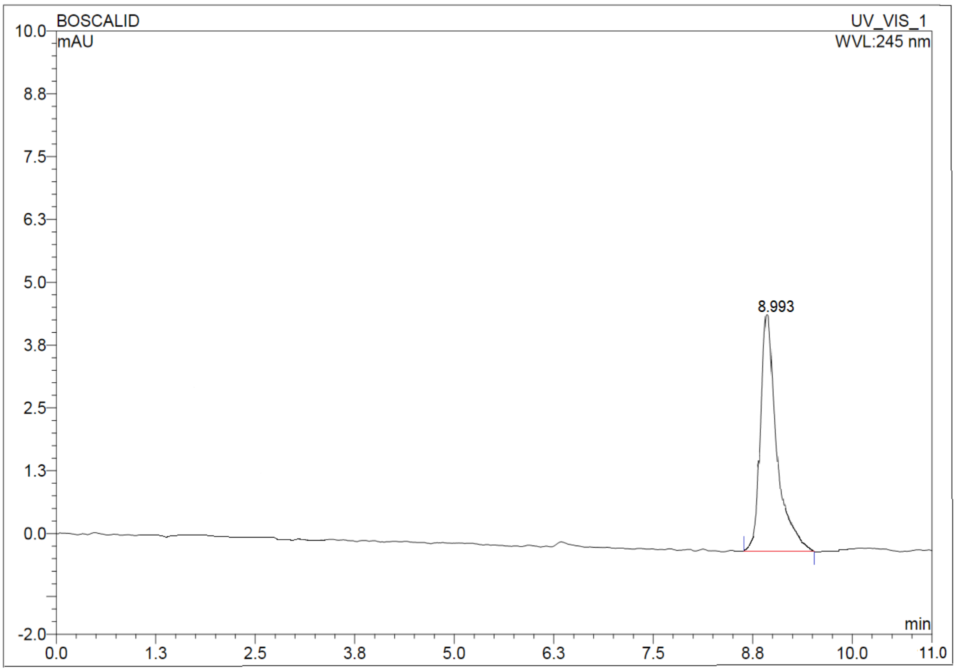


Figure 4.12 Persistence of boscalid in soil sample A @1µg g^{-1} (3rd day)

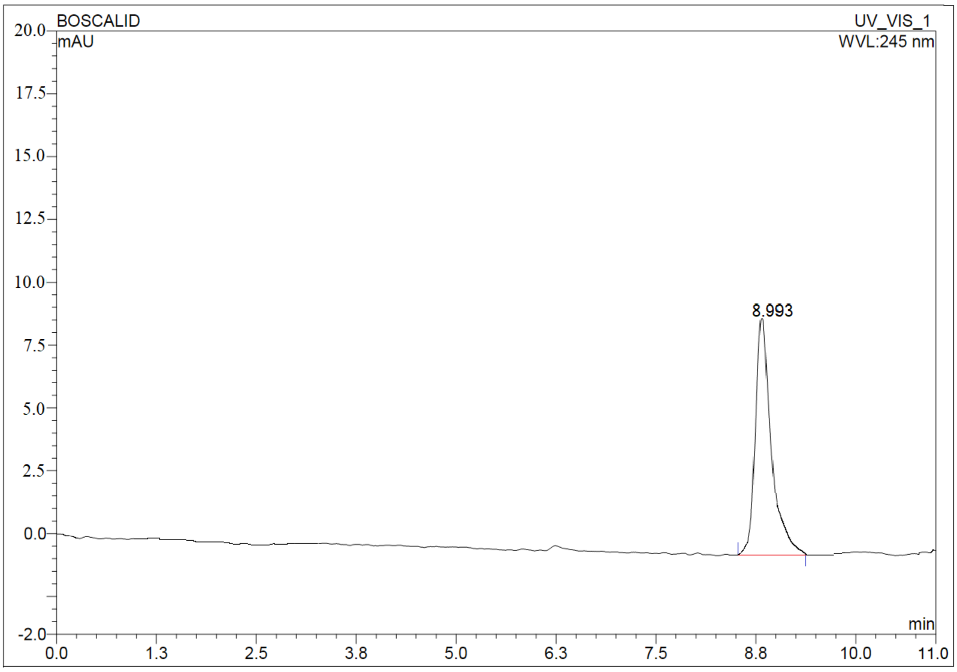


Figure 4.13 Persistence of boscalid in soil sample A @ 2µg g^{-1} (3rd day)

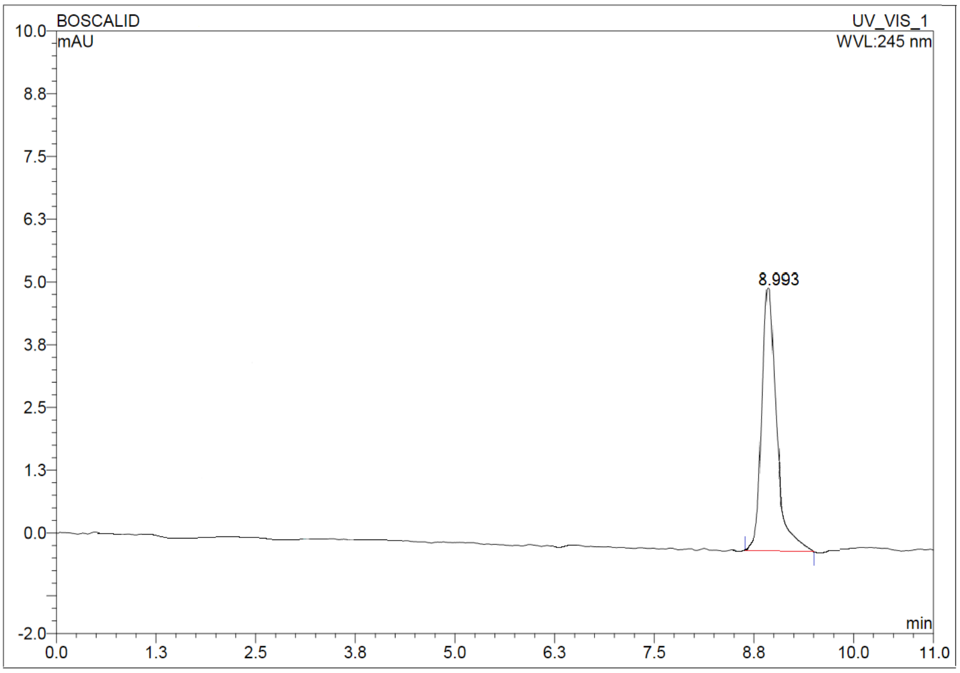


Figure 4.14 Persistence of boscalid in soil sample B @ 1µg g^{-1} (3rd day)

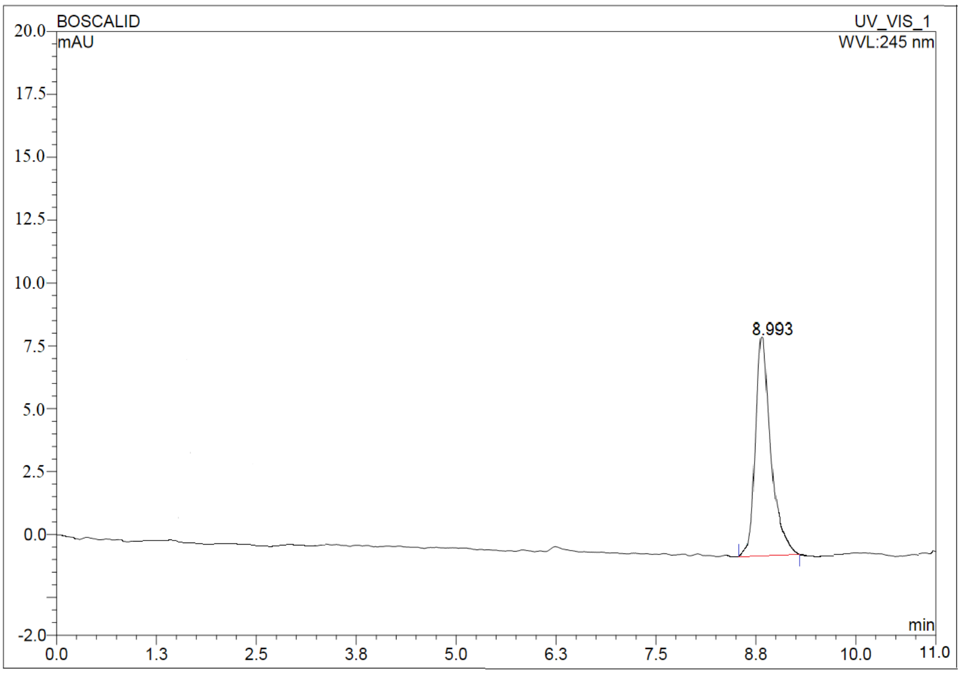


Figure 4.15 Persistence of boscalid in soil sample B @ 2µg g^{-1} (3rd day)

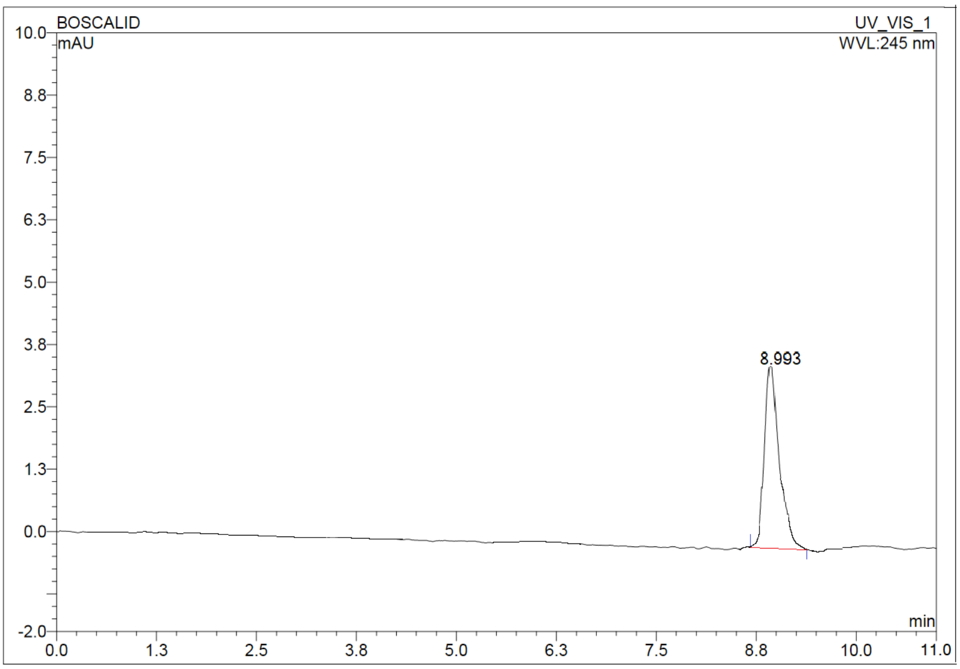


Figure 4.16 Persistence of boscalid in soil sample A @ 1µg g^{-1} (5th day)

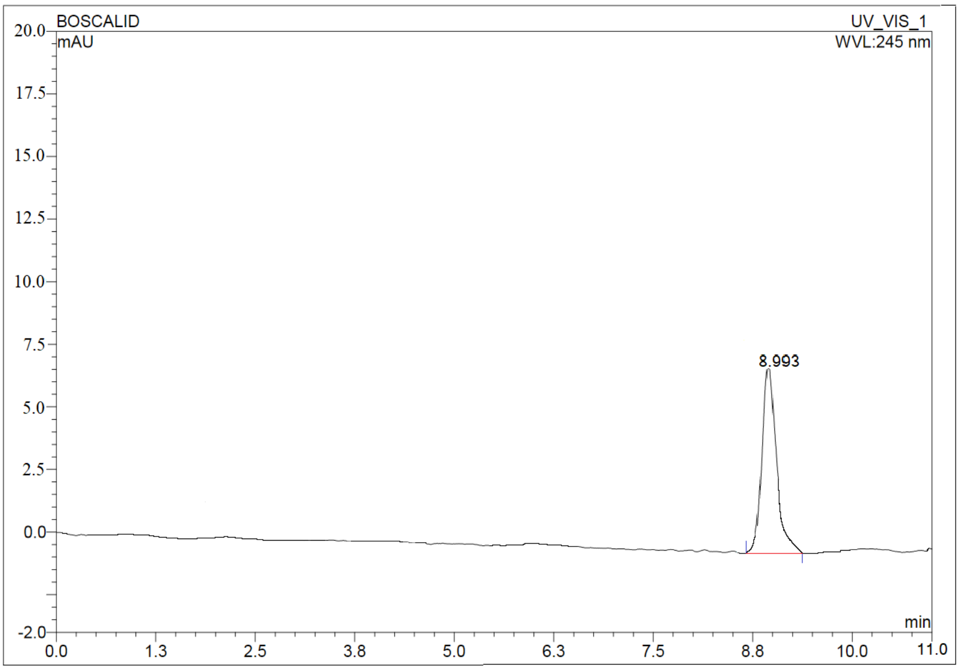


Figure 4.17 Persistence of boscalid in soil sample A @ 2µg g^{-1} (5th day)

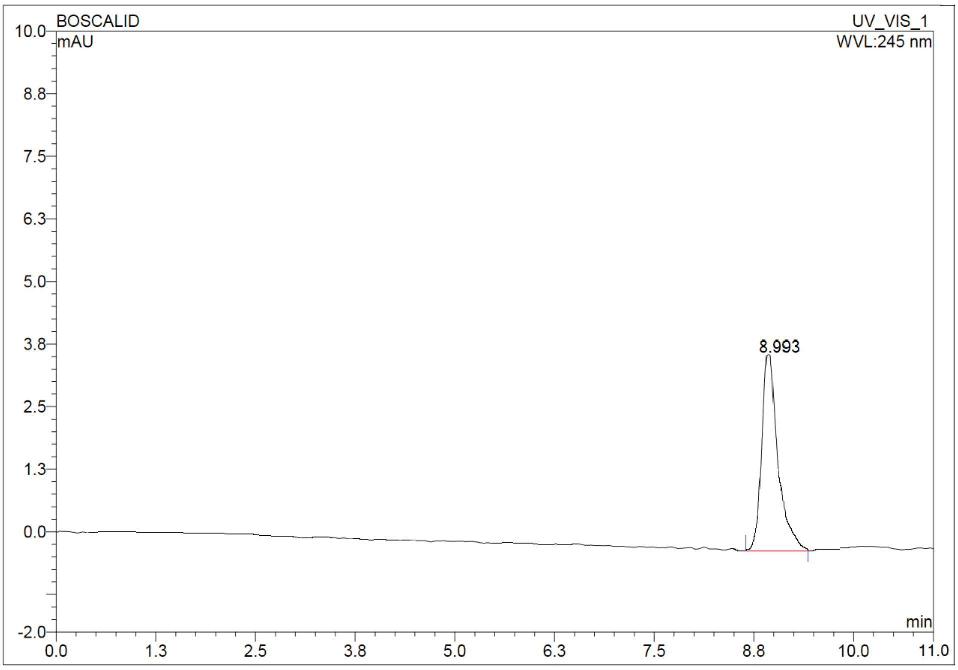


Figure 4.18 Persistence of boscalid in soil sample B @ 1µg g^{-1} (5th day)

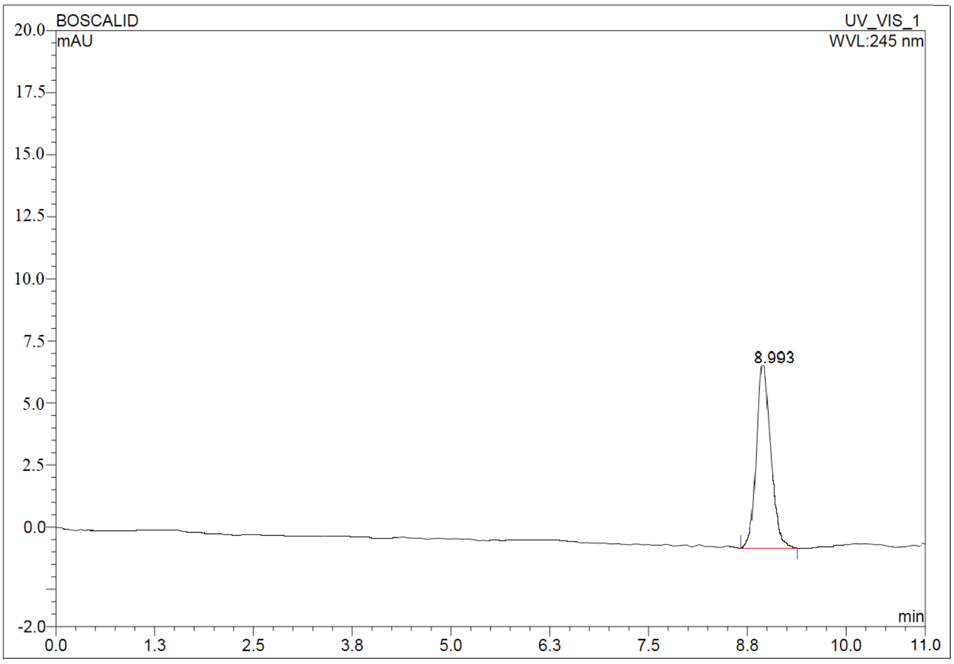


Figure 4.19 Persistence of boscalid in soil sample B @ 2µg g^{-1} (5thday)

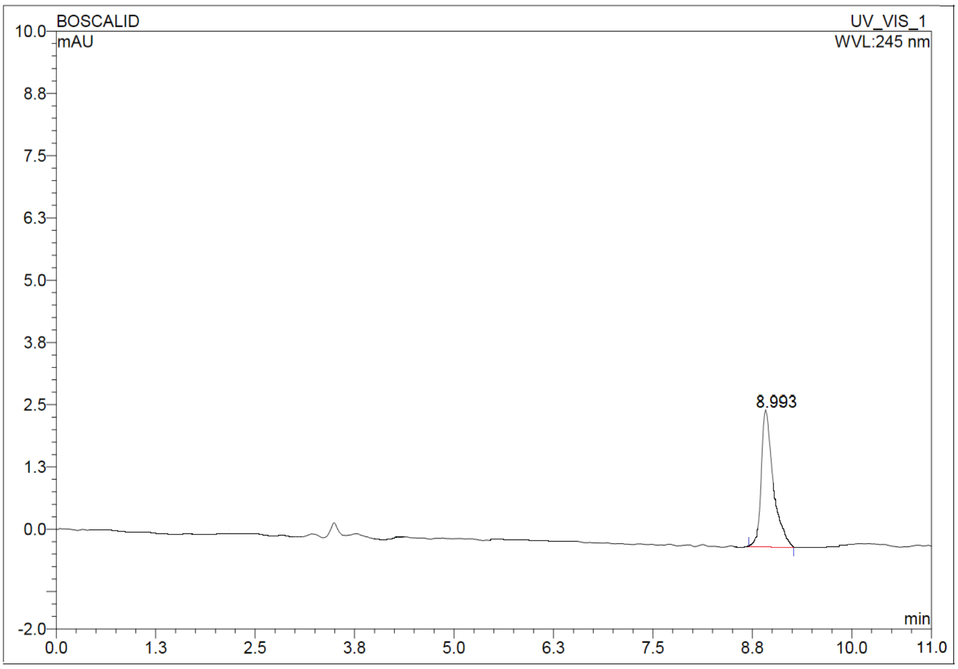


Figure 4.20 Persistence of boscalid in soil sample A @ 1µg g^{-1} (7th day)

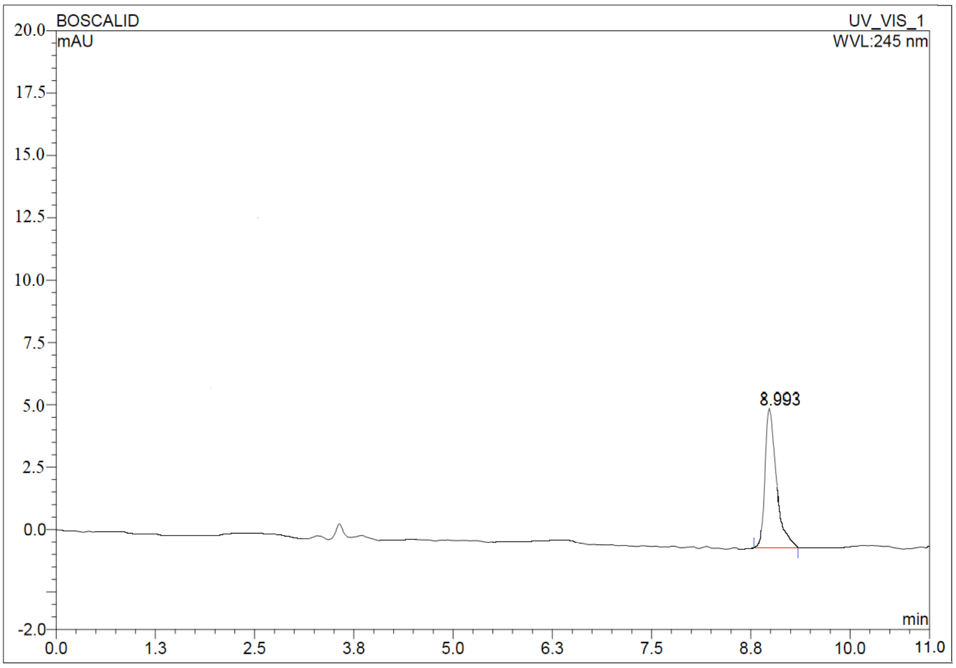


Figure 4.21 Persistence of boscalid in soil sample A @ 2µg g^{-1} (7th day)

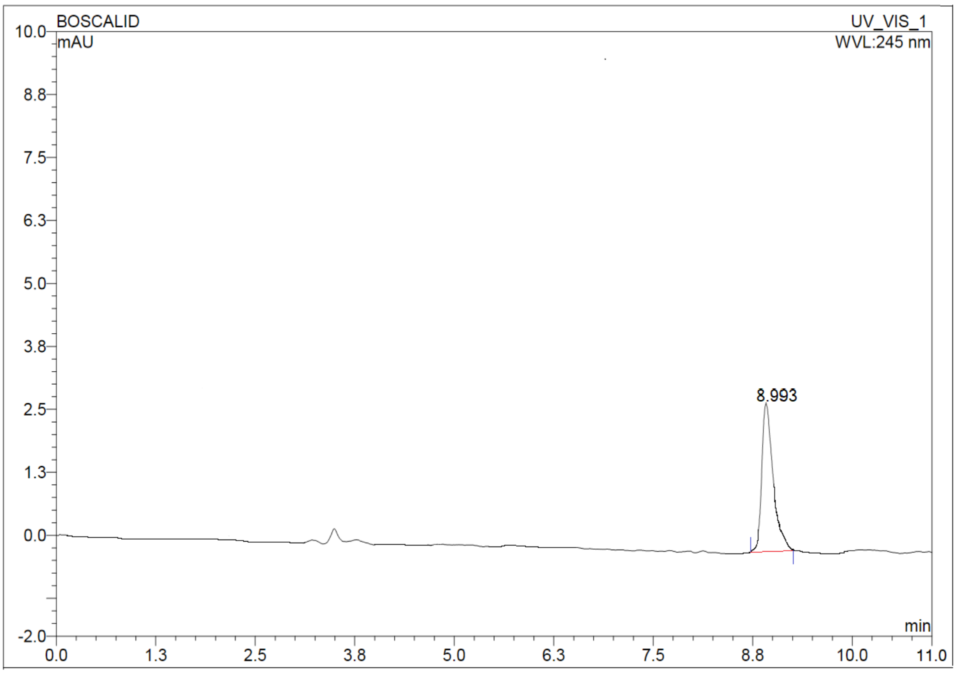


Figure 4.22 Persistence of boscalid in soil sample B @ 1µg g^{-1} (7th day)

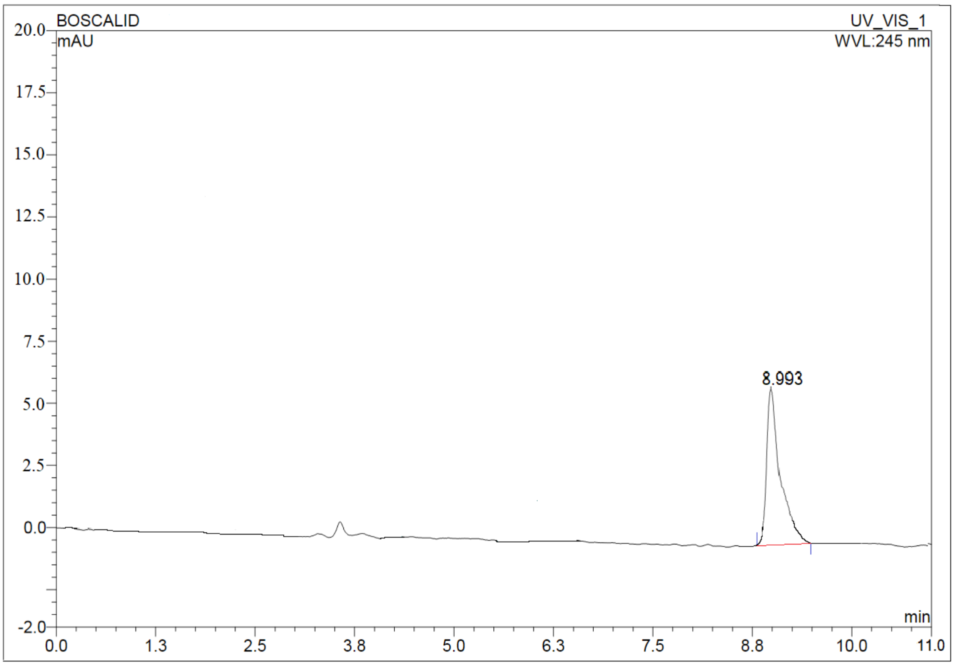


Figure 4.23 Persistence of boscalid in soil sample B @ 2µg g^{-1} (7th day)

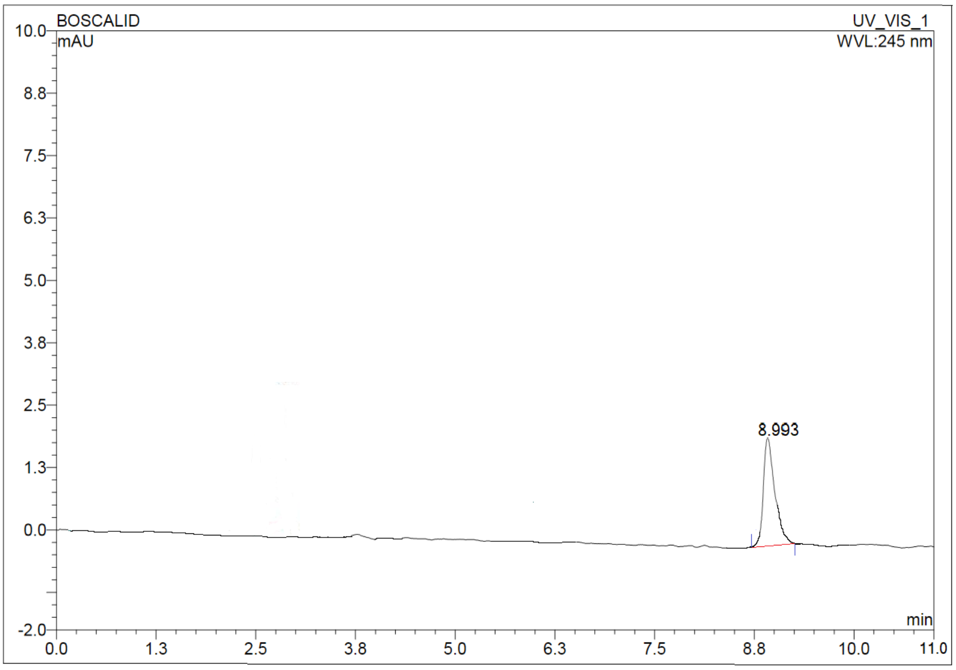


Figure 4.24 Persistence of boscalid in soil sample A @ 1µg g^{-1} (14th day)

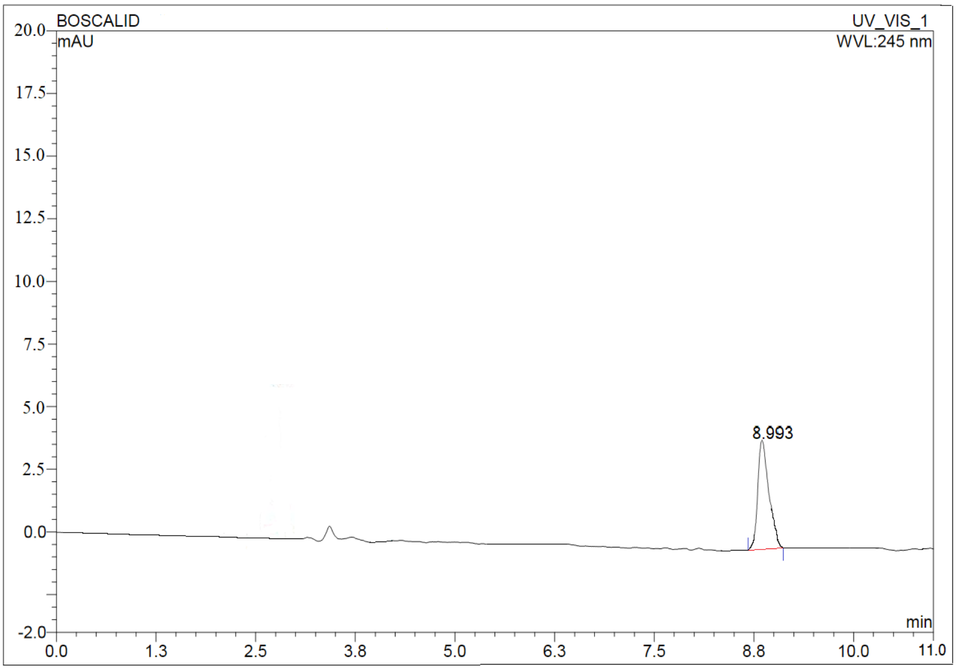


Figure 4.25 Persistence of boscalid in soil sample A @ 2µg g^{-1} (14th day)

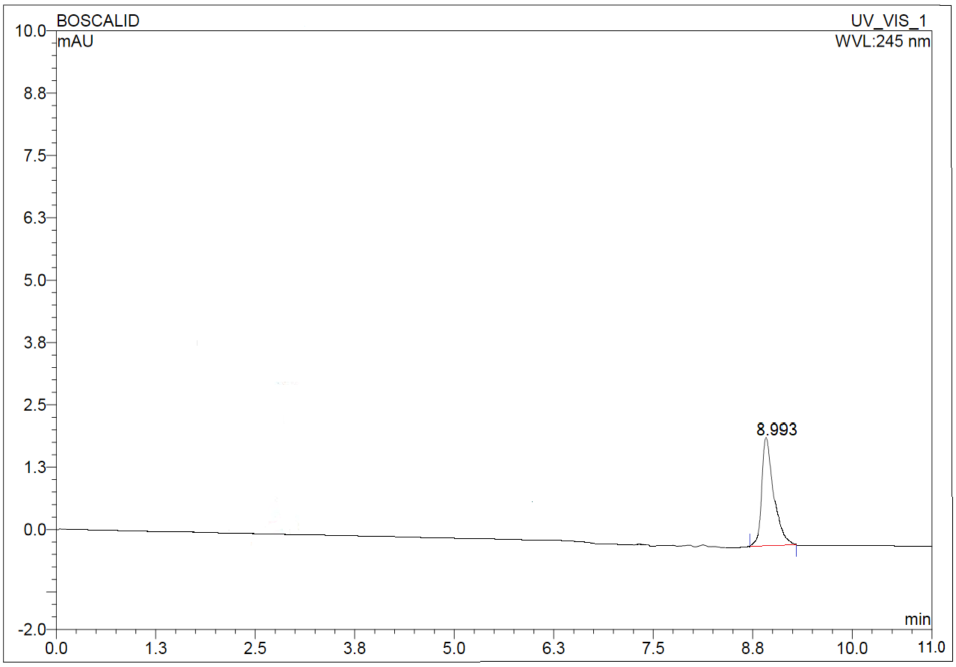


Figure 4.26 Persistence of boscalid in soil sample B @ 1µg g^{-1} (14th day)

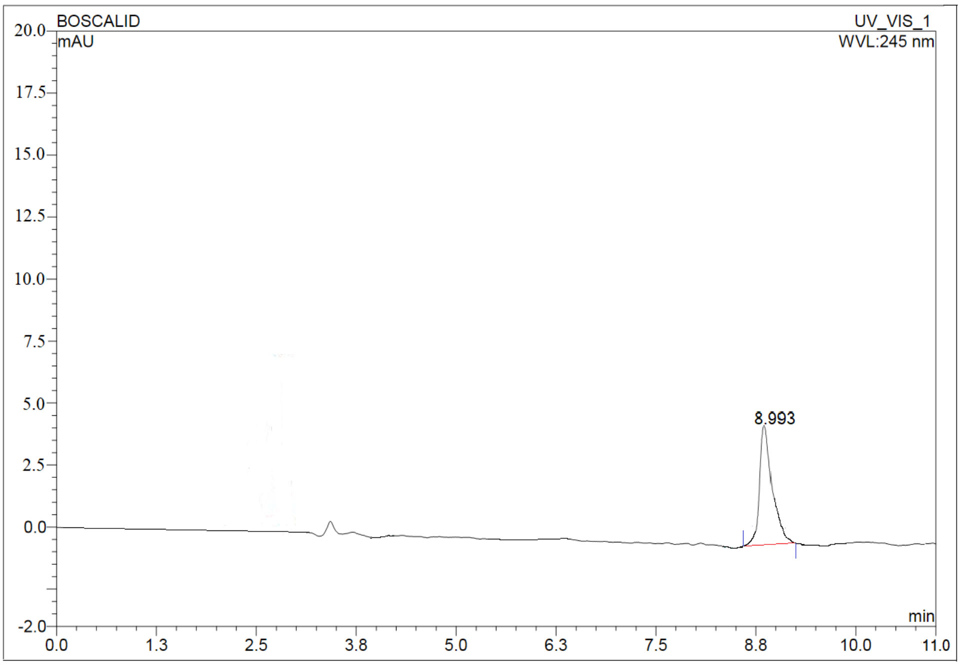


Figure 4.27 Persistence of boscalid in soil sample B @ 2µg g^{-1} (14th day)

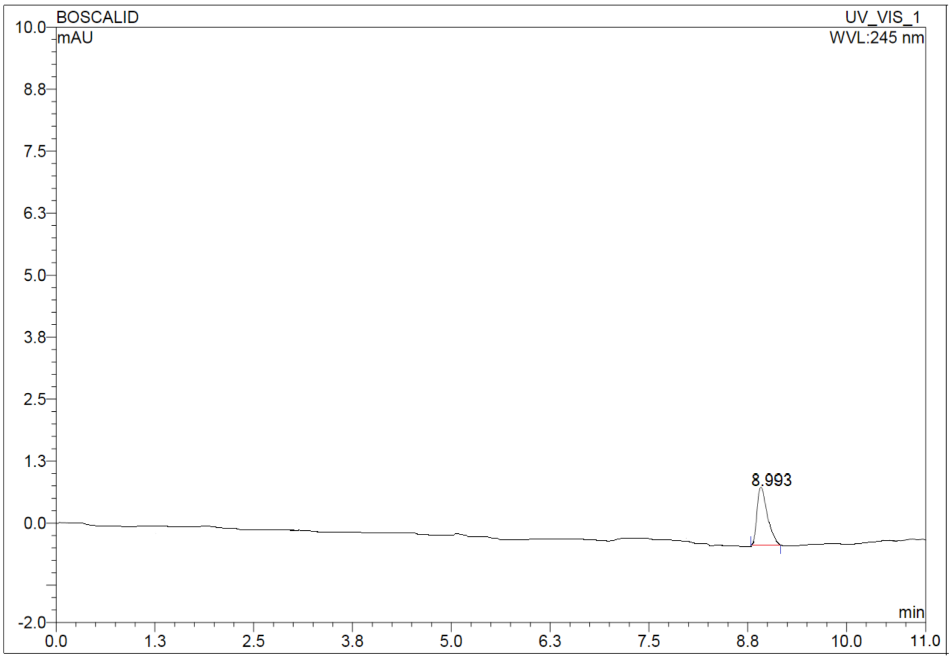


Figure 4.28 Persistence of boscalid in soil sample A @ 1µg g^{-1} (21st day)

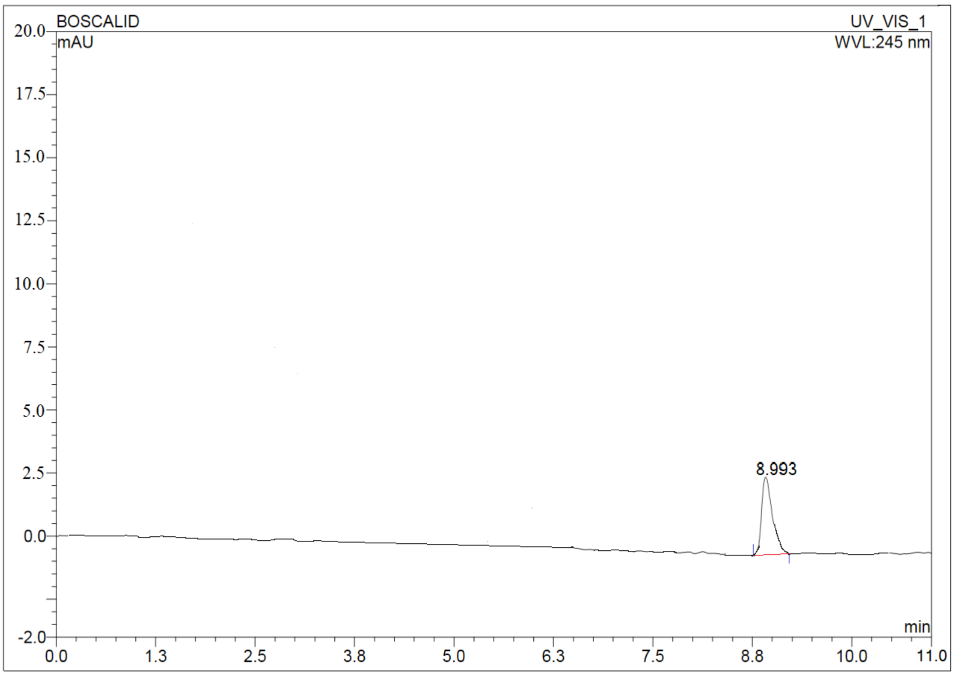


Figure 4.29 Persistence of boscalid in soil sample A @ 2µg g^{-1} (21st day)

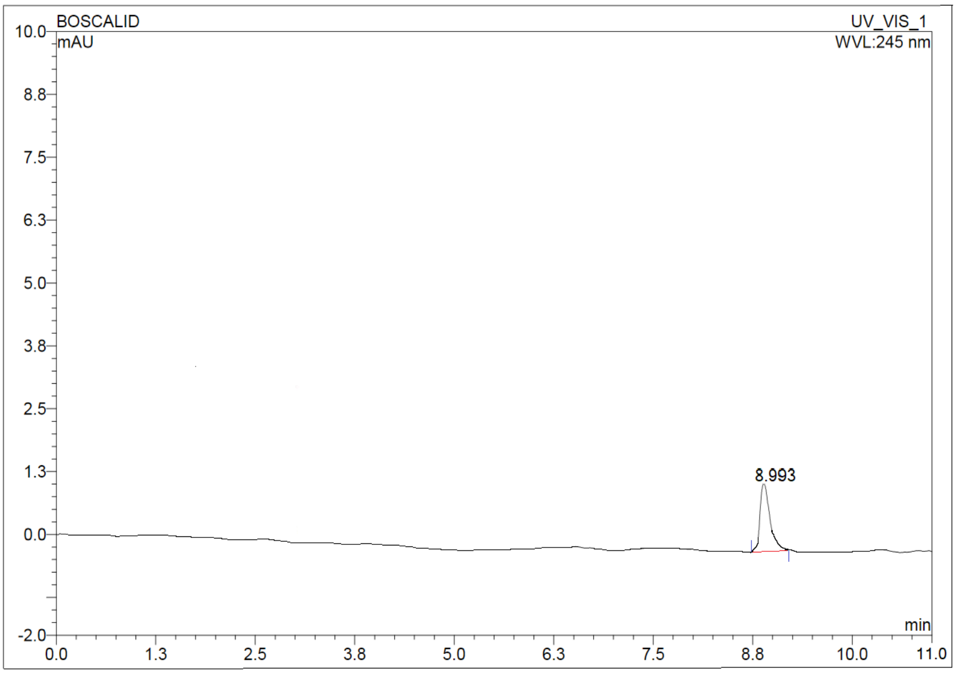


Figure 4.30 Persistence of boscalid in soil sample B @ 1µg g^{-1} (21st day)

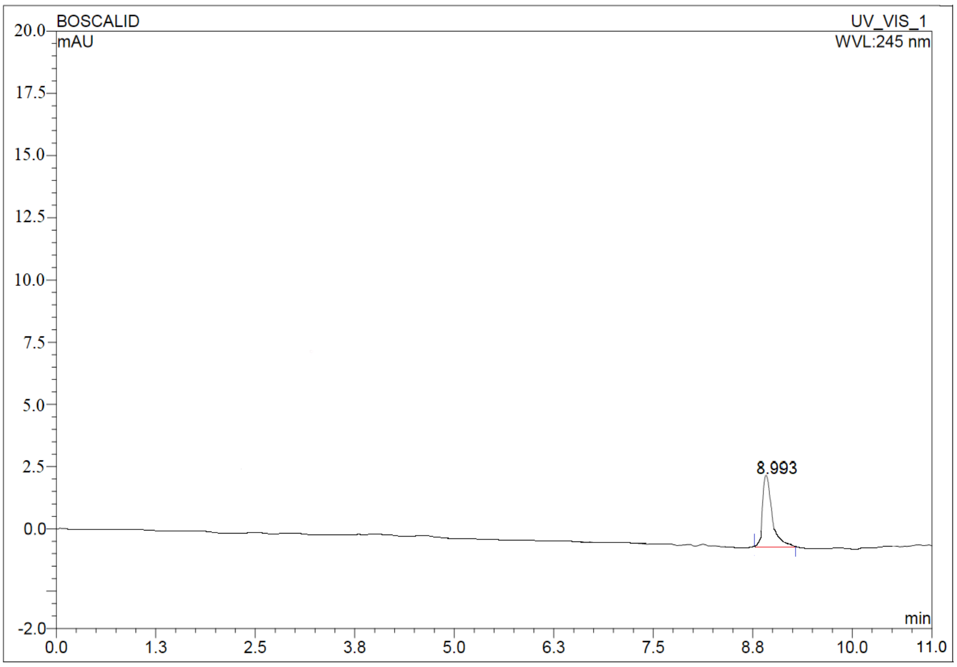


Figure 4.31 Persistence of boscalid in soil sample B @ 2µg g^{-1} (21st day)

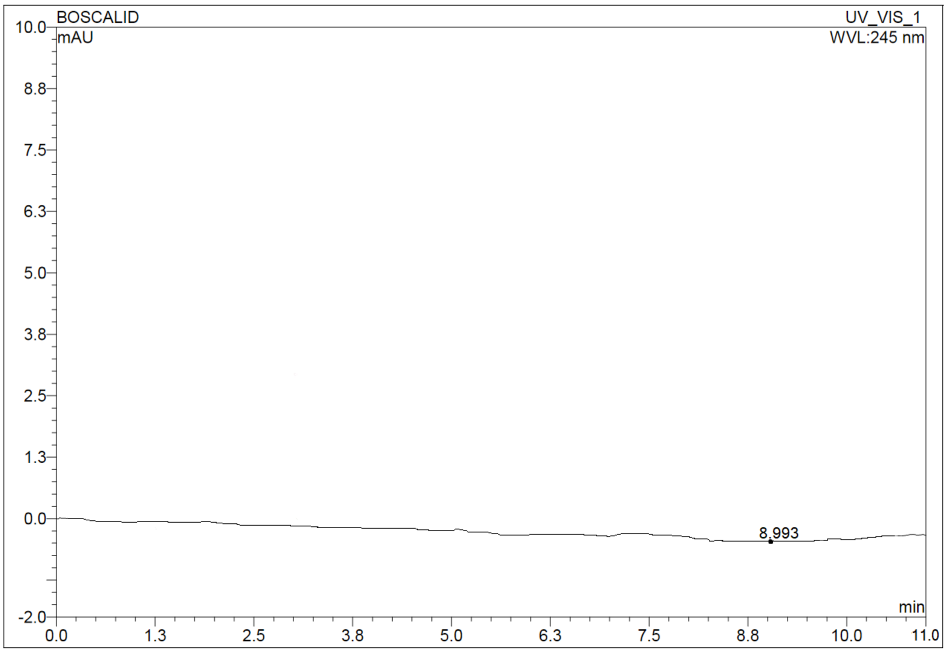


Figure 4.32 Persistence of boscalid in soil sample A @ 1µg g^{-1} (28th day)

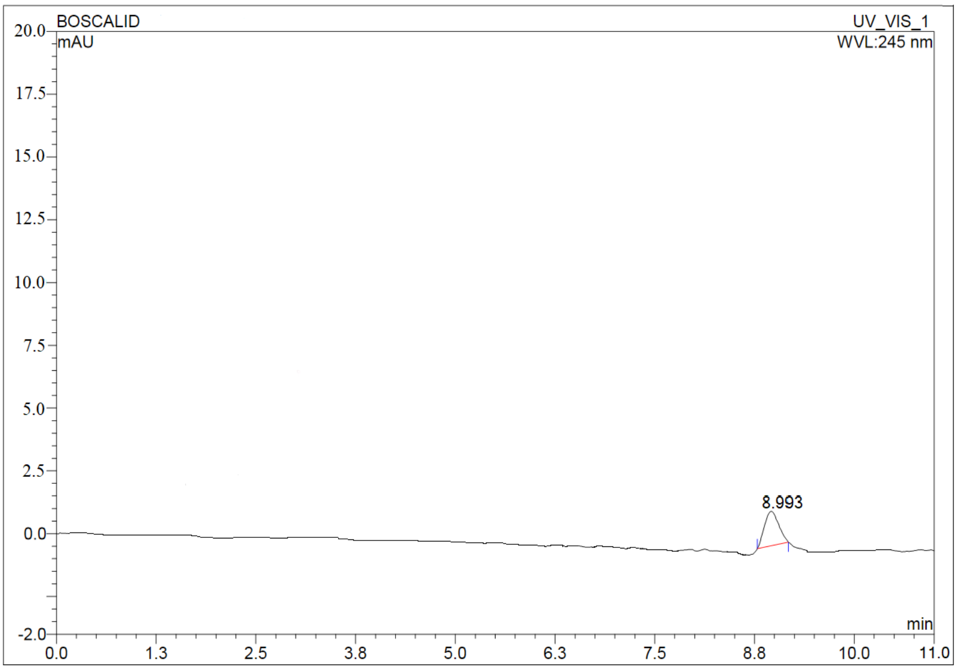


Figure 4.33 Persistence of boscalid in soil sample A @ 2µg g^{-1} (28th day)

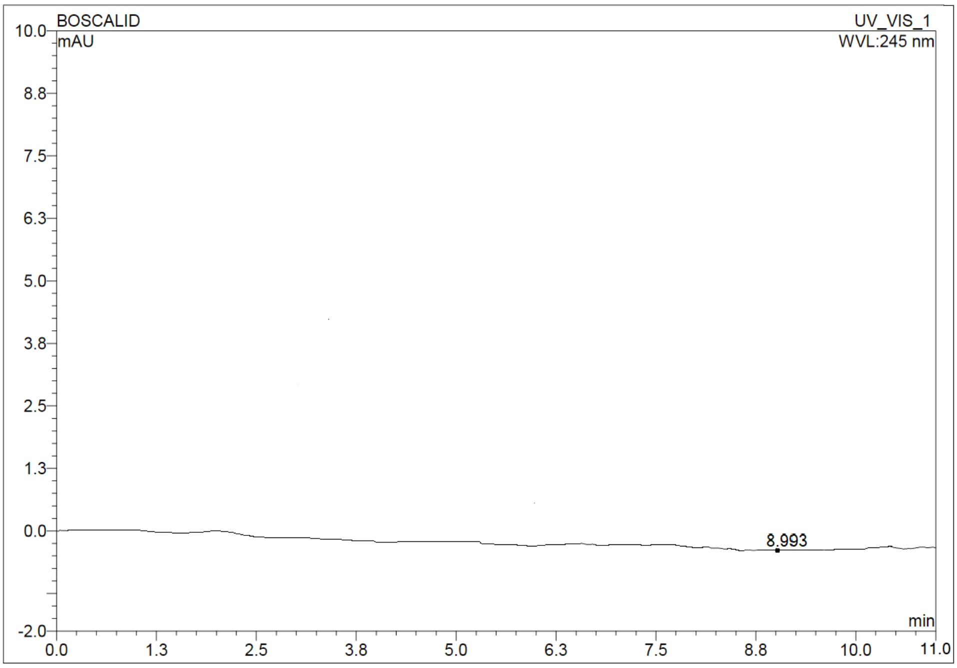


Figure 4.34 Persistence of boscalid in soil sample B @ 1µg g^{-1} (28th day)

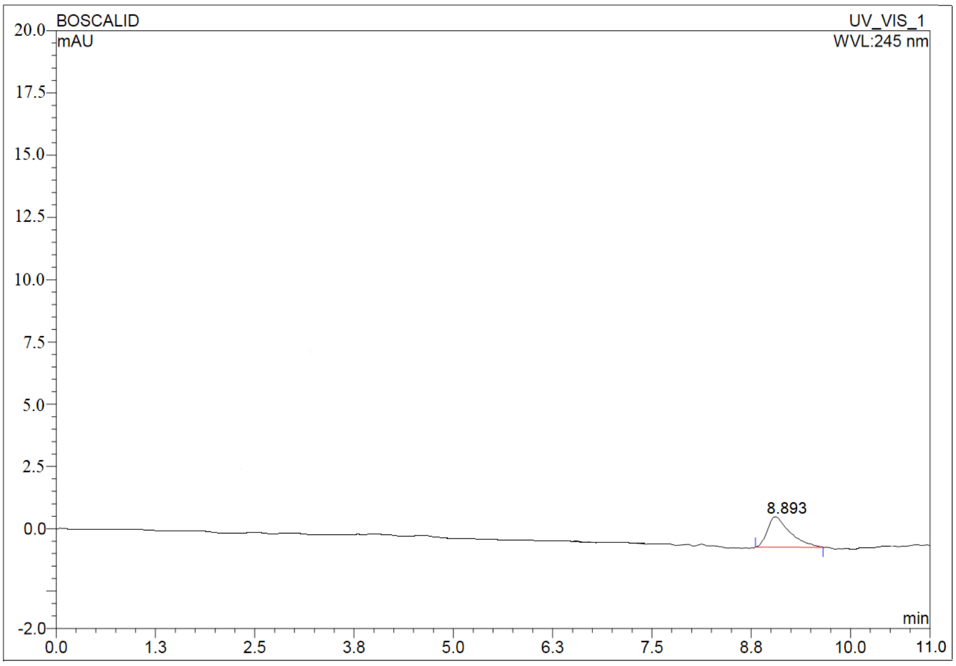


Figure 4.35 Persistence of boscalid in soil sample B @ 2µg g^{-1} (28th day)

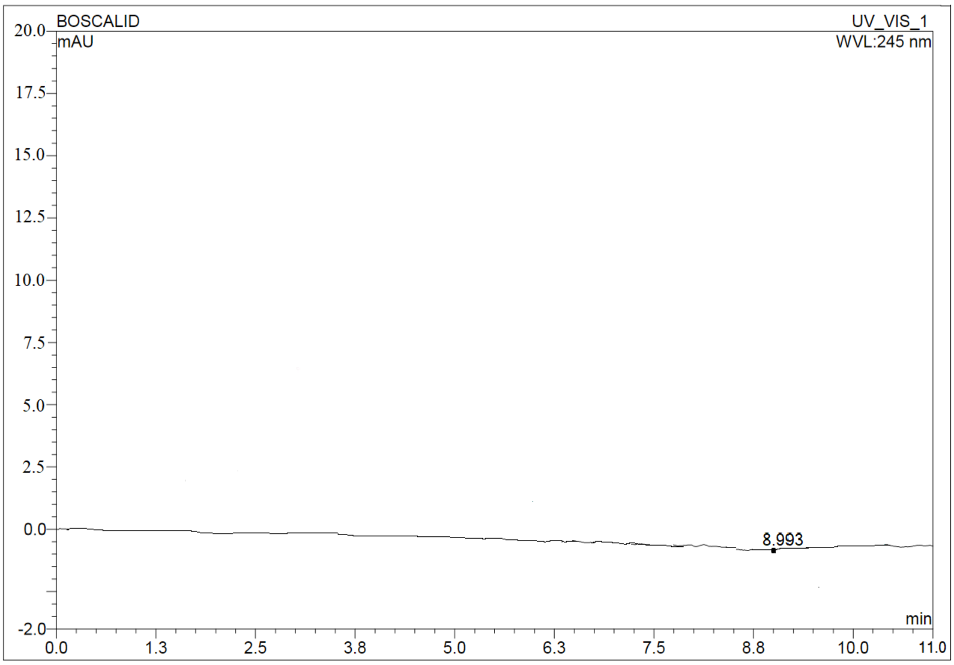


Figure 4.36 Persistence of boscalid in soil sample A @ 2µg g^{-1} (35th day)

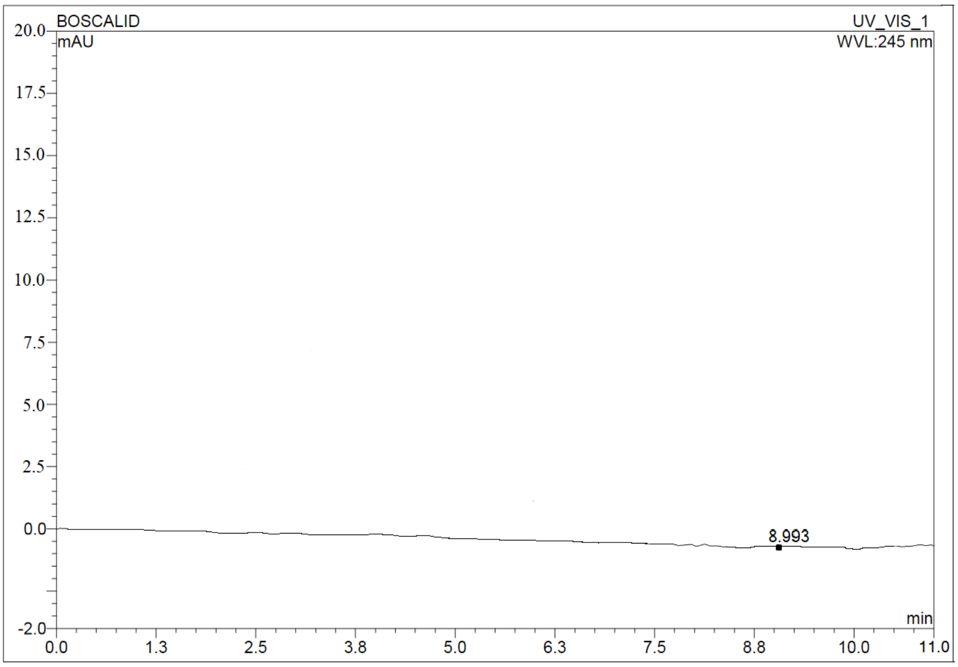


Figure 4.37 Persistence of boscalid in soil sample B @ 2µg g^{-1} (35th day)

4.5 Degradation kinetics of boscalid

The amount of boscalid recovered from soil sample at different time intervals at two treatment rates of 1 and 2µg g^{-1} in two types of soils was determined using the formula

$$C=C_0e^{-kt}$$

Where,

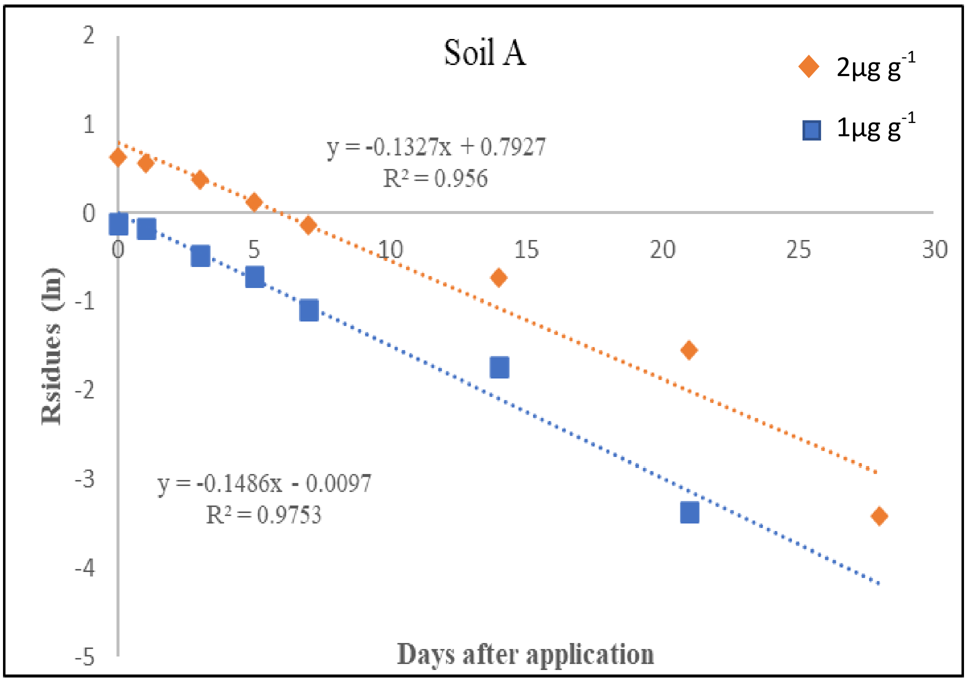
C = Amount of pesticide recovered from samples at time t;

C_0 = Amount of boscalid recovered at t=0;

k = Degradation constant

t = Time in days.

The dissipation data for boscalid were calculated by linear regression from the transformed first order rate reaction. The half-lives of the fungicide in two soils was determined using the equation DT_{50} = ln2/K where K is degradation constant.



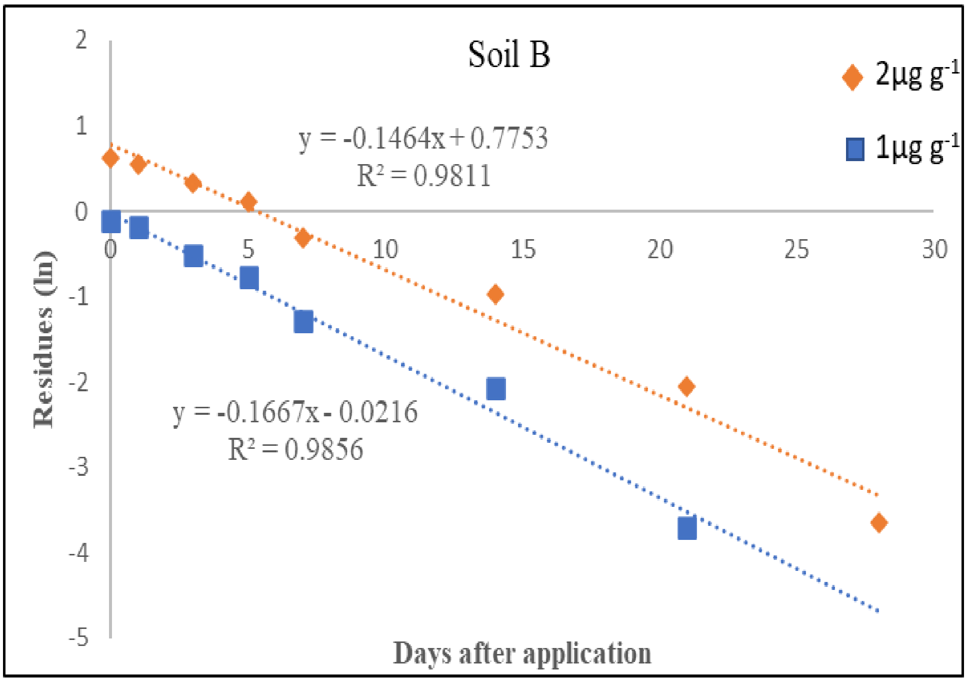


Figure 4.38 **Plots of natural logarithm of boscalid fungicide in soil sample A and B versus time at 1 and 2µg g^{-1}**

Table 4.4 Calculated values of half-life ($t_{1/2}$), degradation constant and coefficient of determination of boscalid following first order kinetics of degradation.

Computed values	**Soil sample A**		**Soil sample B**	
	1µg g^{-1}	**2µg g^{-1}**	**1µg g^{-1}**	**2µg g^{-1}**
Degradation rate constant (λ)	0.149	0.133	0.167	0.146
Half-life ($t_{1/2}$)	4.66 d	5.22 d	4.16 d	4.73 d
Coefficient of determination (R^2)	0.975	0.956	0.985	0.981
Regression equation	$y= -0.1486x - 0.0097$	$y = -0.1327x + 0.7927$	$y = -0.1667x - 0.0216$	$y = -0.1464x + 0.7753$

Table 4.4 shows half-life ($t_{1/2}$), degradation constant (k), coefficient of determination (R^2) and regression equation of boscalid kinetics. At both the rates of boscalid application (1.0 and 2.0 µg g^{-1}) natural logarithm of boscalid residues in two different soils were plotted individually against time **(Figure 4.38).** The distribution of points in two soils at both the levels of treatment recommended that dissipation of boscalid occurred through a single phase conforming that it followed first order kinetics. The computed values of coefficient of determination (R^2) between log residues in two soils, varied from 0.956 to 0.985 and the $t_{1/2}$ values ranged between 4.16 to 5.22 d at the two application rates in soils A and B. The half-life values of boscalid at higher dose were relatively higher in comparison to that at lower application dose and simultaneously the $t_{1/2}$ was greater in soil sample A in comparison to soil sample B.

As evident from data of **Table 4.3** the dissipation of boscalid was faster probably because of high temperatures, relative humidity, sunshine hours and wind velocity rates under the sub tropical climatic conditions prevailing in this region **(Table 3.2).** Such types of climatic conditions also provide favourable conditions for microbial growth and so this might have been another reason for faster boscalid degradation from soils. **Chen and Zhang (2010)** have also reported a half-life range of 6.1 to 8 d of boscalid in soils and suggested that soil property was the key factor for faster degradation. Other studies also with short half-lives of boscalid in crops and soils have been reported in literature **(Lee *et al.*, 2008; Jo *et al.*, 2017).**

Summary and Conclusions

Chapter 5 SUMMARY AND CONCLUSION

The present study was performed to validate a suitable method for extraction of boscalid (a carboxamide fungicide) from soil, perform recovery studies and find out it's dissipation behaviour in two subtropical soils. Dissipation studies were performed in the soils at two different concentration of 1 and 2 μg g^{-1}. The summary and conclusion of whole experiment is presented below:

1. Physicochemical properties of experimental soils
    - The physicochemical properties which consists of pH, EC, Organic carbon content and soil textures of soils were determined by the standard methods.
    - On the basis of soil texture analysis, it was confirmed that soil sample A was sandy loam and soil sample B was sandy clayey loam in texture.
2. The solution of boscalid was scanned at 200-400nm UV range and the maximum absorbance (λ_{max}) as determined by using UV-VIS spectrophotometer was found out to be 245nm.
3. The calibration curve was plotted between different concentrations of (0.2 to 5 μg g^{-1}) of boscalid solution concentration versus peak area was linear in above range.
4. The limit of detection (LOD) and limit of quantification (LOQ) was found to be 0.004 and 0.012 μg/g on basis of S/N ratio.
5. The recovery study of boscalid, performed at two concentrations of 0.5 and 1 μg g^{-1} in both the soils ranged from 80.59 % to 86.87% at 0.5 μg g^{-1} application rate and 90.51% to 98.97% at 1 μg g^{-1} fortification rate in soil samples A and B respectively.
6. Dissipation studies were performed in two soil samples fortified with 1 and 2 μg g^{-1} of boscalid and analysed at different days i.e. 0, 1, 3, 5, 7, 14, 21, 28, 35, 42 and 60 days.

7. The extraction of fungicide was performed through simplified QuEChER’S method.
8. When the extraction of each day was completed, the residue of boscalid was quantified by HPLC (High Performance Liquid Chromatography).
9. The dissipation of boscalid was consistent in both the type of soils.
10. At lower application rate (1µg g^{-1}) boscalid declined uniformly persisting upto 21st days with dissipation of 96.12% in soil sample A and 97.23% in soil sample B and was not detectable on 28th day and thereafter, of boscalid application in both the type of soils.
11. At higher application rate (2µg g^{-1}) the residue of boscalid persisted upto 28th day of boscalid application with dissipation of 98.24% and 98.18% in soil sample A and B respectively. Boscalid was not detectable on 35th and 60th day at higher concentration.
12. The natural logarithm value of boscalid concentration were plotted against time in days in two soils A and B at both concentrations (1 and 2 µg g^{-1}). The persistence data fitted well to first order kinetics. At application rates of 1 and 2 µg g^{-1} the value of coefficient of determination (R^2) were 0.975 and 0.956 in soil sample A and 0.985 and 0.981 in soil sample B respectively.
13. The half life of boscalid was 4.66 and 4.16 days in soil samples A and B at 1 µg g^{-1} and 5.22 and 4.73 d in both the soils at 2 µg g^{-1} application rates.
14. Boscalid does not persist for long time at lower concentration (1µg g^{-1}) and was slightly persistent at higher concentration (2µg g^{-1}) in both subtropical soils.
15. Boscalid is applied for controlling fungal disease in variety of vegetables and field crops like tomato, cucumber, strawberries etc. from where it finds way to the soil also.
16. Thus, it can be concluded that boscalid does not persist in soils of this region and can therefore be safely applied at recommended dosage in crops and subtropical soil.

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ABSTRACT

Boscalid [2-chloro-N-(4'-chlorobiphenyl-2-yl) nicotinamide] is a carboxamide fungicide used in variety of food crops and turf grasses. It comes under the SDHI group of fungicides which inhibit the enzyme succinate dehydrogenase ubiquinone (complex II) which is an important enzyme for energy production in respiratory chain. The dissipation studies of boscalid was performed in different textural soils at 1 and 2 $\mu g\ g^{-1}$. The method of extraction of boscalid residue from soil was validated by following QuEChER’S (Quick, Easy, Cheap, Effective, Rugged and Safe) method and the quantitative analysis was done by HPLC (High performance liquid chromatography). Recovery study of boscalid was performed at two concentration of 1 and 2 $\mu g\ g^{-1}$ and the recoveries obtained ranged from 80.59% to 98.97% in two soils. The dissipation rate of boscalid did not vary much in the two soils. At lower application rates (1$\mu g\ g^{-1}$) boscalid dissipated to 96.12% and 97.23% upto 21st day and was below detectable limit (BDL) on 28th day at lower concentration of 1$\mu g\ g^{-1}$ in both the soils. At higher application rates (2$\mu g\ g^{-1}$) boscalid residue persisted upto 28th day with dissipation upto 98.24% and 98.18% in both the type of soils. It was BDL on 35th and 60th day of fungicide application. Dissipation of boscalid occurred in a single distinct phase following first order kinetics. The half life of boscalid ranged from 4.66 and 4.16 days in soil samples A and B at 1 $\mu g\ g^{-1}$ and 5.22 and 4.73 d in both the soils at 2 $\mu g\ g^{-1}$ application rates. Boscalid is used in various vegetable, field crops and turf grasses for controlling fungal diseases and decreasing the economic damage but since it does not persist in subtropical soils it can be safely applied at recommended dosage in crops and soils.

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शोध का विषय		विभिन्न बनावटों की मिट्टी में बोसकैलिड (एक कार्बोक्सामाइड कवकनाशी) का अपव्यय अध्ययन			
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सारांश

बोस्कालिड [2-क्लोरो-एन- (4'-क्लोरोबिफेनिल-2-वाईएल) निकोटिनमाइड] एक कार्बोक्सामाइड कवकनाशी है जिसका उपयोग विभिन्न प्रकार की खाद्य फसलों और टर्फ घास में किया जाता है। बोस्कालिड कवकनाशी SDHI समूह के अंतर्गत आता है जो एंजाइम succinate dehydrogenase ubiquinone (complex II) को रोकता है जो श्वसन श्रृंखला में ऊर्जा उत्पादन के लिए एक महत्वपूर्ण एंजाइम है। बोस्कालिड का अपव्यय अध्ययन 1 और 2 $\mu g\ g^{-1}$ पर विभिन्न बनावट वाली मिट्टी में किया गया था। बोस्कालिड अवशेषों का निष्कर्षण क्वेचर्स (त्वरित, आसान, सस्ता, प्रभावी, उबड़ और सुरक्षित) विधि द्वारा किया गया था और उच्च प्रदर्शन तरल क्रोमैटोग्राफी (एचपीएलसी) द्वारा मात्रात्मक विश्लेषण किया गया था। दो सांद्रता (1 और 2 $\mu g\ g^{-1}$) पर बोस्कालिड की औसत वसूली दो मिट्टी में 80.59% से 98.97% तक थी। बोस्कालिड की अपव्यय दर दोनो प्रकार की मिट्टी में ज्यादा भिन्न भिन्न नही थी । कम सांद्रता दर (1μg g-1) पर बोस्कालिड 21 वें दिन तक 96.12% और 97.23% अपव्यय हो रहा है और 28 वें दिन पता लगाने योग्य नहीं है। उच्च सांद्रता दर (2$\mu g\ g^{-1}$) पर बोस्कालिड अवशेष 28 वें दिन तक 98.24 प्रतिशत और 98.18% अपव्यय के साथ दोनों प्रकार की मिट्टी में बना रहता है और 35 वें और 60 वें दिन पता लगाने योग्य नहीं है। बोस्कालिड का अपव्यय प्रथम कोटि की गतिक्रिया के साथ एक चरण में हुआ है। बोस्कालिड का अर्द्ध-आयु काल 1 $\mu g\ g^{-1}$ सांद्रता वाली मिट्टी के नमूने ए और बी में 4.66 और 4.16 दिनों से लेकर और 2 $\mu g\ g^{-1}$ वाली दोनों मिट्टी में 5.22 और 4.73 दिन था। बोस्कालिड का उपयोग विभिन्न सब्जियों, खेत की फसलों और टर्फ घासों में कवक रोगों को नियंत्रित करने और आर्थिक क्षति को कम करने के लिए किया जाता है लेकिन चूंकि यह उपोष्णकटिबंधीय मिट्टी में स्थायी नहीं रहता है इसलिए इसे फसलों और मिट्टी में अनुशंसित खुराक पर सुरक्षित रूप से लगाया जा सकता है।

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