DISSIPATION AND DECONTAMINATION STUDIES OF CYANTRANILIPROLE IN CABBAGE AND SOIL

BY

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CERTIFICATE – I

This is to certify that this thesis entitled **"Dissipation and decontamination studies of cyantraniliprole in cabbage and soil"**, submitted for the degree of Master of Science, in the subject of **Chemistry** to Chaudhary Charan Singh Haryana Agricultural University, Hisar, is a bonafide research work carried out by **Ms. Poonam Rani** under my supervision and that no part of this thesis has been submitted for any other degree. The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE – II

This is to certify that this thesis entitled **"Dissipation and decontamination studies of cyantraniliprole in cabbage and soil"** submitted by **Ms. Poonam Rani** to Chaudhary Charan Singh Haryana Agricultural University, Hisar, in partial fulfilment of the requirements for the degree of Master of Science, in the subject of **Chemistry**, has been approved by the Student's Advisory Committee after an oral examination on the same in collaboration with an External Examiner.

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CHAPTER-I

INTRODUCTION

Agriculture, with its allied sectors, is the largest livelihood provider in India, greater in the vast rural areas. It also contributes a significant figure to the Gross Domestic Product (GDP). Agriculture sector employs more than 50 per cent of the total workforce in India and contributes around 17-18 percent to the country's GDP (Sushruth Sunder, 2018). It has been estimated that 50% of total food production is lost due to plant pathogens, insect pests, weeds, rodents, birds, nematodes and during storage (Dureja *et al.*, 2009). Hence, in order to combat the problem of insect pests a lot of pesticides are used. Pesticides along with fertilizers, play a crucial role in agriculture and contribute to enhanced food production worldwide. Pesticide means any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest (insects, nematodes, weeds, rats, mites, etc.) including insecticide, fungicide, herbicide and various other substances used to control pests (EPA, 2009). The requirement for food is related directly with population growth. Annual global net population growth is 80 million and the world population is expected to be 9 billion by 2030. The uncertainty of climatic conditions has further increased outbreak of pests and diseases (Ghosh *et al*., 2015). The unique ability of pesticides to control pests and diseases has acquired enormous attention and has revolutionized agricultural production (Guler *et al*., 2010).

Total amount of pesticide active ingredients now in use is around 2.5 million pounds per year (Elsevier). General concept of "if little is good, a lot more will be better" for pest control has violated the basic scheme of need based application of pesticide and hence have become one major factor for environmental contamination (Ghosh *et al*., 2015). Several issues have resulted from the promiscuous use of pesticides, such as accumulation of toxic contaminants in the environment, emergence of resistance in pests and the resurgence of pests. The acute health problems (McCauley *et al*., 2006) associated with pesticide exposure are nausea, vomiting, dizziness, headache, abdominal pain, skin and eye problems (Ecobichon *et al*., 1996). Their low biodegradability has categorized these pesticides as persistent toxic substances. Biological stability and higher order of lipophilicity in the food commodities pose a significant effect on human and animal health (Tayade *et al*., 2013). Thus, residues of pesticides in soil, water, air and food supplies should be routinely monitored to access possible ecological antagonistic effects.

Vegetables are the fresh and edible portion of the herbaceous plants, important food and highly profitable for health. They contain valuable and essential food ingredients, which can be utilized successfully to build up and repair the body (Kumari, 2008). In India,

vegetables are major constituents of diet as majority of Indians are vegetarian, with a per capita consumption of 135 g per day while the recommended level is 300 g per day. It is still very less than recommended diet level (Ingle and Shyamrao, 2020). However, various factors limit their productivity, mainly insect pests and diseases.

Cruciferous vegetables have a significant position among *rabi* crops grown in India. Cabbage (*Brassica oleracea* var. *capitata*) is a leafy green, red (purple) or white (pale green) vegetable crop which is grown for its dense-leaved heads and belongs to "Cole crops". It is a prominent vegetable that has appreciable nutritional and economic value and is grown in all the states of India (Jat *et al.*, 2017). It belongs to family *Brassicaceae* and source of chemoprotective phytochemicals (Sharma *et al*., 2018). Green leafy vegetables can be used as natural fortificants of iron as these are rich sources of iron in vegetarian diets (Chiplonkar *et al*., 1999). Approximately 6.3 kg of Brassica vegetables are consumed per person annually (Jordbruksverket, 2003). Cabbage is used in many different ways for eating such as they can be pickled, fermented, steamed, braised or eaten raw. Cabbage is a good source of vitamin K, vitamin C and dietary fibre. Cabbage plants thrive in a relatively cool and humid condition. It is predominantly grown as a winter crop in plains whereas in the hills it is grown as a spring and early summer crop. For an early maturing crop sandy loam soil is generally considered most suitable but where higher yield is the main emphasis clay loam or silt loam soil is more suitable. In highly acidic soils it doesn't flourish well. The optimum pH range for cabbage is between 5.5-6.5 (NCPAH, INDIA). Six critical disease of cabbage found worldwide are black rot, clubroot, wirestem, black spot (dark spot), downy mildew and watery soft rot (Sharma *et al*., 2018).

It is one of India's most prominent winter vegetables. In India, cabbage is grown in an area of 4.06 m ha with a production of 8.97 MT with an average productivity of 25 t ha^{-1} (NHB, 2018). Productivity of cabbage in India is much lower attributing to many causes and among them insect pests are the major constraints (Biradar *et al*., 2020). It has been anticipated that insect pests alone cause 40-100% yield loss annually (Hasan and Ansari, 2011). The leading producer states of cabbage are West Bengal, Uttar Pradesh, Orissa, Bihar, Maharashtra and Karnataka. West Bengal contributes 2.042 Mmt of cabbage from 74,100 ha area with an average yield of 27.6 mt/ha (NCPAH, INDIA). The Food and Agriculture Organization of the United Nations (FAO) reported world production of cabbage and other Brassicas for 2014 was 71.8 million metric tonnes, of which 47% accounted by China. Several diseases in cabbage are caused by insects, fungi, bacteria, slugs and snails etc. (Jat *et al.*, 2017). Due to its anti-inflammatory, antioxidant and antibacterial properties cabbage has extensive utility in traditionary medicine, in alleviation of symptoms associated with gastrointestinal disorders (peptic and duodenal ulcers, gastritis, irritable bowel syndrome) as well as in treatment of minor cuts and wounds and mastitis (Rokayya *et al*., 2013).

According to the report of World Bank database, Faostat and registration authorities in respective countries (2019) India is the second biggest producer of agricultural products after China. India uses much less pesticides both in volume and variety. In China 681 pesticide molecules are registered while in India 282 molecules are registered. Pesticide use during 2017 in China was 17,63000 tonnes while in India it was 52,750 tonnes. The ability of insects to rapidly develop resistance to conventional pesticides poses an immense problem for effective pest management. The discovery of control agents that work by new biochemical mechanisms is therefore of critical importance in crop protection. Calcium channel regulation and in particular, the ryanodine receptor (RyR) represents a new biochemical target for insect control and thus offers excellent promise in integrated pest management strategies.

Cyantraniliprole [3-bromo-1-(3-chloro-2-pyridyl)-4'-cyano-2'-methyl-6'-(methylcarbamoyl) pyrazole-5-carboxanilide] is an anthranilic diamide insecticide developed by DuPont Crop Protection. It possesses a Molecular Formula of $C_{19}H_{14}BrClN_6O_2$ and Molecular Weight of 473.715 g/mol. It controls pests through activation of insect ryanodine receptors which are indispensable for muscle contraction (Lahm *et al*., 2007). The ryanodine receptor (RyR) is a non-voltage-gated calcium channel located in the sarcoplasmic reticulum of muscle cells and endoplasmic reticulum of non-muscle cells. Its name is derived from the natural insecticide ryanodine, a plant metabolite from *Ryania speciosa* that has been found to affect calcium release by locking channels in a partially opened state. It is achieved by replacing a cyano group of the 4-halo substituent of former anthranilic diamide chlorantraniliprole (Feng *et al*., 2010).

Activation of ryanodine receptors in insects affects calcium homeostasis by unregulated release of internal calcium in the cell leading to feeding cessation, muscle paralysis, lethargy and ultimately death of insect (Cordova *et al*., 2006; Jacobson and Kennedy *et al*., 2011; Lahm *et al*., 2005; Selby *et al*., 2013). It exhibits remarkable selectivity and low toxicity to mammals based on structural differences between insect and mammalian ryanodine receptors, moreover, improved plant mobility and increased spectrum has been reported (Lahm *et al*., 2009; Dong *et al*., 2012). Cyantraniliprole shows exceptional cross-spectrum activity on a broad range of Lepidoptera, Coleoptera, Isoptera and Dipteran insects. It has root systemic activity with some translaminar movement and effective against the larval stages of lepidopteran insects and also on thrips, aphids and some other chewing and sucking insects. Compared with chlorantraniliprole it is reported to be active against wider insect spectrum (Chai *et al*., 2010). For controlling insect pests in fruit crops, oil seed crop, tree nuts, cotton, grapes, rice, vegetables, ornamentals and turf around the world cyantraniliprole is utilised (WHO, 2013). Cyantraniliprole use may leads to residues in pollen and nectar but oral honeybee risk assessments indicate low risk for honeybees through oral exposure. It was determined that intended utilization of DuPont cyantraniliprole formulations pose low risk for pollinators (Dinter and Samel, 2015).

Chlorantraniliprole (CLAP) and cyantraniliprole (CNAP) are two analogous products from anthranilic diamides class of compounds. In their structure they have minor differences while there is a marked distinction in the insects affected by these products (Sharma *et al*., 2014). Eminent mammalian safety along with a broad spectrum of crop uses is leading to extensive applications of these products. Use of these products on a wide scale has also prompted reports on degradation of these products in food commodities (Gaddamiddi *et al*., 2011) as well as interest from the academic community in further scrutiny of these compounds (Lavtizar., 2013). The hydrolytic stability of these compounds was explored in sterile buffer solutions under acidic and alkaline conditions at environmentally relevant concentrations to establish the path of degradation of these compounds in water. Hydrolysis under conditions that imitate food processing and sterilization was also scrutinized for degradations at 90−120°C for short periods (Sharma *et al*., 2014). Although we expected the formation of free carboxylic acids in the degradation reactions to prevail, the reactions observed showed some unusual cyclic reactions. The formulated products for the evaluation were 'DPX-HGW86 100 g/l OD' an oil dispersion formulation, 'DPX-HGW86 100 g/l SE' a suspo-emulsion, 'DPX-HGW86 200 g/l SC' a suspension concentrate and 'A16971 B' a water dispersible formulation (WG) containing 400 g/kg cyantraniliprole. All formulations are used as spray applications except the SC formulation which is by drip irrigation or in hydroponics to a wide range of outdoor and protected crops (EFSA, 2014). The toxicokinetics of cyantraniliprole showed an oral absorption value of 70%, an extensive distribution in the body, no prominent bioaccumulation and an excretion occurring chiefly within 48 h after administration.

Through the evaluation of leaching potential of anthranilamide insecticides through the soil mobility of two relatively new anthranilic diamide insecticides, cyantraniliprole (CY) and chlorantraniliprole (CH) was examined in soil (Vela *et al*., 2017). It was performed by means of disturbed columns loaded with a typical semiarid Mediterranean soil under laboratory conditions. It was found that both insecticides appeared in leachates with 41% of CH and 52% of CY of the initial mass added. For CH recoveries were 33%, 22% and for CY these were 21% and 19% from upper and bottom layers of the soil respectively. As per the calculated half-lives (27 and 29 days for CH and CY, respectively) and their log K_{oc} (about 2.5 for both), the calculated Groundwater Ubiquity Score (GUS) index was higher than 5 for both which indicates that they have the potential to leach. Two transformation products, $C_{13}H_9Cl_2N_2O$ (IN-ECD73) and $C_{19}H_{12}BrClN_6O$ (IN-J9Z38) corresponding to the deterioration of CH and CY, respectively were also identified and detected in soil and leachates (Vela *et al*., 2017).

Residues on crops are a major concern specifically when raw vegetables are consumed and the persistence of pesticides in soil further increases the chances of their uptake in subsequent crops. Pesticide residues in food must be at the lowest level as much possible

and should be safe for consumers. For acquiring the minimum amount of pesticides level to achieve the desired pest control efficiency, MRL values are set. Therefore, evaluating the consequences of cyantraniliprole usage from a residue perspective on consumer health and its persistence in soil from an environmental point of view is mandatory. According to the best of our knowledge, there is no prior information available on the residues of cyantraniliprole and their dissipation in cabbage plant and soil under cultivation. Being a new insecticide little information is available on persistence and dissipation behaviour of cyantraniliprole in some other crops. Therefore, present study is planned with following objectives to assess the potential risk to the consumer and environment:

1. To study the dissipation and persistence behaviour of cyantraniliprole in cabbage and soil
2. To study the effect of household processing methods on reduction of residues of cyantraniliprole

CHAPTER-II

REVIEW OF LITERATURE

In this chapter an attempt was made to review the work carried out in India and abroad on "Dissipation and decontamination studies of Cyantraniliprole in cabbage and soil". Cyantraniliprole [3-bromo-1-(3-chloro-2-pyridyl)-4'-cyano-2'-methyl-6'-(methylcarbamoyl) pyrazole-5-carboxanilide] is an anthranilic diamide insecticide which activates the insect ryanodine receptors causing an imbalance of calcium ions in the body of insect. Cyantraniliprole is a xylem systemic insecticide and it is the first compound in the diamide class that has demonstrated cross-spectrum activity on chewing and sucking arthropod pests (Lahm *et al*., 2005). Cyantraniliprole exhibits remarkable selectivity and low toxicity for mammals based on structural variations between insect and mammalian ryanodine receptors, along with increased plant mobility and spectrum (Lahm *et al*., 2009; Dong *et al*., 2012). Its activity against a wider spectrum of insects than chlorantraniliprole is identified (Chai *et al*., 2010). Pesticides are used in agriculture to protect crops against destructive pests both in the field and during storage. Pesticides may contaminate the environment and accumulate in the food chain thereby posing hazard to human health (Blasco *et al.*, 2003). These studies were carried out mainly to examine the magnitude of cyantraniliprole residues in cabbage and soil. These studies also considered the effect of various culinary processes in reducing the residues of cyantraniliprole. In order to be succinct and descriptive, the literature examined was divided primarily into three parts.

2.1 Residue analysis techniques

2.2 Dissipation of cyantraniliprole in various crops and soil

2.3 Impact of various processing methods in dislodging the residues of cyantraniliprole in various crops

2.1 Residue analysis techniques

For the purpose of analysis of pesticides in given matrices it is absolutely essential to extract them in a suitable solvent. During the process of extraction of pesticide of interest many other contaminants such as plant pigments, waxes, fats and also other pesticides come along with the desired extract. Therefore, it is required to remove all these impurities from the extract to enhance the sensitivity and reliability of the process by the process called clean-up. Extent of clean-up depends upon the sensitivity and selectivity of the adopted method for sample analysis, nature of sample, scope of analysis. In the current study pesticide of interest is cyantraniliprole, belongs to anthranilic diamide class of insecticides which are efficient at low doses. Therefore, they require a sensitive method for their analysis. The increasing rate of

its usage in agriculture and its novelty has prompted researchers for its residue estimation in various crops and soil. For its residue detection various quantification methods like HPLC, UPLC -MS/MS etc. and clean-up processes were used by researchers. The research work of different workers has been reviewed.

Dong *et al*., (2012) used ultra-performance liquid chromatography/tandem mass spectrometry (UPLC-MS / MS) to study the cyantraniliprole and its major metabolite residues J9Z38 in vegetable and soil. QuEChERS method was applied for estimating cyantraniliprole and its major metabolite in tomato, cucumber and soil samples. Extraction was done with the acetonitrile solvent and clean-up with primary and secondary amine (PSA) sorbent. Mobile phase consists of 2 mM ammonium acetate aqueous solution (solvent A) and ACN (solvent B). UPLC system was coupled with a triple-quadrupole mass spectrometer equipped with electrospray ionization (ESI) source. Solvent flow was maintained @ 0.5 mL/min. and gradient elution programming was followed. Run time of cyantraniliprole and J9Z38 separation was 3.5 min. LOD and LOQ for cyantraniliprole were found to be 2 µg/kg and 5 µg/kg respectively.

Sun *et al*., (2012) studied cyantraniliprole (SC, 10 %) and its primary metabolites in Pakchoi and soil using ultra performance liquid chromatography-tandem mass spectrometry. The technique used for sample preparation was quick, easy, cheap, effective, rugged and safe i.e. QuEChERS. With chromatographic grade acetonitrile, samples were extracted and before analysis samples were cleaned up with dispersive primary and secondary amine (PSA) sorbent and filtered with 0.22 µm filter membrane for pesticide analysis using Thermo TSQ Quantum UPLC-MS/MS equipped with a Betasil C18 column (2.1 mm x 100 mm x 5 µm). For the activity of the LC gradient, two eluent components acetonitrile/0.2 percent acetic acid and water/0.1 percent acetic acid were used. Volume injected was 10 µL, column temperature kept at 40°C and flow rate of solvents remained constant @ 0.2 mL/min.

MS/ MS detection was carried out in the selected reaction monitoring (SRM) mode using electrospray ionization (ESI) source in the positive ion mode. The cyantraniliprole and J9Z38 retention times were 1.82 and 2.42 minutes, respectively. The quantification limit for cyantraniliprole and J9Z38 in Pakchoi and soil was 0.01 mg/kg.

Zhang *et al*., (2013) studied dissipation dynamics of cyantraniliprole and its metabolite J9Z38 in rice field ecosystem using Waters Acquity UPLC system. They developed a reliable and easy method to analyse the residues of cyantraniliprole in rice straw, brown rice, paddy water and soil. Cyantraniliprole 10% oil suspension formulation was sprayed @ 150 g a.i. hm^{-2} which was 1.5 times of the recommended dose. Acetonitrile was used for extracting the target compounds. Clean-up was done with silica gel or strong anion exchange column and analyzed by ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). Column used was Waters Acquity UPLC BEH C18 (2.1 mm x

50 mm, 1.7 µm particle size). Column and sample manager temperature set at 40°C and 10°C respectively. The LC separation was performed by injecting 2 µL of sample @ 0.20 mL min^{-1}. Retention time (R_t) of cyantraniliprole was found to be 0.89 min. The LOQ of cyantraniliprole and J9Z38 were 18 and 39 µg/kg for rice straw, 4.3 and 6.3 µg/kg for brown rice, 2.8 and 5.0 µg/kg for paddy water and 3.9 and 5.3 µg/kg for paddy soil.

Hu *et al*., (2013) investigated residues of cyantraniliprole (10% oil suspension) and its main metabolite in watermelon and soil using UPLC-MS/MS. Dosage of cyantraniliprole used was 1.5 x recommended dosage i.e. @ 100 g a.i. Extraction was done with acetonitrile-water and clean up on silica gel column. Waters Acquity UPLC system was utilised for the chromatographic separation of cyantraniliprole and J9Z38. Using 0.22 µm pore filter mobile phase solvents were distilled and passed. LODs estimated for cyantraniliprole in watermelon and soil were 0.000063 and 0.000045 mg/kg respectively. LOQs for cyantraniliprole were determined to be 0.00021 and 0.00015 mg/kg and for J9Z38 were 0.0010 and 0.00090 mg/kg respectively for watermelon and soil. Injection volume was 5 µl, flow rate was 0.20 mL/minute. The column maintained at 40ºC and temperature in the sample manager was set at 10ºC.

Sample analysis was performed on a triple-quadrupole mass spectrometer using the positive electrospray ionization mode. The high-purity argon collision gas was managed to hold at 0.15 mL/min. Retention times (R_t) of cyantraniliprole and J9Z38 were found 0.84 and 1.03 min. respectively.

Hong-Mei *et al.*, (2014) analysed residues of cyantraniliprole and its major metabolite J9Z38 in pepper and soil with gradient ultra-performance liquid chromatography (UPLC-MS/MS) with electrospray ionization in positive mode (ESI+). Extraction of target compounds was done with acetonitrile and clean-up with C-18 cartridge. Column used was UPLC BEH C-18 and LOQs for cyantraniliprole and J9Z38 were 0.1 and 0.2 m µg/kg respectively.

Pan *et al*., (2015) standardized protocol for simultaneous determination of cyantraniliprole and chlorantraniliprole in fruits, vegetables and cereals using UHPLC-MS/MS. A good strategy i.e. isotope labelled internal standard (ILIS) for overcoming the negative effects of ion suppression or enhancement on the quantitative results was adopted. QuEChERS method was used for analysis of pesticides. Clean-up effect based on different sorbents- primary secondary amine (PSA), graphitized carbon black (GCB) and octadecylsilane (C_{18}) was evaluated and compared. The mobile phase consists of ACN and 0.1% (v/v) formic acid in water. Using gradient mode elution was performed and total run time was 4 minutes. Acquity UPLC HSS T3 analytical column (2.1 mm x 50 mm, 1.8 µm particle size) was used, temperature of column oven set at 30 ºC and of sample manager at 5 ºC. Injection volume was 10 µL and LOD of the two analytes lies between 0.015-0.085 µg/kg.

For all the matrices LOQ ranges from 0.05-0.28 µg/kg.

Vann *et al.*, (2017) investigated cyantraniliprole and spinosad residues in flue-cured tobacco for establishing maximum expected residues on cured tobacco leaf which would result from application of specific compound at recommended level. Under a research program at North Carolina State University, cyantraniliprole and spinosad were evaluated over a period of 3 years in three environments. Cyantraniliprole was applied in a single tray drench application immediately before transplantation. The operating pressure was 138 kPa, material was delivered to 1 tray of transplants through a 2-L solution (1.98 L water + 0.02L cyantraniliprole) i.e. a total of 197.51 g a.i./ha.

With a CO_2 pressurized applicator having a TG-3 nozzle both applications were completed and spinosad was applied through 6 foliar treatments. Four replications were taken and arranged in a randomized complete block design in all growing environments.

Malhat *et al.*, (2018) determined degradation dynamics of cyantraniliprole in tomato. Technique of high-performance liquid chromatography coupled with photo-diode array detector (HPLC-DAD) was used for analysing cyantraniliprole residues in tomato. Cyantraniliprole formulation, Benevia 10 percent OD was applied to tomato plants @ 75 g a.i./h (recommended dose) using knapsack sprayers for 40 min. Sampling from the treated plots was performed randomly at various intervals (0, 1, 3, 7, 10 and 14 days) after spray. Homogenisation of tomato samples was carried out using a HOBART Food Processor. Sample preparation involved single step extraction with ACN and NaCl was used for partitioning. For the clearance of extract florisil solid phase extraction cartridge was utilized.

An Agilent 1260 series HPLC-DAD was employed to conduct quantitative analysis of cyantraniliprole residues. The column used was Zorbax XDB C18 (4.6 mm x 250 mm x 5 µm), water and acetonitrile (60:40, v / v) were used as solvents @ 0.8 mL/min, HPLC was used in isocratic mode. Injection volume was 20 µL, column temperature kept at 35 ºC and λ_{max} was 280 nm. LOQ was estimated at 0.05 mg/kg.

Lee *et al.*, (2019) studied about dissipation pattern of cyantraniliprole and its metabolite IN-J9Z38 in Proso Millet during cultivation using the citrate-buffered QuEChERS method and ultra-high-performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS) for multiple reaction monitoring of target compounds. According to the dates of pesticide application the experimental field was divided into four plots, each plot contained three replicates of 10 m^2. Before harvest, spray of an oil dispersion (OD) formulation of pesticide was done twice. Column used was C18 (100 mm x 2.1 mm, 2.6 µm) and its temperature kept at 40 ºC. Mobile phase involved solvents- deionized water and methanol, both containing 0.1% HCOOH (formic acid) and 5 mM ammonium formate, flow maintained @ 0.2 mL/min. and gradient programming applied. Total run time of samples was

10 min. and injection volume were 5 µL.

Retention time of cyantraniliprole was 4.68 min. and 4.97 min. for IN-J9Z38. Instrumental limit of quantification (ILOQ) for cyantraniliprole and IN-J9Z38 was 0.0025 µg/mL in the matrix matched standard solution in both straw and grain.

Zhang *et al.,* (2018) resolute seed treatment efficiency of cyantraniliprole against *Agrotis ipsilon* (Lepidoptera: Noctuidae) and residue concentrations in corn plants and soil. Starting experiment was carried out in plastic garden pots containing 40% sand, 40% clay and 20% organic matter in a greenhouse with a controlled environment having temperature of 25 ºC, relative humidity 70% and a 14:10 (L:D) photoperiod. Two seed treatments were applied @ 2 and 4 g a.i. kg^{-1}. Extraction was performed by QuEChERS method. Chromatographic separation of cyantraniliprole and J9Z38 was done by Waters Acquity UPLC System including a Waters Acquity UPLC manager. With a triple quadrupole mass spectrometer equipped with an Electrospray ionisation (ESI) source, UPLC system was coupled. Capillary voltage set at 3 kV while the desolvation temperature and source temperature was kept at 400 ºC and 150 ºC respectively.

Mobile phase consists of CH_3OH (solvent A) and 0.1 % formic acid in water (solvent B) and the flow rate was maintained at 0.35 mL min^{-1}. Injection volume was 2 µL, column temperature was 35 ºC, collision gas used was 99.99 % argon and nebulizer gas were 99.95% nitrogen. R_t of cyantraniliprole and J9Z38 were found to be 1.88 min. and 2.30 min. respectively. For detection of cyantraniliprole and J9Z38 the MRM mode was used. At 3 different fortification levels (0.01, 0.10 and 1.00 mg kg^{-1}) recovery experiments were performed from blank corn stalk and soil samples.

2.2 Dissipation of cyantraniliprole in various crops and soil

Dong *et al*., (2012) examined cyantraniliprole in vegetable and soil and its main metabolite residues (J9Z38). Level of average recoveries for cucumber, tomato and soil at three levels (10, 50 and 100 µg/kg) lies between 74.7-96.2 % with intra-day relative standard deviation (RSD) of 2.6-15.1%. Inter-day RSD was found to be 3.4-13.3%. Dissipation of cyantraniliprole residues was mathematically explained by a pseudo first order rate equation. The half-lives of cyantraniliprole reported were 2.2, 2.8 and 9.5 days in cucumber, tomato and soil respectively in Zhejiang. The residue level was found < 0.01 mg/Kg after 10 days of treatment. Dissipation of cyantraniliprole in soil was slower than in tomato and cucumber.

Sun *et al*., (2012) studied cyantraniliprole and its major metabolite residues in Pakchoi and soil by using UPLC-MS/MS. Mean recoveries at three spiked levels (0.01, 0.05, 0.1 mg/kg) for cyantraniliprole and J9Z38 ranged from 77.8-102.5% with RSDs differ from 1.6-8.9%. The half-life of cyantraniliprole lies between 2.9 to 6.4 days in Pakchoi and 8.7-18.2 days in soil. Final level of residues of cyantraniliprole in Pakchoi and soil from Guangdong were < 0.20 mg/kg and from Shanghai were below 0.10 mg/kg. Also, final

residue level of J9Z38 in Pakchoi and soil from Guangdong and Shanghai were < 0.07 and 0.01 mg kg^{-1}. These results would be helpful for setting maximum residue limit guidance for cyantraniliprole in Pakchoi.

Zhang *et al*., (2013) studied dissipation dynamics of cyantraniliprole and its metabolite J9Z38 in rice field ecosystem. Level of recoveries for all matrices ranged from 79.0-108.6 % with relative standard deviations of 1.1-10.6%. The results showed that half-lives ($T_{1/2}$) of cyantraniliprole were 6.3, 4.4 and 3.2 days in rice straw and 6.2, 2.0, 4.9 days in paddy water in Shandong, Hunan and Zhejiang respectively. After 14 days of pre-harvest interval (PHI) final residues of cyantraniliprole and J9Z38 were lower than 0.05 and 0.02 mg kg^{-1} respectively. Maximum residue limit (MRL) of cyantraniliprole at 0.1 mg kg^{-1} and dosage of 100 g a.i. hm^{-2} was recommended and considered as harmless for human beings and animals.

Hu *et al*., (2013) investigated residues of cyantraniliprole and its major metabolite in watermelon and soil using UPLC-MS/MS. Range of average recoveries of cyantraniliprole and J9Z38 in watermelon and soil at 3 distinct fortifications levels (0.01, 0.1 and 0.5 mg/kg) lies from 85.71 to 105.74% with RSDs of 0.90-6.34%. Residues of cyantraniliprole in watermelon degraded from 0.079 to 0.01 mg kg^{-1} in Zhejiang and from 0.037 to 0.01 mg kg^{-1} in Hunan over 5 days. Residue level in soil degraded from 0.52 to 0.019 mg kg^{-1} after 21 days of treatment in Zhejiang. The half-lives reported were 1.1 and 2.7 days in watermelon in Zhejiang and Hunan respectively and 4.1 and 2.6 days in soil of Zhejiang and Hunan respectively. The residues reached below quantification level of 0.01 mg/Kg within 14 days interval after treatment.

Hong-Mei *et al.*, (2014) analyzed residues of cyantraniliprole and its primary metabolite J9Z38 in pepper and soil. At three spiking concentrations (0.01, 0.10 and 1.00 mg/kg) intra-day mean recoveries lies between 88.6-105.7% with relative standard deviations of 3.8-15.1%. Levels of inter-day mean recoveries of cyantraniliprole and J9Z38 were obtained between 91.4-105.7% and RSDs in range of 4.9-12.3%. Experimental results showed that half-life of cyantraniliprole ranged from 9.2-11.2 days in pepper and for soil lies between 9.2 to 20.8 days. Correlation coefficient (r) > 0.9992 was obtained in the concentration range of 2.0-128.0 µg/L. J9Z38 residues in pepper were below LOQ and in soil with half-life of 9.4 days. With increase of precipitation, rate of degradation of cyantraniliprole increased.

Pan *et al*., (2015) standardized protocol for simultaneous determination of cyantraniliprole and chlorantraniliprole in fruits, vegetables and cereals using UPLC-MS/MS. In accordance with findings of this experiment, a new, sensitive ILIS method was successfully developed and achieved for residue analysis. Recoveries of two native analytes in the six matrices (apple, grape, cucumber, tomato, rice and wheat) were evaluated. At three

spiking levels (10, 20 and 50 µg/kg) overall average recoveries procured were 95.5-106.2% with relative standard deviations < 14.4% for all the analytes.

Vann *et al.,* (2017) determined residues of cyantraniliprole and spinosad in flue-cured tobacco. Field experiment conducted during 2013, 2014 and 2015 at North Carolina State University showed that residues of cyantraniliprole in all environments were below the limit of quantification (0.125 mg/kg). Spinosad residues were detected and reported by individual year and stalk position due to effective environment x treatment interaction. Tolerance limits of cyantraniliprole in tobacco ranges from 0.02-1.0 mg/kg.

Malhat *et al*., (2018) conducted a study to access magnitude of cyantraniliprole residues in tomato following open field application. Evaluation of this novel method was performed by comparing the analytical results with those found in case of QuEChERS. This approach outbalanced QuEChERS in the study with respect to matrix interferences thus meeting all of the guideline requirements. Satisfactory results were obtained at three spiking concentrations (0.05, 0.1 and 1.0 mg/kg) with 88.9-96.5% recoveries and RSDs ranged from 9.9-15.2%. Its MRL in tomato is fixed by the Joint FAO/WHO Codex Alimentarius Commission (Codex 2016) at 0.3 mg/kg and that by the EU at 1 mg/kg (MRL-Cyantraniliprole-EU 2017). Half-life was 2.6 days for cyantraniliprole degradation. PHI for cyantraniliprole was 3 days for tomatoes, relying on Codex MRL (0.3 mg / kg).

Lee *et al*., (2019) investigated dissipation patterns of insecticide cyantraniliprole and its metabolite IN-J9Z38 in proso millet during cultivation under open field conditions. For all fortification levels (0.1 and 0.5 mg/kg) and matrices (grain and straw) sufficient good recoveries of 91.2-105.3% for cyantraniliprole and 88.8-108.2% for IN-J9Z38 were obtained. RSD was < 5.9% for all the results. They observed that cyantraniliprole residues in straw and grain showed a decline of 91.1% and 89.1% respectively, between plot A and plot D from the initial residues. IN-J9Z38 level gradually increased with time which signal that during cultivation cyantraniliprole transformed into IN-J9Z38. Biological half-lives ($T_{1/2}$) of cyantraniliprole were 9.4 and 11.3 days respectively for straw and grain. The outcome of this study will inform the regulation and management of pesticide for minor crop proso millet.

Zhang *et al.,* (2018) determined seed treatment efficiency of cyantraniliprole against *Agrotis ipsilon* (Lepidoptera: Noctuidae) and residue concentrations in corn plants and soil. At three different fortification levels (0.01, 0.10 and 1.00 mg kg^{-1}) recovery experiments were conducted from blank corn stalk and soil samples and for each level replication was done three times. Experiments conducted in plant pots showed that mortality of *A. ipsilon* was greater than 92 % and < 24% of seedlings were damaged when treatment of corn seeds with cyantraniliprole was done @ 2 and 4 g a.i. kg^{-1} seed. Seed treatment with cyantraniliprole leads to better control efficiency of *A.ipsilon* in spring than in summer.

Residues of cyantraniliprole in stem bases of corn plants degraded from 1.50 to 0.02 mg kg^{-1} in the summer and from 8.73 to 0.18 mg kg^{-1} in the spring after 34 days of treatment. The half-life period ($T_{1/2}$) of cyantraniliprole in stem bases of spring corn plants was 3.21 days and in summer corn plants it was 3.02 days. Similarly, in the soil residue concentration of cyantraniliprole and J9Z38 gradually declined over the sampling period. Half-life ($t_{1/2}$) of cyantraniliprole in soil was 6.76 days and 5.03 days in spring and summer respectively.

2.3 Impact of various processing methods in dislodging the residues of cyantraniliprole in various crops

Large number of pesticides are used for reducing the risk of disease caused by numerous pests in various commodities. But the residue deposit on these commodities may remain in excess than their acceptable MRL level. We can reduce the level of residues of these pesticides to some extent by application of various household processing methods such as washing, boiling, peeling etc.

Ahlawat *et al*., (2019) determined the impact of culinary processes on the discharge of chlorantraniliprole residues in chilli and found that washing with 5% brine solution was most efficacious than washing with hot water (59.40–58.54%) and washing under tap water (53.79–50.80%) with 62.02 and 67.94% reduction in chlorantraniliprole residues regardless of the dose on 0 day.

Kar *et al*., (2012) reported decontamination of chlorantraniliprole in cauliflower and cabbage. Washing with tap water results into 17– 40% elimination of residues while boiling declined the residue level 100% in both cabbage and cauliflower.

Kaushik *et al*., (2019) determined impact of various culinary practices in reduction of residues of tetraniliprole in tomato crop in India. Tetraniliprole was sprayed on tomato crop @ 60 g a.i. ha^{-1} and 120 g a.i. ha^{-1}. Washing of tomato fruits reduced the residues up to 37.63% and washing with lukewarm and saline water removed the residues by 44.67% and 61.49% respectively. Open pan cooking declined the residues by 72.21% and microwave cooking of tomato provided -12% better reduction of residues in comparison with open pan cooking.

Chen *et al.,* (2015) evaluated the outcome of washing with tap water and processing time on residue removal of chlorantraniliprole in cowpea fruits. Observed reduction rate was 12.79 % and processing factor was 0.8721 in case of 2-min. washing. The preliminary 9.293 mg kg^{-1} deposit reduced to 8.1048 mg kg^{-1} and decontamination level enhanced with increase in washing time. After 15 min. washing 68.42% reduction in residues occurred.

Reddy *et al.,* (2018) determined the effect of various processing methods on reduction of residues of different insecticides including chlorantraniliprole 20% SC @ 30 g a.i. ha^{-1} in field bean. Among these different decontamination methods treatment with formula 1 (4% acetic acid + 0.1% $NaHCO_3$ + 1 lemon) reduced residues of different insecticides to greater extent ranging from 67.60-74.90 % followed by treatment with 2% salt solution.

Vijaysree *et al*., (2015) studied the effect of various decontamination processes (treatment with 2% slaked lime, 2% NaCl solution, 1% turmeric solution, 2% vinegar solution, 2% baking soda, 2% tamarind and scrubbing) on elimination of chlorantraniliprole residues in okra and brinjal. Chlorantraniliprole residues reduced to 0.051 and 0.068 mg kg^{-1} from starting residue level of 0.073 mg kg^{-1} after treatment with 2% slaked lime and 2% NaCl solution respectively recording greater than 90% reduction in brinjal after 2h of spray. Better results were obtained for okra by treatment with vinegar, scrubbing, slaked lime and turmeric and >80% reduction in beginning residue level after 2h of spray.

CHAPTER-III

MATERIALS AND METHODS

The material used and the technique opted to accomplish the objectives of present research entitled, "Dissipation and decontamination studies of cyantraniliprole in cabbage and soil" are explained in this chapter. Contagion of food commodities with trace amounts of pesticides has become a growing source of concern for the general population. Extensive use of pesticides may lead to their accumulation in the agricultural produce. These chemicals are classified as persistent toxic substances due to their low biodegradability (Tayade *et al*., 2013). In present work cabbage, which is pretentious by diverse diseases like black rot, clubroot, downy mildew, watery soft rot (white mould), black spot (dark spot) and wirestem results in less production is treated with an insecticide cyantraniliprole (Benevia 10.26% OD). To observe the dissipation pattern of cyantraniliprole the field experiment was carried out in research farm of Entomology, CCS HAU, Hisar, using randomized block design (RBD) and analysis was performed in pesticide residue laboratory under All India Network Project (AINP) on Pesticide Residue. For any pesticide that is used by the farmers on a large scale such studies play a crucial role in safety from consumer point of view. Therefore, present study was conducted with the following objectives:

(1) Standardization of analytical techniques for quantification of cyantraniliprole residues in cabbage and soil using HPLC system.
(2) To study the dissipation kinetics of cyantraniliprole residues in cabbage and soil
(3) To examine the effects of decontamination/culinary processes on reduction of cyantraniliprole residues in cabbage

Cyantraniliprole:

Cyantraniliprole [3-bromo-1-(3-chloro-2-pyridyl)-4'-cyano-2'-methyl-6'-(methylcarbamoyl) pyrazole-5-carboxanilide] is an anthranilic diamide insecticide proposed by DuPont Crop Protection. It possesses a Molecular Formula of $C_{19}H_{14}BrClN_6O_2$ and Molecular Weight of 473.715 g/mol (EFSA, 2014). It controls pests through activation of insect ryanodine receptors which are indispensable for muscle contraction (Lahm *et al*., 2007). For studying the dissipation kinetics of cyantraniliprole, the insecticide was sprayed on cabbage at recommended and double of recommended dose by Central Insecticides Board (CIB) and experiment was conducted during 2019-2020. The field experiment was performed using Randomized Block Design (RBD) at Research Farm of Entomology, CCS HAU, Hisar. The laboratory tests for the recovery study were performed at Pesticide Residue Laboratory, Entomology Department, CCS HAU, Hisar. Cyantraniliprole 10.26% OD was applied @ 60 g

a.i. ha^{-1} (T_1) and 120 g a.i. ha^{-1} (T_2) at the fruit setting stage and one plot for each treatment was kept as control in which no pesticide was applied. Cyantraniliprole formulation was screened for active ingredient before spraying the insecticide, on the basis of which proper amount of insecticide necessity for each treatment was calculated. Further information of the experiment is provided in the following text:

3.1 Location

The research trials were conducted at Research Farm of department of Entomology CCS HAU, Hisar during *Rabi* season in 2019-2020. The physico-chemical properties of soil are given in Table-1

Table 1: Physico-chemical properties of soil

Soil Property	Sandy Soil
Texture	Sandy Loam
pH	7.6
EC_e (dSm^{-1})	2.0
O.C. (%)	0.67
P_2O_5 (kg ha^{-1})	15

3.1.1 Crop variety and fertilisers applied:

Cabbage (*Brassica oleracea* var. *capitata)* of variety "Golden Acre" during *Rabi* season by 2019-2020 was planted, following efficient and good agronomic practices. Field experiment details are provided in table-2.

Table 2: Layout of field experiment for Cabbage crop

Location	Pesticide applied	Crop	Variety	Year
Research Farm, Department of Entomology, CCS HAU HISAR	Cyantraniliprole	Cabbage	Golden Acre	2019-2020
Sub plot size (m^2)	**Date of sowing of seed**	**Date of seedling transplantation**	**Date of spray application**	
25	16-10-2019	11-11-2019	03-02-2020	

Table 3: Details of sampling for cabbage and soil

Days of sampling (after spray application)	Date of sampling
0 (1h)	3-02-2020
1	4-02-2020
3	6-02-2020
5	8-02-2020
7	10-02-2020
10	13-02-2020
15	18-02-2020

3.1.2 Meteorological data

Data on maximum and minimum temperature, relative humidity, wind speed, sunshine hours and rainfall recorded at meteorological centre of CCS HAU, HISAR are utilised and compiled in Table 4.

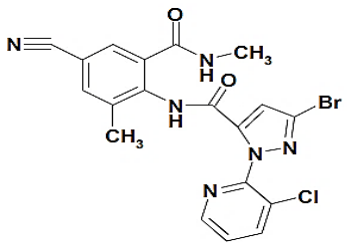
Table 4: Meteorological data during the crop period

DEPARTMENT OF AGRICULTURAL METEOROLOGY, CCS HAU, HISAR
Weekly data from 15 Oct 2019 to 18 feb 2020

Location LAT: 29° 10'N LONG: 75° 46'E, ALT: 215.2m 0727 LMT 1427 LMT

WEEKLY VALUES							
WEEK No.	**Temperature (°C)**		**Relative Humidity**		**Average Wind Speed (KM/H)**	**Bright Sun Shine Hours**	**Rainfall (mm)**
	MAX	**MIN**	**M**	**E**			
42	34.1	18.4	79	34	4.5	7.3	0
43	31.9	14.9	79	31	2.1	6.5	0
44	30.7	16.2	90	40	1.3	1.8	0
45	28.4	12.7	85	36	4.1	6.6	0.3
46	26.8	12.7	86	41	3.1	2.5	0
47	26.7	10.9	88	42	2.5	4.7	0
48	22.6	12.1	92	62	3.5	2.8	12
49	23.1	6.0	88	47	1.5	6.2	0
50	19.2	8.3	95	74	3.8	2.2	4.5
51	13.7	6.1	99	81	3.2	1.1	0
52	11.9	2.6	97	75	3.1	1.7	0
1	17.3	5.7	96	60	3.1	3.5	0
2	17.7	5.7	96	64	3.3	3.3	3.2
3	13.4	4.7	100	82	2.8	2.1	0
4	19.2	5.0	97	56	3.9	5.9	7.2
5	18.8	3.9	98	61	3.4	6.3	0
6	20.1	2.8	93	46	2.6	7.2	0
7	24.7	4.8	93	37	3.6	8.7	0

Table 5: Details of Cyantraniliprole (FAO, 2015)

ISO Common name	Cyantraniliprole
Trade Name	Benevia
Chemical Name	3-bromo-1-(3-chloro-2-pyridyl)-4'-cyano-2'-methyl-6'-(methylcarbamoyl)pyrazole-5-carboxanilide
Function	Insecticide
Formulation	Oil Dispersion (10.26% OD)
Chemical family	Anthranilic diamide
Molecular formula	$C_{19}H_{14}BrClN_6O_2$
Molecular weight	473.715 g/mol
Melting point	217-219 °C
Vapour pressure	5.133 × 10–15 Pa (20 °C) 1.787 × 10–14 Pa (25 °C)
Solubility in water (20° C)	(pH 4)- 17.43 mg/L (pH 7)- 12.33 mg/L (pH 9)- 5.94 mg/L
Relative density	1.497 (98.4 %) at 20 °C
Mode of action	Systemic insecticide Activator of insect Ryanodine Receptor (RyR)
Oral LD_{50} (rat)	>5000 $mgkg^{-1}$
Appearance	off-white, mild oily odour
Photolysis	Rapid
Hydrolysis	Temperature and pH dependent
Dissociation constant (pKa)	8.8 at 20 °C
pH (1% dispersion)	5.61
Structure	

3.1.3 Fate of cyantraniliprole (FAO, 2015):

Degradation in soil:

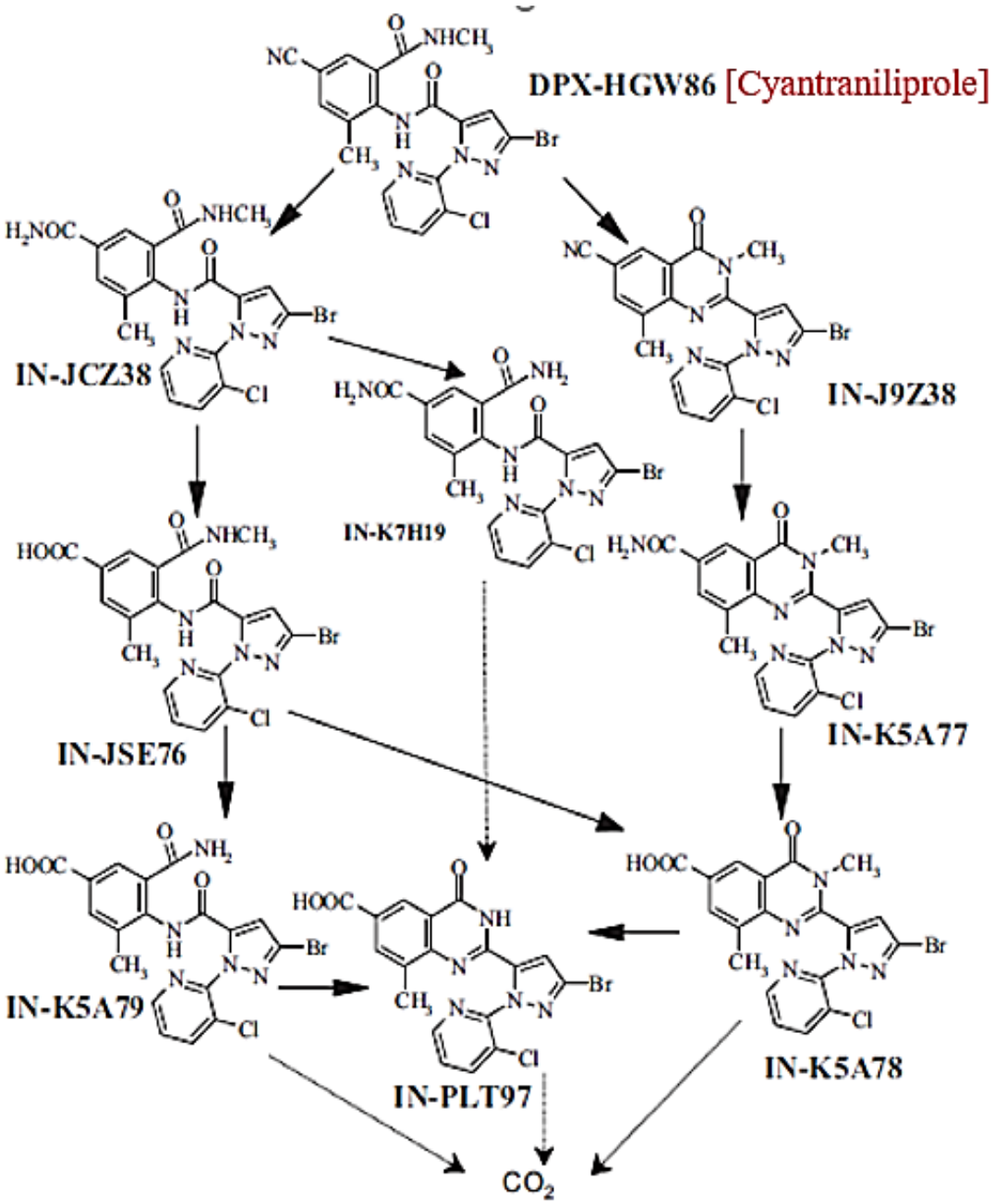


Figure 1: Proposed cyantraniliprole degradation pathway in soil under aerobic conditions

Degradation in Plant: Metabolic pathway of cyantraniliprole has been studied in cotton, rice, tomato and lettuce plants. Metabolism of cyantraniliprole was extensive, consisting of hydroxylation, N-dealkylation, oxidation and conjugation processes (APVMA, 2013).

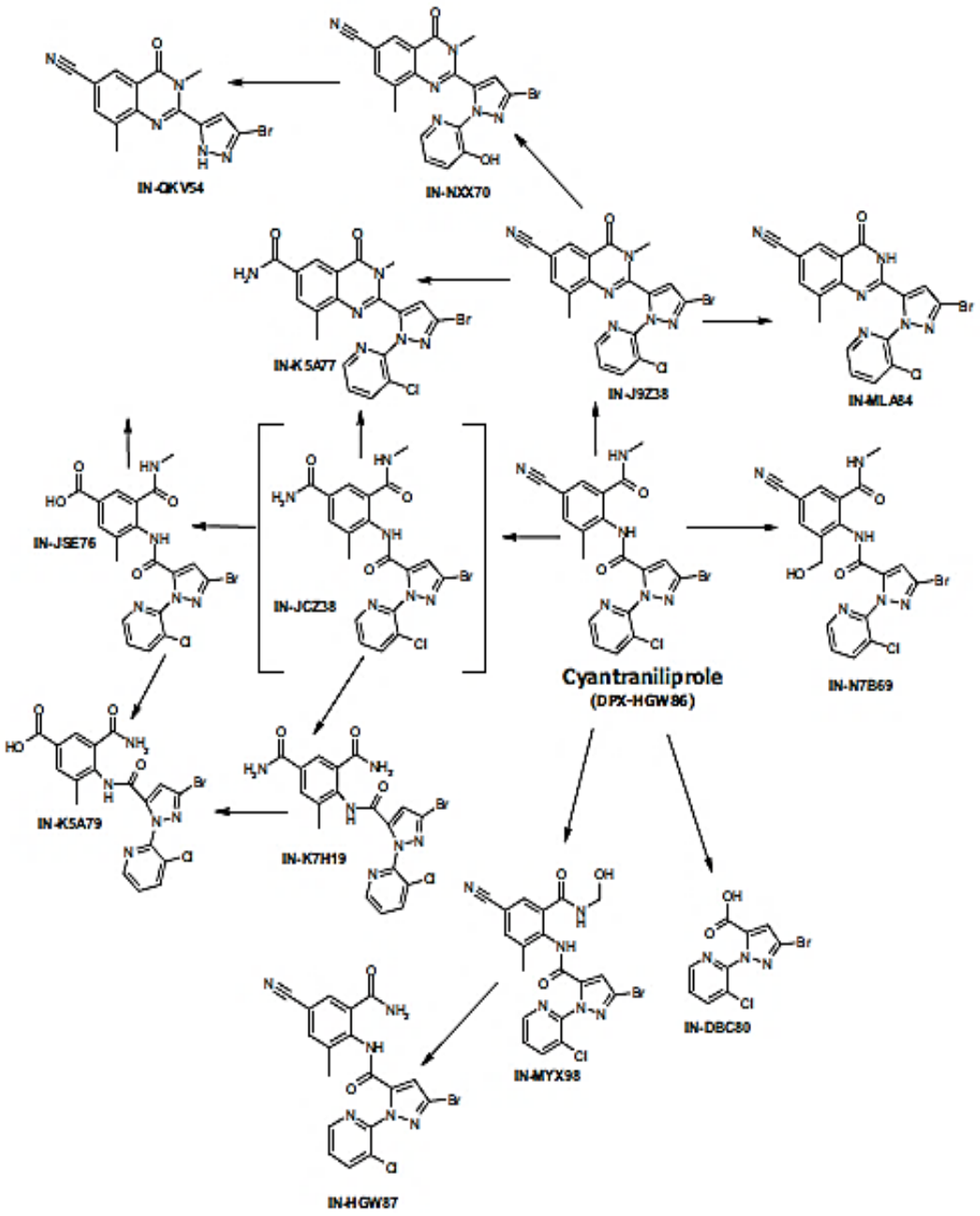


Figure 2: **Proposed Metabolic pathway of cyantraniliprole in plant (lettuce)**

Degradation in Water: Hydrolysis of cyantraniliprole was slow at pH 4 with minimum amount of degradate IN-J9Z38 formed mainly at lower temperatures. It was observed that transformation of cyantraniliprole proceeded at the fastest rate at higher temperature of 35°C and the parent compound disappeared completely within 5 days. Degradates were identified by comparing the retention time (R_t) of radioactive peak with an authentic standard (Sharma *et al*., 2014).

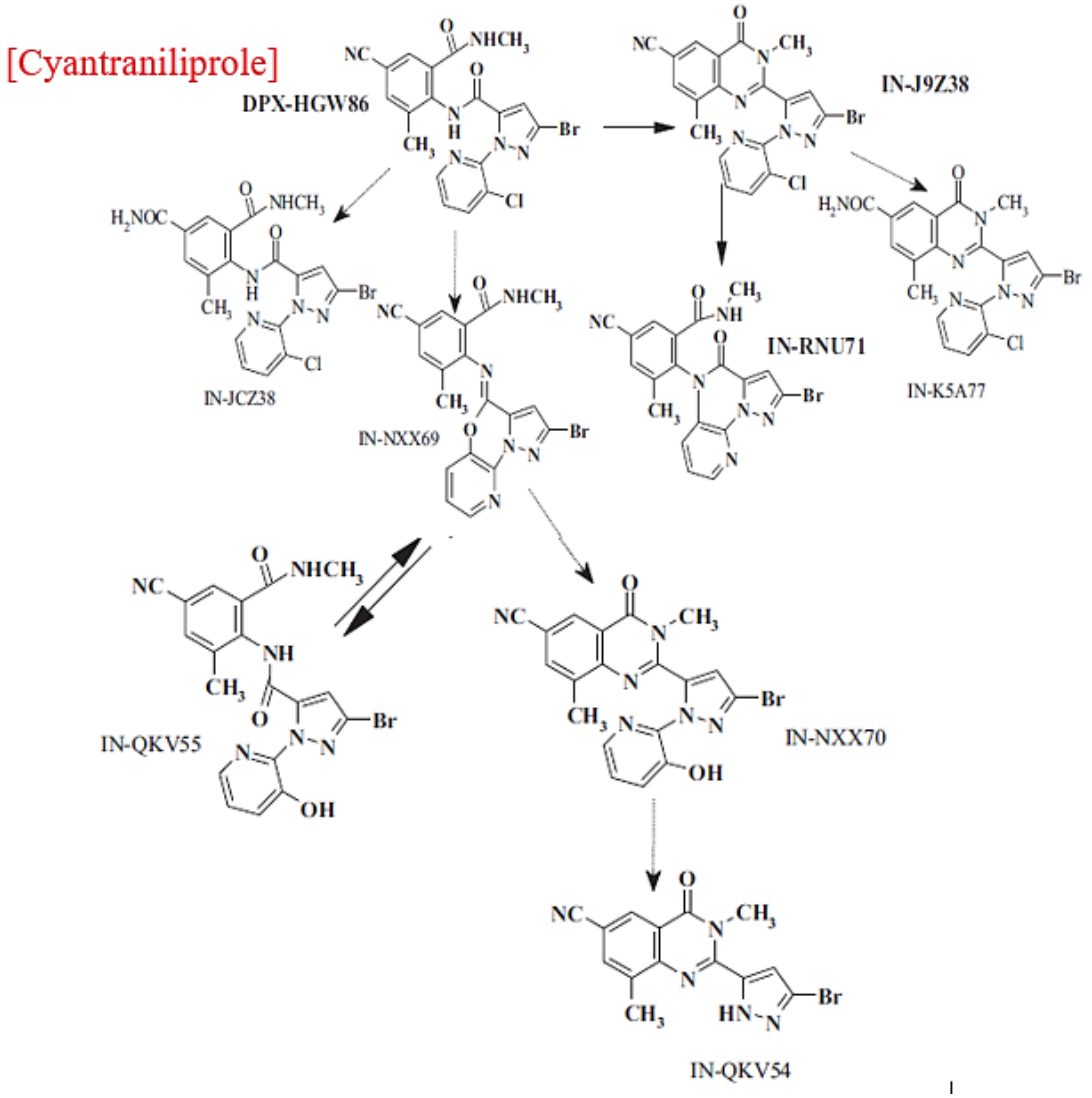


Fig. 3: Proposed metabolic pathway of cyantraniliprole in water

3.2 Persistence behaviour of cyantraniliprole insecticide in cabbage and soil

3.2.1 Method of field application of cyantraniliprole insecticide

Cyantraniliprole was sprayed in the field on the cabbage crop at the fruit setting stage using knapsack sprayer in plots of 5m x 5m size. Plots were differently used for the recommended (60 g a.i. ha^{-1}) and double of recommended dose (120 g a.i. ha^{-1}) while one plot was left untreated for control. Samples were collected in triplicate from all plots. Samples were taken randomly from each treated plot at 0 (1 h after spray), 1, 3, 5, 7, 10 and 15 days after the insecticide application. Soil samples were also collected in triplicate at 0 (1 h after spray), 1, 3, 5, 7, 10 and 15 days after the application of insecticide from top 15 cm of soil profile, from 5-6 separate spots within each treated plot. After sampling the samples were brought to the laboratory for their further processing in polythene bags. Extraction of cabbage samples was carried out on the same day of sampling. Soil samples were first dried under

natural conditions (air dried), ground and sieved to remove the stones and extra debris. Then, sample processing was performed for determining the residue level.

3.3 Processing of samples

Samples in triplicates from treated plots were processed on 0 (1 hr after spray), 1, 3, 5, 7, 10 and 15 days after the spray for evaluating dissipation pattern of cyantraniliprole and effect of decontamination processes like washing under tap water, washing with 5% NaCl solution and washing followed by boiling. Residue level was also estimated in soil at both doses on respective days.

3.3.1 Extraction of cabbage heads

All the samples were extracted fresh. Each sample was divided into two parts. One part was used for residue analysis and another part further divided into three parts. Out of these 3 parts, one part washed with tap water and processed, second part washed with 5% solution of NaCl (5g/100 mL) and third part washed with tap water and then boiled and processed following method of Walter *et al*., (2000). All the samples were extracted by three methods.

Method 1:

Liquid – Liquid partitioning (Kumari *et al.*, 2001)

- The representative 20 g of sample was taken in flask and 100 mL of acetone was added.
- The flask was shaken on a mechanical shaker for one and half hour.
  Filter the extracted sample in separating funnel followed by rinsing with approximately 10 ml acetone in two lots.
- Added 600 mL brine solution (10% NaCl solution) and partitioned twice with dichloromethane (DCM) and hexane (100, 50 mL) by vigorous shaking for 1 minute to remove the non-emulsifying impurities. Each time, organic phase was collected and passed through the bed of anhydrous sodium sulphate to remove the trace amount of moisture and pooled together.
- To the extract 0.3 mg activated charcoal was added (to absorb the coloured impurities present in it) and left for 4 hours.
- The clear extract thus obtained was concentrated twice near to dryness on rotary vacuum evaporator to eliminate last traces of moisture and remake 3-5 ml in acetonitrile.

Clean-up

- Preparation of column: A cotton plug was placed at the bottom of the clean and dried glass column (60 cm x 22 mm i.d.) over which 1 cm film of anhydrous sodium sulphate (Na_2SO_4) was placed. After gentle tapping, adsorbent mixture i.e. Florisil: activated charcoal (3:0.3 w/w) mixed invariably and transferred into column and tapped again to ensure compact packing of the column.

- One cm film of anhydrous Na_2SO_4 was added over the adsorbent mixture and the column was prewashed with n-hexane (40 mL).
- Transferred the extract quantitatively to the column and eluted with 125 mL solution of hexane: acetone (9:1 V/V).
- Concentrated the elute near to dryness using rotary vacuum evaporator and reconstitute the final volume to 3-5 mL using acetonitrile.
- Analysis was performed using HPLC.

Method 2: QuEChERS

Samples were extracted using the modified QuEChERS method (Anastassiades *et al*., 2003)

- The representative 15 g macerated cabbage sample was taken into 50 mL centrifuge tube.
- Added 30 ml of acetonitrile (ACN) in the sample and homogenise the mixture using low volume homogenizer (Heidolph) for 3-4 min at 14000 rpm.
- For water and acetonitrile phase separation 5 g sodium chloride was added to the above extract and vortexed for 2 min.
- Then centrifuge the vortexed sample for 3 min at 2500-3000 rpm and upper 18 ml layer of acetonitrile was transferred in another centrifuge tube by passing through anhydrous sodium sulphate to remove traces of moisture.

Clean – up

Dispersive solid phase extraction (DSPE) technique was used for clean–up as mentioned below:

- Weighed 0.4 g primary secondary amine (PSA) and 1.15 g magnesium sulphate ($MgSO_4$) as adsorbent into 15 mL centrifuge tubes.
- Then 11 mL of extract was transferred into 15 mL centrifuge tubes and shaken for 30 second. Then again centrifuged the sample at 2500 rpm for 5 min.
- Transfer 6 mL of the extract into test tube and then concentrated the extract near to dryness using rotary vacuum evaporator.
- Reconstituted the final volume to 3-5 ml using acetonitrile and filtered using 0.2 µm filter before HPLC analysis.

Method 3: Modified QuEChERS- without clean-up (Malhat *et al*., 2018)

- Ten grams of macerated samples were weighed in 50 mL centrifuge tubes.
- Extraction was done with 20 mL acetonitrile (ACN) and 5 g sodium chloride.
- The samples were homogenized on a low volume homogenizer (Heidolph) at 14000 rpm for 3-4 min.
- Then centrifugation of samples was performed for 10 min. at 4000 rpm. Upper 10 mL layer was moved to a round bottom flask and evaporated to 4 mL on rotary vacuum

evaporator. Then filtration of extract was done using a 0.2 µm PTFE syringe filter before HPLC analysis.

3.3.2 Extraction cum clean–up of soil

For processing of soil samples methods of Kumari (2008) were employed.

- Distillation of all useful chemicals was done before use, also adsorbents were acetone washed and activated before using.
- The indicative 15 g sample of fully dried soil was taken in a 100 mL beaker, 0.5 mL of ammonia solution was added to it and kept undisturbed for about 1 h till ammonical smell fades away.
- Florisil and activated charcoal (0.3 g each) was added into it and mixed properly.
- For preparing column steps followed were: Bottom of the column (60 cm x 22 mm in diameter) was blocked by a cotton plug. Then one cm layer of anhydrous Na_2SO_4 was placed over the plug. Gradually the column was tapped, after that mixture of adsorbent i.e. Florisil: activated charcoal (3:0.3 w/w) was completely mixed and permeated down the column. The column was jam packed by tapping it once again. Following this a one cm anhydrous sodium sulphate layer over soil mixture was added.
- For eluting the column 125 mL solution of acetone: hexane (1:9 v/v) was used.
- The elute was concentrated near to dryness on rotary vacuum evaporator.
- Reconstitute the final volume to 3-5 mL using acetonitrile and further analyzed on High Performance Liquid Chromatography (HPLC).

3.4 Impact of culinary processes on reduction of residues of cyantraniliprole

Cyantraniliprole was sprayed on the cabbage crop as explained above. Samples were collected in triplicate from each treated plot together with control on 0 (1h after spray), 1, 3, 5 and 7 days after spray and decontamination processes like washing with simple tap water, washing with 5% NaCl solution and washing + boiling were employed. Further by adopting modified QuEChERS method (Malhat *et al*., 2018) the processed samples were extracted and cleaned. Principle and details of method of extraction and clean-up were given in section 3.3.1. Quantification of cyantraniliprole residues was carried out on HPLC.

3.4.1 Impact of washing with tap water

First of all, cabbage was taken under running tap water, washing was done with gentle rubbing for about three minutes. To eliminate the excess of water washed samples were placed on blotting paper following method of Walter *et al*., (2000). Further the samples were extracted, cleaned and analysed following the modified QuEChERS method.

3.4.2 Impact of washing with 5% NaCl solution

About 1000 mL of 5% solution of NaCl was prepared and cabbage head was dipped in it for 3 min. with gentle rubbing by hand. For removing excess water these samples were

placed on blotting paper. Then extraction and clean–up of samples was conducted by modified QuEChERS method (Malhat *et al*., 2018) and further analysed on HPLC.

3.4.3 Effect of washing followed by boiling

Wash the cabbage head in running tap water as explained in 3.4.1. To evacuate the excess of water these washed samples were placed on blotting paper and cabbage was chopped into small pieces. Then water was added to these and boiled over an induction coil until it becomes soft. Finally, make a fine paste of it by using a high-volume homogenizer. Then extraction and clean–up of samples was carried out using modified QuEChERS method (Malhat *et al*., 2018) and further analysed on HPLC.

3.5 Quantification of cyantraniliprole residues

High Performance Liquid Chromatography (HPLC) was used for quantification of cyantraniliprole residues in cabbage heads. Details of method are explained in following section:

3.5.1 Principle of method

Cabbage samples were extracted using modified QuEChERS method as explained before. The obtained extract was concentrated to dryness using rotary vacuum evaporator till the volume remained 3-5 ml. Then this extract was filtered using a 0.2 µm PTFE syringe filter and then analysed on HPLC.

3.5.2 Recovery experiments

Samples were collected from control and macerated to make the fine paste. The indicative 10 g of macerated cabbage sample and soil under the crop was taken from the control plot in 250 mL Erlenmeyer flasks and fortified @ 0.05, 0.50 and 1.00 mg kg^{-1} with Sigma Aldrich purity CRM (Certified Reference Material), Batch number − BCCB7455. These flasks were placed uninterrupted overnight. On next day, extraction and analysis were carried out as explained in section 3.3.

Table 6: HPLC Parameters

	HPLC Specification	
1.	**Software:**	Chem Station
2.	**Column:**	ZORBAX Eclipse plus C_{18} (5µm) column (4.6 mm x 250 mm)
3.	**Column oven temp:**	30 ºC
4.	**Detector**	Diode Array Detector (DAD)
5.	**Injection volume:**	20 µL
6.	**Column flow:**	0.8 mL min^{-1}
7.	**LOQ:** **LOD:**	0.05 mg kg^{-1} 0.01 mg kg^{-1}
8.	**R_t of CYN**	15.351 min.
9.	**λ_{max}**	230 nm

3.5.3 Glassware and Equipment

- ✓ Graduated glass vials, 2 mL
- ✓ Glass beakers 100, 200, 500 mL
- ✓ Homogenizer (Heidolph)
- ✓ Rotary vacuum evaporator
- ✓ Digital analytical balance having efficiency of 0.001 g certainty for weighing analytical standard
- ✓ Volumetric flask 10, 100, 150 mL
- ✓ Pipette 1, 5, 10 ml
- ✓ Measuring cylinder 10, 50, 100, 500 mL
- ✓ Centrifugation machine

3.5.4 Reagents and Adsorbents

- ✓ Cyantraniliprole (CRM) of Sigma Aldrich purity (≥ 95.0 %)
- ✓ Benevia 10.26% OD formulation of Cyantraniliprole (Cyazypyr)
- ✓ Acetone (Suprasole)
- ✓ Hexane (Suprasole)
- ✓ HPLC grade Acetonitrile (ACN)
- ✓ Anhydrous sodium sulphate (Na_2SO_4)
- ✓ Activated charcoal
- ✓ Florisil
- ✓ Cotton

3.6 Methodology

In the following section, the analytical methodology employed for analysing the cabbage heads and soil containing cyantraniliprole insecticide is described in details:

3.6.1 Preparation of Analytical Standard Solutions

Initially the method was validated to check the efficiency of the extraction and clean-up processes adopted and also to standardise the procedure adopted for residue estimation in cabbage heads. Standard solution of cyantraniliprole was constituted by adding 10 mg of analytical standard of cyantraniliprole (sigma Aldrich purity) of ≥ 95.0 % purity in 100 ml cyantraniliprole in a volumetric flask. Further working solutions were prepared by using the resultant 100 ppm stock solution of cyantraniliprole. Standard and working solutions of cyantraniliprole were placed in refrigerator at -4ºC.

3.6.2 Preparation of Standard Solution for Sample Fortification

For the fortification of control samples, solutions of different concentrations of range 0.01–5.00 mg kg^{-1} were prepared by using cyantraniliprole stock solution by serial dilution technique and used for recovery experiments and calibration curve.

3.6.3 Preparation of HPLC Calibration Solutions

With sequential dilutions working standard solutions of lower concentrations were obtained from the stock solution. For preparation of 10 mg kg^{-1} standard solution of cyantraniliprole, 10 mL of 100 mg kg^{-1} stock solution was delivered to 100 mL volumetric flask and made volume with acetonitrile. Further working solutions were prepared using the resultant 100 ppm stock solution of cyantraniliprole with acetonitrile (ACN) and kept in refrigerator.

3.6.4 Method Validation

Method was fully validated according to selectivity, precision, accuracy, linearity, robustness (ruggedness), LOD and LOQ.

Selectivity of method was assessed by comparing the HPLC chromatogram of a set of five different concentrations at the LOQ level (0.05 mg/kg) for HPLC detection. The peak in the control sample didn't interfere with peak of spiked sample at retention time (R_t) of 15.351 minutes.

3.7 Interpretation of data

3.7.1 Calculation for the determination of residues in the test samples

$$\text{Residues (mg kg}^{-1}) = \frac{SA_{inj}}{ASA} \times \frac{A_s}{V_{s\ inj}\ (\mu l)} \times \frac{V_f\ (ml)}{V_i\ (ml)} \times \frac{V_{inc}}{W\ (g)} \times \frac{100}{\%\ R}$$

Where,

SA_{inj} = Standard amount injected

ASA = Peak area of standard

A_s = Area of the sample

$V_{s\ inj}$ = Volume of sample injected

V_f = Final volume of sample extract

V_i = Sample extract aliquot processed

W = Analytical sample weight

% R = Per cent Recovery

$$\text{Per cent mean Recovery} = \frac{\text{Amount recovered}}{\text{Amount added}} \times 100$$

3.7.2 Calculation for Regression Equations, Rate Constants and Residue Half-life

With determination of linear regression equation half-life of residue was calculated between log [residue (mg kg^{-1}) x 10^3] and days after spray. The log [residue (mg kg^{-1}) x 10^3] was taken as abscissa (y-axis) dependent on days after spray taken as ordinate (x-axis). For finding out the slope (b) of y and x-axis the least square method was used (Ragupathy and Dhamu, 1990).

$$t_{1/2} = \frac{e}{b} = \frac{0.301}{b} \; [e = \log 2 = 0.301)$$

$$b = \frac{SP_{xy}}{SS_x}$$

$$SP_{xy} = \frac{\sum X\,Y - (\sum X)(\sum Y)}{n}$$

$$SS_x = \frac{\sum X^2 - (\sum X)^2}{n}$$

$$SS_y = \frac{\sum Y^2 - (\sum Y)^2}{n}$$

$$r = \frac{SP_{xy}}{\sqrt{SS_x . SS_y}}$$

$$\bar{y} = a + b\,\bar{x}$$

$$a = y - bx$$

$$k = \frac{0.693}{t_{1/2}}$$

$$\overline{X} = \frac{\sum x}{n}$$

$$\overline{Y} = \frac{\sum y}{n}$$

where,

n = number of observations

x = number of days

y = log [residues (mg kg^{-1}) x 10^3]

CHAPTER-IV

RESULTS

The findings obtained from the current research have been illustrated under the following sections for cabbage heads:

4.1 Standardization of analytical techniques/methods for assessment of residues of cyantraniliprole in cabbage heads and soil using HPLC system.

4.2 Recovery tests for cyantraniliprole from cabbage heads and soil

4.3 Persistence, dissipation and impact of culinary processes in dislodging residues of cyantraniliprole in cabbage heads and soil

4.1 Standardization of analytical techniques/methods for assessment of residues of cyantraniliprole in cabbage heads and soil using HPLC system

HPLC system, based on chromatographic technique, was initially standardized for the investigation of cyantraniliprole micro-quantities to analyse the test samples. Details about the methods/techniques implemented for residue estimation in above mentioned part have been discussed under 'Materials and Methods'. The experimental details of technique are given here under:

4.1.1 Construction of calibration curve for cyantraniliprole

The peak area recorded for 0.01 to 5.00 mg kg^{-1} of cyantraniliprole standards is represented in table-7. The analogous calibration curve illustrating concentration against peak area is shown in fig. 4. The curve was showing linear relationship between 0.01 to 5.00 mg kg^{-1} of cyantraniliprole and their corresponding peak area. The observed retention time (R_t) value for cyantraniliprole was 15.351 min. Chromatogram depicting R_t value and peak area of cyantraniliprole has been shown in Fig. 5.

Table 7: Standard curve data of cyantraniliprole

Concentration (mg kg^{-1})	Area
0.01	10
0.02	18
1.25	93
2.50	162
5.00	287

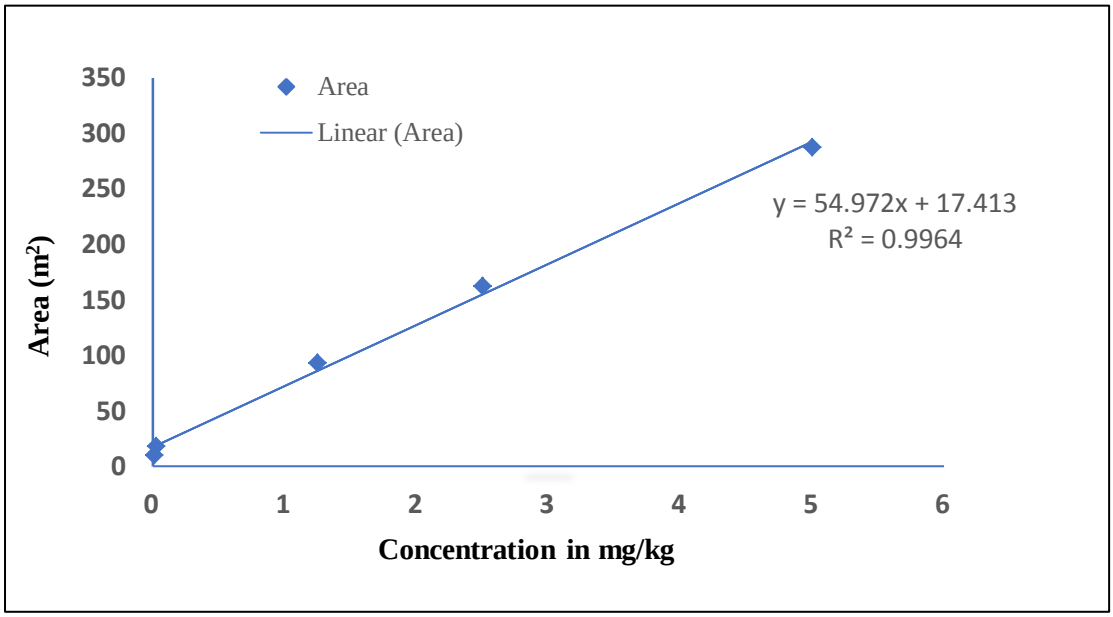


Fig. 4: Standard curve of cyantraniliprole

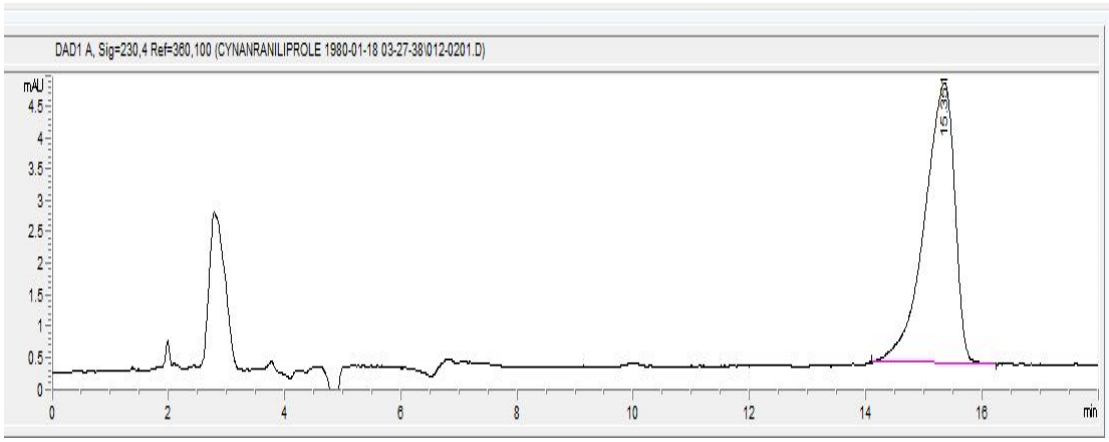


Fig. 5: Chromatogram of standard cyantraniliprole on HPLC

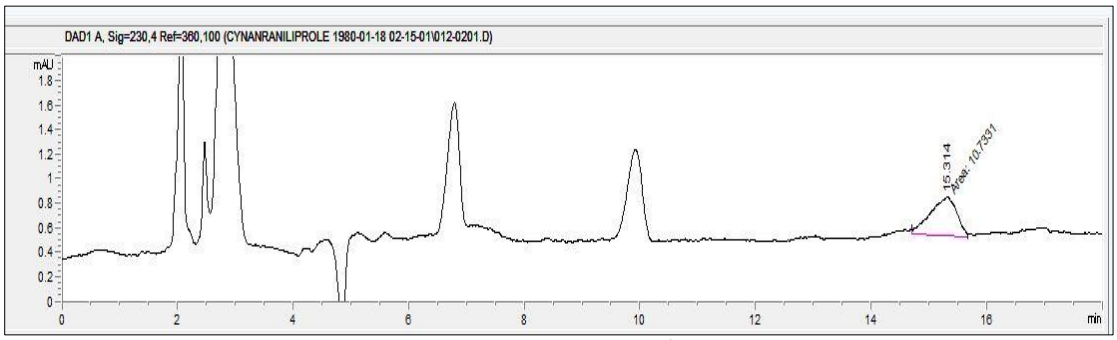
4.1.2 Linearity, LOD and LOQ for HPLC

Analysis of linearity of the adopted method was done by plotting calibration curve at five concentration levels ranging from 0.01 mg kg^{-1} to 5.00 mg kg^{-1}. Calibration curve was designed by plotting concentration against area under the peak as shown in fig. 4 using the data represented in table – 7. Linearity of the curve was explained from regression coefficient (0.996 very closer to 1). The LOD and LOQ were determined using control samples fortified with standard solution of different concentrations. Twenty micro litre (µL) of the working standard solution @ 0.01, 0.02, 1.25, 2.50 and 5.00 mg kg^{-1} was injected in HPLC. Now the lowest concentration for which signal to noise ratio (S/N) was greater than 3 was considered as limit of detection (LOD) for that specific compound.

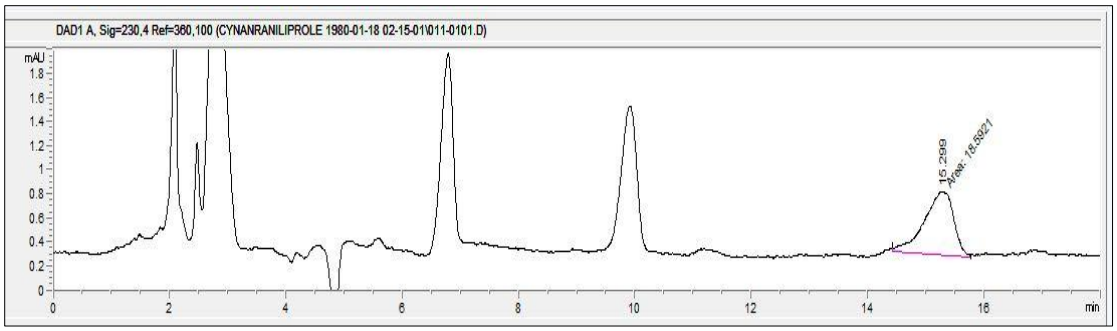
In the present study the LOD arrived was 0.01 mg kg^{-1}. The limit of quantification was taken 5 times of LOD to consider the effect of co-extractive (matrix effect). Thus, LOQ

was 0.05 mg kg^{-1} and limit of detection (LOD) being 0.01 mg kg^{-1}. Cyantraniliprole residues were analysed on HPLC due to its high sensitivity, reproducibility and reliability.

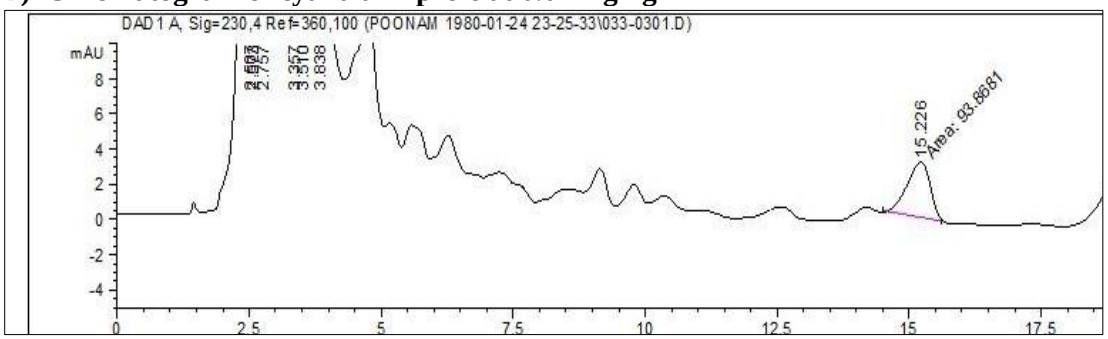
The samples were screened for range of wavelength and minimum matrix interference was found to be at 230 nm.



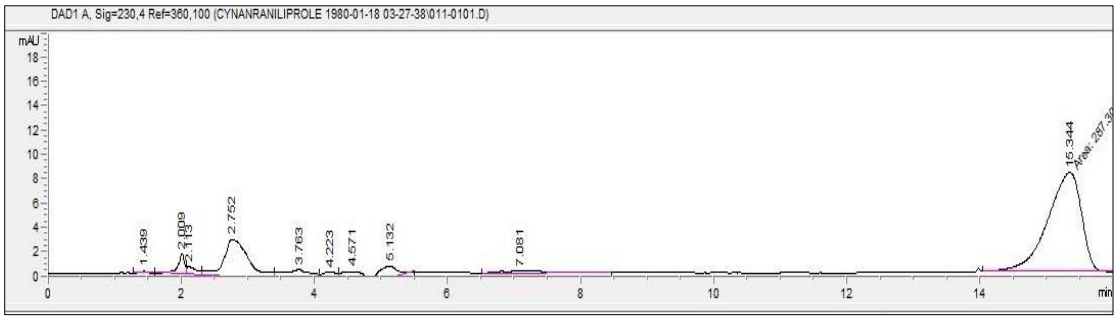
A) Chromatogram of cyantraniliprole at 0.01 mg kg^{-1}



B) Chromatogram of cyantraniliprole at 0.02 mg kg^{-1}



C) Chromatogram of cyantraniliprole at 1.25 mg kg^{-1}



D) Chromatogram of cyantraniliprole at 5.00 mg kg^{-1}

Fig.6: Chromatograms of cyantraniliprole at different concentrations

A (0.01 mg kg^{-1})**, B** (0.02 mg kg^{-1})**, C** (1.25 mg kg^{-1}) and **D** (5.00 mg kg^{-1}) on HPLC

4.1.3 Robustness of the method

Robustness of the method was observed by performing same analysis with minor changes in chromatographic conditions i.e. temperature of column and injector, flow rate of mobile phase etc. Due to these changes the variations in the HPLC analysis was ≤ 1.66 (less than 5%) indicates the robustness of the method.

4.2 Recovery Tests for cyantraniliprole from cabbage heads and soil

To analyse the validity and accuracy of methods, for determination of residues in cabbage and soil sample, recovery experiments were conducted. Untreated cabbage heads (control) and soil sample were fortified with cyantraniliprole standard solution @ 0.05, 0.50 and 1.00 mg kg^{-1} spiking levels. Fortified samples were extracted, cleaned and analysed as per details given in 'Materials and Methods'. Adequacy of three methods was tested by recovery experiments at different fortification levels. Modified QuEChERS method (86.11 – 89.54 %) was found better as compared to liquid-liquid partitioning and QuEChERS (original), both have recoveries < 40% (Table 8 and 9). Therefore, modified QuEChERS method of Malhat *et al*., (2018) was adopted for further investigation of dissipation and decontamination of cyantraniliprole in cabbage heads.

4.2.1 Cabbage heads

As clear from data represented in Table–10, using modified QuEChERS method average recoveries of cyantraniliprole from samples of cabbage fortified @ 0.05, 0.50 and 1.00 mg/kg were 86.11, 88.60 and 89.54 percent, respectively with standard deviation less than 2.10. Recoveries were efficient (>80%) so the adopted method was best suited method and used without correction factor.

Table 8: Percent recovery of cyantraniliprole from cabbage heads using Liquid-Liquid Partitioning

Spiking levels (mg kg^{-1})	Recovery (%)			Average* Recovery (%) ± SD	RSD (%)
	R_1	R_2	R_3		
0.05	33.70	34.43	33.27	33.80 ± 0.42	1.24
0.50	35.21	36.24	33.86	35.10 ± 0.83	2.36
1.00	37.86	36.65	35.12	36.54 ± 0.95	2.60

***Average of three replicates**

Table 9: Percent recovery of cyantraniliprole from cabbage heads using QuEChERS (original) method

Spiking levels (mg kg^{-1})	Recovery (%)			Average* Recovery (%) ± SD	RSD (%)
	R_1	R_2	R_3		
0.05	34.90	35.20	36.11	35.40 ± 0.47	1.33
0.50	35.81	37.05	38.30	37.05 ± 0.83	2.24
1.00	38.98	38.04	36.80	37.94 ± 0.76	2.00

***Average of three replicates**

Table 10: Percent recovery of cyantraniliprole from cabbage heads using Modified QuEChERS method (without clean-up)

Spiking levels (mg kg^{-1})	Recovery (%)			Average* Recovery (%) ± SD	RSD (%)
	R_1	R_2	R_3		
0.05	88.00	85.22	85.11	86.11 ± 1.26	1.46
0.50	90.74	86.90	88.15	88.60 ± 1.43	1.61
1.00	90.67	91.54	86.40	89.54 ± 2.09	2.33

***Average of three replicates**

4.2.2 Soil

Soil sample under the crop were taken from control plot and spiked @ 0.05, 0.50 and 1.00 mg kg^{-1} with cyantraniliprole. Extraction and clean–up was followed as described in "Material and Method". The average recoveries obtained were 85.60, 86.32 and 88.05 percent at 0.05, 0.50 and 1.00 mg kg^{-1} fortification level respectively with standard deviation < 1.96 as represented in table–11. Hence the result showed that the recovery for cyantraniliprole in soil sample (>85%) was satisfactory and the results obtained by applying the adopted method were represented without any correction factor.

Table 11: Percent recovery of cyantraniliprole insecticide from soil

Spiking levels (mg kg^{-1})	Recovery (%)			Average* Recovery (%) ± SD	RSD (%)
	R_1	R_2	R_3		
0.05	85.33	84.00	87.47	85.60 ± 1.25	1.46
0.50	87.92	85.21	85.82	86.32 ± 1.07	1.24
1.00	85.13	88.08	90.95	88.05 ± 1.95	2.21

***Average of three replicates**

4.3 Persistence and dissipation kinetics of cyantraniliprole in cabbage heads

In order to know the magnitude of persistence of cyantraniliprole residues, its dissipation kinetics and dislodging of residues by using various culinary processes, sampling of cabbage heads was done on 0 (1h), 1, 3, 5, 7, 10 and 15 days after treatment under field conditions.

4.3.1 Persistence and degradation kinetics of cyantraniliprole residues in cabbage heads and soil

The residues and statistically observed data for cabbage obtained after spray at recommended and twice of recommended dose by central insecticide board (CIB) i.e. 60 g a.i. ha^{-1} (T_1) and 120 g a.i. ha^{-1} (T_2) respectively are presented in Table–12 and 13 and Fig. 7 and 8. Residue data exposed that initial deposit of 0.449 mg kg^{-1} from single dose (T_1) at 0 (1 h after spray) day degraded to 0.320, 0.151 and 0.070 mg kg^{-1} on 1, 3 and 5 days after spray respectively (Table-12), thereby observing 28.73, 66.37 and 84.41 percent dissipation in this duration. Further level of residue declined to below quantification level (LOQ) i.e. 0.05 mg kg^{-1} in 7 days at single dose (T_1) hence showing > 84.41% dissipation.

At T_2 (120 g a.i. ha^{-1}), initial residues of 0.576 mg kg^{-1} declined to 0.435, 0.291, 0.101, 0.06 mg kg^{-1} on 1, 3, 5 and 7 days after spray respectively (Table-13). Therefore, observing 24.48, 49.48, 82.46 and 89.58 percent dissipation of residues in this duration. After 10 days of spray, the residues reached below quantification limit (LOQ) showing 100% dissipation.

Table 12: Cyantraniliprole residues (mg kg^{-1}) in cabbage heads at single dose

Days after treatment	Residue (mg kg^{-1})					
	T_1 (60 g a.i. ha^{-1})					
	R_1	R_2	R_3	Average ± SD	RSD (%)	% Dissipation
0 (1 h)	0.473	0.429	0.445	0.449 ± 0.016	3.56	–
1	0.315	0.310	0.335	0.320 ± 0.010	3.12	28.73
3	0.157	0.149	0.147	0.151 ± 0.004	2.65	66.37
5	0.074	0.066	0.069	0.070 ± 0.003	4.28	84.41
7	<LOQ	<LOQ	<LOQ	<LOQ	-	100
	Correlation coefficient r = -0.99954 **Regression equation y = -0.1631x + 2.6619** **R^2 = 0.9991** **$T_{1/2}$ = 1.91 days**					

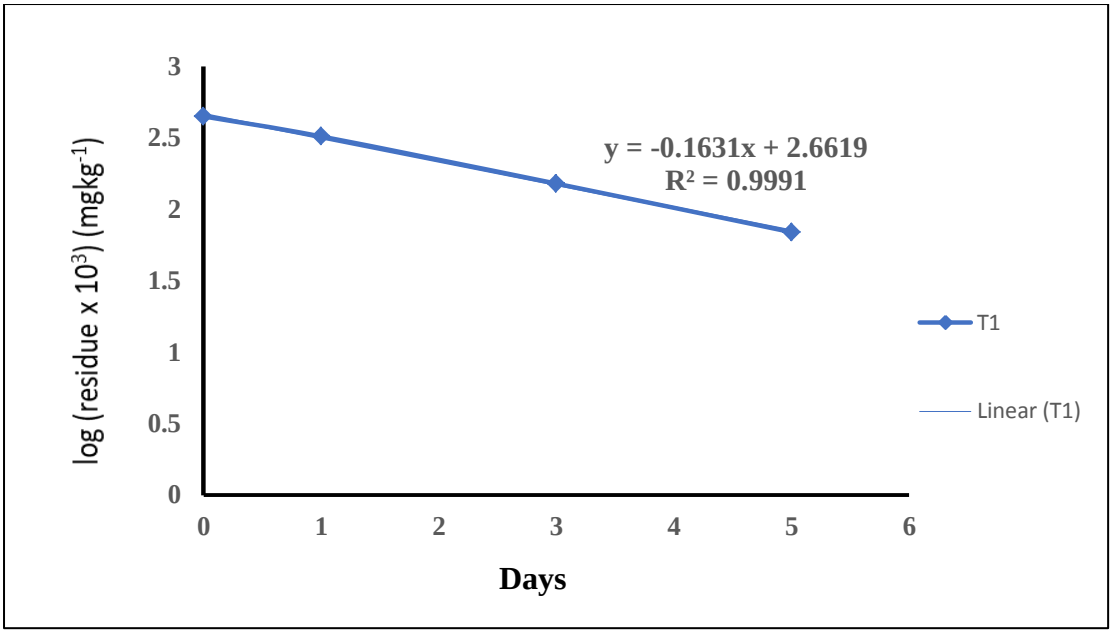


Fig. 7: Degradation kinetics of cyantraniliprole in cabbage heads at single dose

Table 13: Cyantraniliprole residues (mg kg^{-1}) in cabbage heads at double dose

Days after treatment	Residue (mg kg^{-1})					
	T_2 (120 g a.i./ha)					
	R_1	R_2	R_3	Average ± SD	RSD (%)	% Dissipation
0	0.570	0.564	0.594	0.576 ± 0.012	2.08	–
1	0.451	0.420	0.433	0.435 ± 0.011	2.53	24.48
3	0.295	0.301	0.278	0.291 ± 0.009	3.09	49.48
5	0.102	0.105	0.097	0.101 ± 0.003	2.97	82.46
7	0.063	0.057	0.060	0.060 ± 0.002	3.33	89.58
10	<LOQ	<LOQ	<LOQ	<LOQ	–	100
	Correlation coefficient r = -0.98853 **Regression equation y = -0.1454x + 2.7932** **R^2 = 0.9772** **$T_{1/2}$ = 2.29 days**					

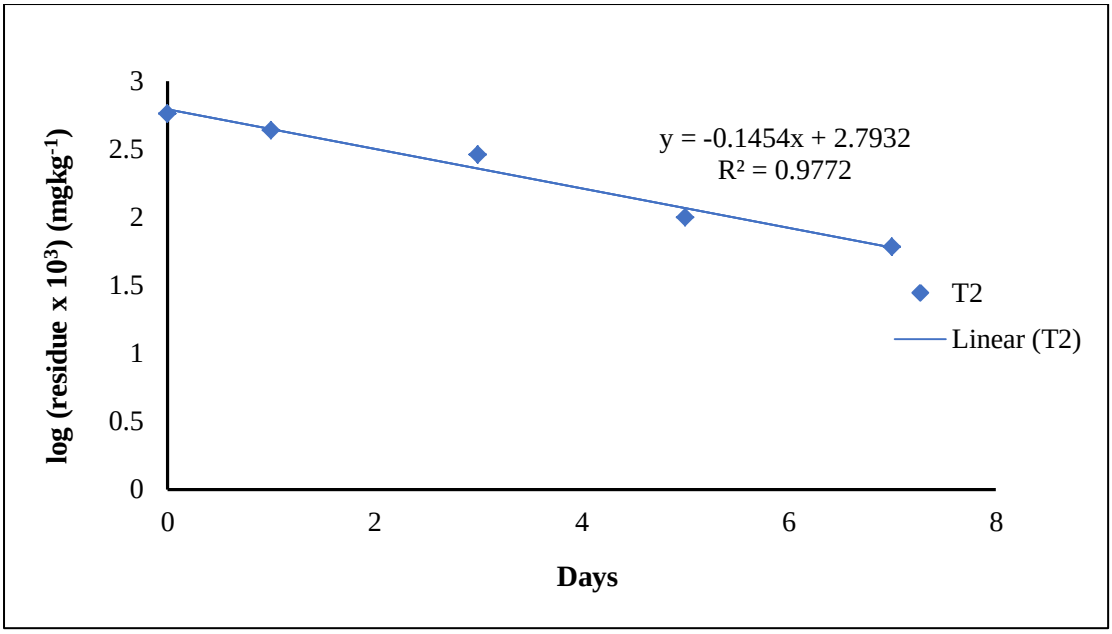


Fig. 8: Degradation kinetics of cyantraniliprole in cabbage heads at double dose

Table 14: Cyantraniliprole residues (mg kg^{-1}) in cabbage heads at single and double doses

Days	Residue (mg kg^{-1})			
	T_1 (60 g a.i. ha^{-1})		T_2 (120 g a.i. ha^{-1})	
	Average ± SD	% Dissipation	Average ± SD	% Dissipation
0	0.449 ± 0.016	–	0.576 ± 0.012	–
1	0.320 ± 0.010	28.73	0.435 ± 0.011	24.48
3	0.151 ± 0.004	66.37	0.291 ± 0.009	49.48
5	0.070 ± 0.002	84.41	0.101 ± 0.003	82.46
7	< LOQ	100	0.060 ± 0.002	89.58
10	< LOQ	100	<LOQ	100
	Correlation coefficient r = -0.99954 **Regression equation y = -0.1631x +2.6619** **R^2 = 0.9991** **$T_{1/2}$ = 1.91 days**		**Correlation coefficient r = -0.98853** **Regression equation y = -0.1454x + 2.7932** **R^2 = 0.9772** **$T_{1/2}$ = 2.29 days**	

CD (α = 0.05) for dose= 0.002; for days = 0.003; for dose x days = 0.005
For regression equation x = days and y = log [residue (mg kg^{-1}) x 10^3]
LOD: Limit of detection (0.01 mg kg^{-1})
LOQ: Limit of quantification (0.05 mg kg^{-1})

Statistically evaluated data using ANOVA depicts that regardless of time period and process at single dose (T_1) significantly less residue (0.449 mg kg^{-1}) were reported than double dose (0.576 mg kg^{-1}) (CD = 0.002; $\alpha = 0.05$). With increase in duration after spray, residues declined significantly (CD = 0.003; $\alpha = 0.05$). Dependence of time period and doses with each other was also significant (CD = 0.005; $\alpha = 0.05$). So, we can conclude that in case of single dose residue level was low as compared to double dose and also level of residues declined significantly in both doses with increase in duration resulting into residue levels of cyantraniliprole below LOQ after 5th and 7th days of treatment in single (T_1) and double (T_2) dose respectively from cabbage heads.

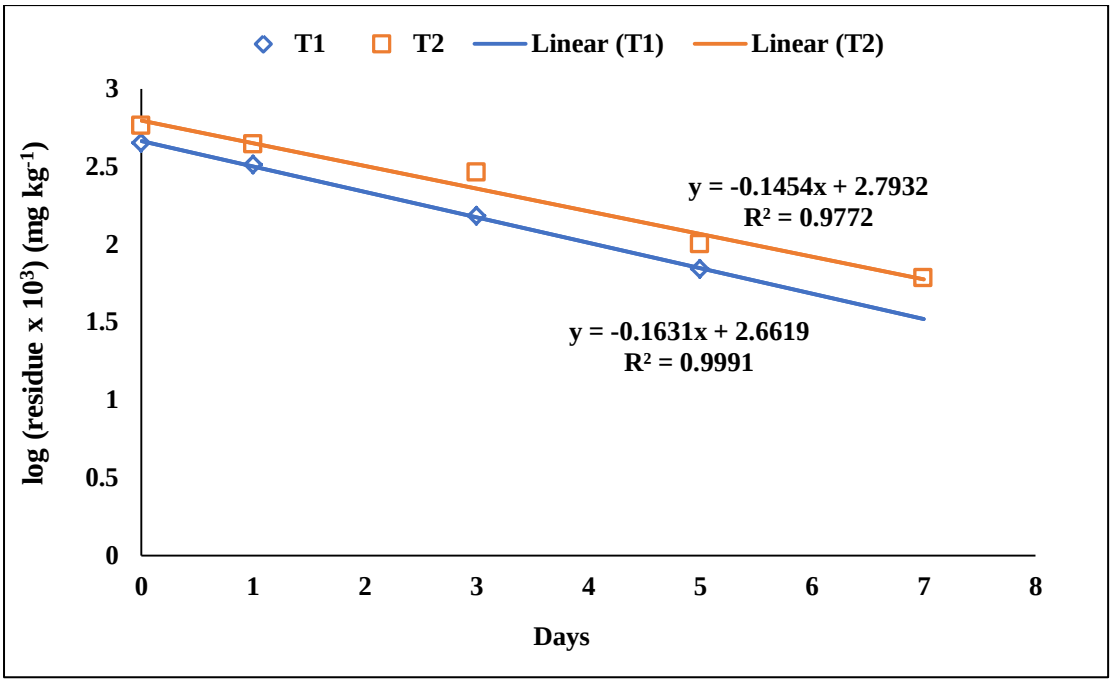


Fig. 9: Degradation kinetics of cyantraniliprole in cabbage heads at single and double dose

4.3.2 Degradation kinetics of cyantraniliprole insecticide and Half-Life values in cabbage heads

The data represented in Table-15 shows the half-life values, regression equations, correlation coefficient and degradation rate constant of cyantraniliprole residues degradation at single (T_1) and double dose (T_2). Graph was plotted for log [Residues (mg kg^{-1}) x 10^3] against time (in days) to examine the degradation kinetics of cyantraniliprole residues in cabbage heads (Fig. 7, 8 and 9). Half-life ($T_{1/2}$) of cyantraniliprole in cabbage heads was 1.91 and 2.29 days for T_1 (60 g a.i. ha^{-1}) and T_2 (120 g a.i. ha^{-1}) dose respectively.

The dissipation rate constant (k) was 0.363 and 0.303 day^{-1} for single and double dose respectively. The correlation coefficient for degradation kinetics in cabbage heads

experiments were -0.999 and -0.988 for single (T_1) and double (T_2) dose respectively. All the results reveal that degradation of cyantraniliprole follows first order kinetics.

Table 15: Values of degradation rate constant, half-life values, correlation coefficient and regression equation for the dissipation of cyantraniliprole residues in cabbage heads

Commodity	Doses (Cyantraniliprole)	Regression equation (y = ax + b)	Degradation rate Constant (k) (day^{-1})	Correlation Coefficient (r)	Half – life (days)
Cabbage	60 g a.i. ha^{-1}	y = -0.1631x + 2.6619	0.363	-0.999	1.91
Cabbage	120 g a.i. ha^{-1}	y = -0.1454x + 2.7932	0.303	-0.988	2.29

4.3.2 Persistence and Dissipation Kinetics of Cyantraniliprole Residues in soil under cabbage crop

Residue level in soil under cabbage crop was found below LOQ at both single and double doses on the same day (0 day) of spray application and also on successive sampling days.

4.3.3 Impact of culinary processes on the dislodging of cyantraniliprole residues in cabbage heads

For studying magnitude of reduction of residues of cyantraniliprole in cabbage heads by using different culinary processes like washing with simple tap water, washing with 5% NaCl solution and washing + boiling, samples were collected at 0 (1h after spray), 1, 3, 5 and 7 days after spray at recommended and double of recommended dose under field conditions.

4.3.3.1 Impact of Washing with simple Tap Water

The data shown in Tables 16 and 17 and Figs. 10 and 11 exposed that initial residues of 0.449, 0.320 mg kg^{-1} at single dose (T_1) were declined to 0.068, 0.051 mg kg^{-1} respectively on 0 (1h after spray) and 1 day after spray due to washing of cabbage heads with simple tap water resulting in 84.86% and 84.06% reduction respectively. For double dose (T_2), the Initial deposit of 0.576, 0.435 and 0.291 mg kg^{-1} were declined to 0.080, 0.065 and 0.051 mg kg^{-1} after washing with tap water on 0 (1h after spray), 1 and 3 days after spray respectively and thus showed 86.11, 85.06 and 82.47% loss of cyantraniliprole residues at corresponding intervals.

4.3.3.2 Impact of washing with 5% NaCl solution

For single dose initial residues of 0.449 decreased to 0.062 mg kg^{-1} on the 0 day of treatment showing 86.19% reduction from initial deposit. Initial residues of 0.576, 0.435 mg kg^{-1} for double dose reduced to 0.079 and 0.061 mg kg^{-1} respectively on 0 and 1 day after application of spray showing 86.28 and 85.98 % dissipation.

4.3.3.2 Impact of washing followed by boiling

The data shown in Tables 16 and 17 and Figs. 10 and 11 exposed that initial residues of both single and double doses reached < LOQ value after boiling showing 100% reduction from initial deposits.

Table 16: Impact of culinary processes on reduction of cyantraniliprole residues (mg/kg) in cabbage at single dose

Days	Residue (mg kg^{-1}) T_1 (60 g a.i./ ha)						
	Unwashed	Washing with tap water	% Reduction	Washing with 5 % NaCl	% Reduction	Washing + Boiling	% Reduction
0 (1h)	0.449 ± 0.016	0.068 ± 0.002	84.86	0.062 ± 0.003	86.19	< LOQ	–
1	0.320 ± 0.010	0.051 ± 0.001	84.06	< LOQ	–	< LOQ	–
3	0.151 ± 0.004	< LOQ	–	< LOQ	–	< LOQ	–
5	0.070 ± 0.002	< LOQ	–	< LOQ	–	–	–
7	< LOQ	< LOQ	–	–	–	–	–

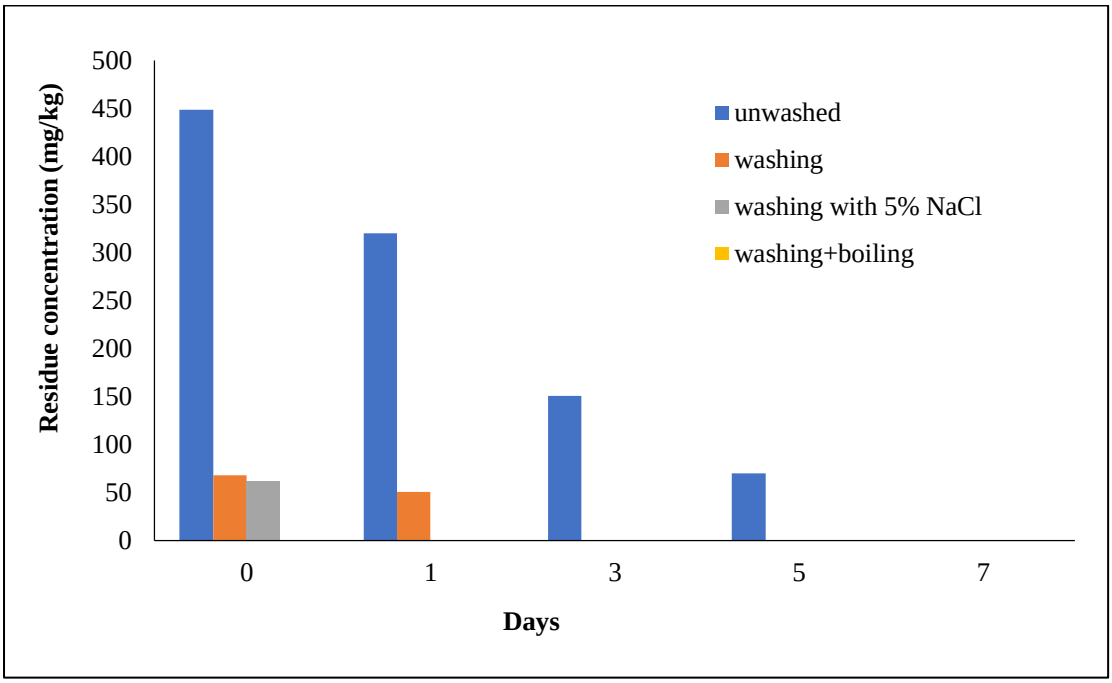


Fig. 10: Impact of culinary processes on reduction of cyantraniliprole residues (mg kg^{-1}) in cabbage at single dose

Table 17: Impact of culinary processes on reduction of cyantraniliprole residues (mg/kg) in cabbage at double dose

DAYS	Residue (mg/kg) T_2 (120 g a.i. / ha)						
	Unwashed	Washing with tap water	% Reduction	Washing with 5% NaCl solution	% Reduction	Washing +Boiling	% Reduction
0	0.576 ± 0.012	0.080 ± 0.003	86.11	0.079 ± 0.002	86.28	< LOQ	-
1	0.435 ± 0.011	0.065 ± 0.002	85.06	0.061 ± 0.001	85.98	< LOQ	–
3	0.291 ± 0.009	0.051 ± 0.001	82.47	< LOQ	-	–	–
5	0.101 ± 0.003	< LOQ	-	< LOQ	–	–	–
7	0.060 ± 0.002	< LOQ	-	–	–	–	–
10	< LOQ	–	–	–	–	–	–

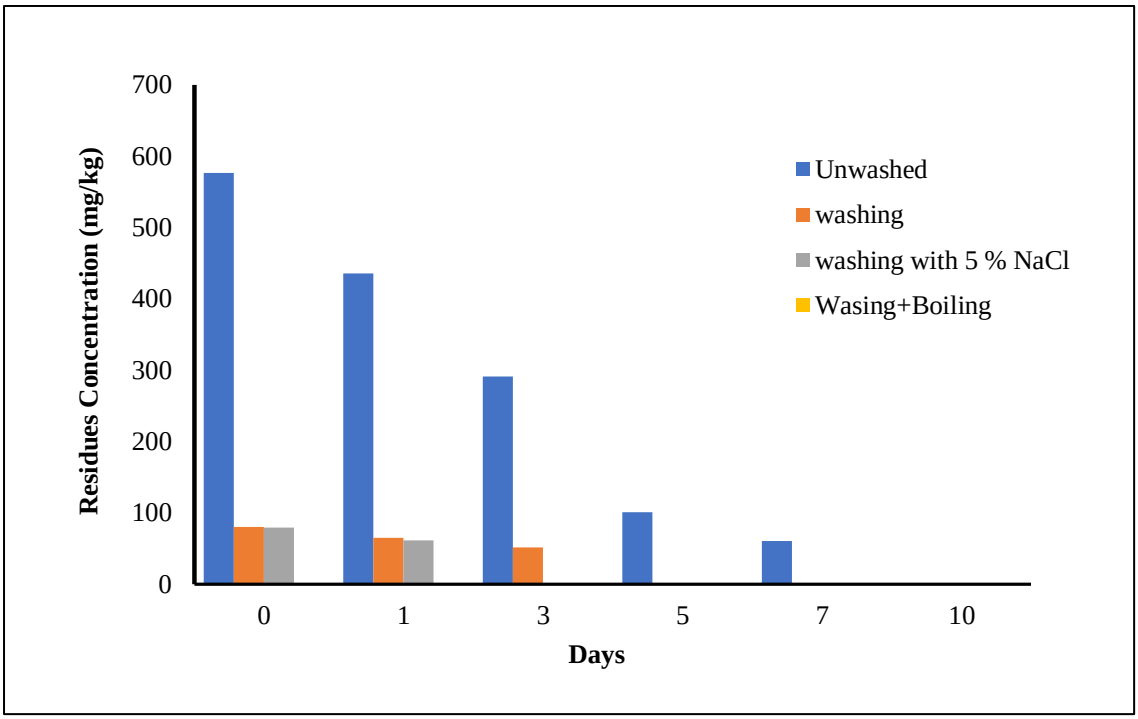


Fig. 11: Impact of culinary processes on reduction of cyantraniliprole residues (mg kg^{-1}) in cabbage at double dose

Statistically evaluated data using ANOVA for culinary processes was described in table -18 for cyantraniliprole individually described that regardless of the time period and process, at single dose, significantly less residues (0.059 mg kg^{-1}) were observed as compared to double dose (0.090 mg kg^{-1}) (CD = 0.002; $\alpha = 0.05$). With increase in time period, level of residues decreases (CD = 0.003; $\alpha = 0.05$). Among culinary processes washing + boiling was found significantly effective than washing with salt solution and washing with simple tap water in dislodging the residues. Level of residues in case of washing observed (0.032 mg kg^{-1}) was more as compared to washing with salt solution (0.020 mg kg^{-1}) and washing + boiling (< LOQ) (CD = 0.003; $\alpha = 0.05$). Residue level was maximum (0.164 mg kg^{-1}) on 0 day and reduce significantly to 0.116, 0.062, 0.022 and 0.008 mg kg^{-1} on 1, 3, 5 and 7 days after spray of insecticide. Interaction among processing and duration was also significant (CD = 0.007; $\alpha = 0.05$) which demonstrate that washing followed by boiling effectively removed the residues (-90%) from the 0 day of spray and treatment with 5 % NaCl solution leads to significant reduction of residues from cabbage heads at 3 days after treatment and 5 days after treatment, the level of residues becomes below limit of quantification (LOQ) i.e. 0.05 mg kg^{-1}. Similarly interaction between dose and duration (CD = 0.005; $\alpha = 0.05$) and between dose and processing (CD = 0.004; $\alpha = 0.05$) were also significant which show that at single dose (60 g a.i. ha^{-1}) due to processing there is significantly less percent reduction in residue level as compared to double dose (120 g a.i. ha^{-1}). Level of residues found to be significantly different at each dose, duration and processing (CD = 0.010; $\alpha = 0.05$).

Table 18: Effect of processing on reduction of cyantraniliprole in cabbage heads

Processing	Average Residues (mg kg^{-1})																	Pooled mean
	0 Day			1 Day			3 day			5 Day			7 day			Mean		
Unwashed	SD	DD	M	SD	DD	M	SD	DD	M	SD	DD	M	SD	DD	M	SD	DD	
Washing	0.449	0.576	0.512	0.320	0.435	0.378	0.151	0.291	0.221	0.070	0.101	0.086	-	0.060	0.03	0.198	0.293	0.246
Washing with salt solution	0.068	0.080	0.074	0.051	0.065	0.058	-	0.051	0.026	-	-	-	-	-	-	0.024	0.039	0.032
W + B	0.062	0.079	0.070	-	0.061	0.030	-	-	-	-	-	-	-	-	-	0.012	0.028	0.020
Mean	-	-		-	-		-	-	-	-	-	-	-	-	-	-	-	-
Pooled mean (days)	0.145	0.184		0.093	0.140		0.038	0.086		0.018	0.025		-	0.015				
Pooled mean (dose)			0.164			0.116			0.062			0.022			0.008			
																0.059	0.090	

CD (α = 0.05), Dose = 0.002; processing = 0.003; days = 0.003; dose x processing = 0.004; processing x days = 0.007; days x dose = 0.005; dose x days x processing = 0.010
SD: Single dose; DD: Double dose; M: Mean; W + B: Washing + Boiling

CHAPTER-V

DISCUSSION

For evaluating the impact of numerous pesticides on environment, human health and other living organisms it is essential to analyse the effect of these pesticides under diverse environmental conditions. During and after application on crop, pesticides migrate to various components of environment by natural processes like leaching, volatilization, translocation etc. During foliar application some amount of pesticides enter into the air and soil environment. Further, from soil some pesticide residues leaches into ground water which ultimately affect the aquatic life and pollute every aspect of our environment. The level of migration depends upon environmental conditions, kind of soil and type of pesticide. According to best of our knowledge there is no literature available on the dissipation and decontamination behaviour of cyantraniliprole in cabbage heads and soil (under crop). Current research has been discussed under the following sections:

5.1 Standardization of analytical techniques/methods

5.2 Recovery tests for cyantraniliprole in cabbage heads and soil

5.3 Persistence and dissipation of cyantraniliprole in cabbage heads and soil

5.4 Effect of culinary processes on the reduction of cyantraniliprole residues in cabbage heads

5.5 Risk assessment of cyantraniliprole in cabbage

5.1 Standardization of Analytical Techniques /Methods

The method opted in the current study is satisfying the terms of linearity, robustness, accuracy and sensitivity. The calibration curve for concentration of cyantraniliprole (Fig. 4) in range 0.001 to 5.00 mg kg^{-1} against peak area using data presented in Table-7 indicates a linear relationship between these parameters. Retention time (R_t) of cyantraniliprole peak is 15.351 min. Limit of quantification (LOQ) was 0.05 mg kg^{-1} and limit of detection (LOD) was 0.01 mg kg^{-1}. Using HPLC (isocratic mode) analysis of the samples was carried out. The column used was ZORBAX Eclipse plus C_{18} (5µm) column (4.6 mm x 250 mm), column oven temperature was kept at 30ºC.

Similar results were obtained by Malhat *et al.*, (2018) who observed the dissipation pattern of cyantraniliprole in tomato under open field conditions using florisil as clean-up reagent. They employed high performance liquid chromatography (HPLC-DAD) and observed retention time for cyantraniliprole was 12.65 min. The novel method outbalanced QuEChERS (traditional) regarding matrix interferences in the analysis while fulfilling all the baseline criteria.

Sun *et al*., (2012) determined cyantraniliprole and its major metabolite J9Z38 residues in Pakchoi and soil, the sample preparation method used was QuEChERS. Samples were taken from open field conditions and the experimental results showed retention time (R_t) values were 1.82 and 2.42 min. respectively for cyantraniliprole and J9Z38. Analysis was performed using UPLC-MS/MS and MS/MS detection using electrospray ionization (ESI) source in positive ion mode.

Analysis of cyantraniliprole and its major metabolite residues in cucumber, tomato and soil was performed by Dong *et al.*, (2012) using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). Two pairs of precursor product ion transitions for cyantraniliprole and J9Z38 were measured and evaluated. The values of R_t measured were found to be 2.28, 2.28 and 2.26 min. respectively for cucumber, tomato and soil, all spiked at 10 µg/kg.

Considering the repeatability of the responses, sharp and well resolved peak of cyantraniliprole the operative HPLC parameters were found satisfactory.

5.2 Recovery Tests for cyantraniliprole in cabbage heads and soil

Before assessment of test samples of cabbage heads and soil for quantification of residues recovery tests were carried out, for validation of analytical methodology. The recovery data obtained at diverse spiking levels in different samples have been discussed below.

Average recovery level of cyantraniliprole in cabbage heads and soil at the spiking levels of 0.05, 0.5 and 1.0 mg kg^{-1} were 86.11, 88.60 and 89.54 percent and 85.60, 86.32 and 88.05 percent respectively in the present studies. The recoveries obtained were >80% for both soil and cabbage heads. Hence, indicated that the adopted method was satisfactory and used without any correction factor.

Similar results were obtained by Malhat *et al*., (2018) who performed recovery experiment on tomato samples spiked with cyantraniliprole @ 0.05, 0.1 and 1.0 mg/kg. The recovery values obtained were 88.9-96.5%. The observations were in striking similitude with the result of present study.

Work done by Lee *et al*., (2019) showed that at fortification levels of 0.1 and 0.5 mg/kg in both grain and straw of proso millet, average recovery rates obtained were 91.2-105.3% for cyantraniliprole and from 88.8-108.2% in its metabolite IN-J9Z38. The RSD observed was < 5.9% for all the results.

Research carried out by Pan *et al*., (2015) for determination of cyantraniliprole and chlorantraniliprole in fruits (apple and grape), cereals (wheat and rice) and vegetables (tomato, cucumber) at spiking levels of 10, 20 and 50 µg/kg the mean relative recoveries of the two analytes lies between 95.5-106.2%.

Our results found consistency with Hong-Mei *et al*., (2014) where intra-day mean recovery levels in pepper and soil at spiking levels @ 0.01, 0.10 and 1.00 mg/kg were found between 88.6-105.7% with RSD of 3.8-15.1%. Inter-day mean recoveries of cyantraniliprole and J9Z38 ranges between 91.4-105.3%.

Zhang *et al*., (2013) conducted recovery experiments for cyantraniliprole in rice straw, paddy water, brown rice and paddy soil at concentrations of 0.05, 0.50 and 1.00 mg/kg, recoveries obtained lies between 88.8-97.5%, 91.6-104.4%, 94.4-103.4% and 90.8-102.8% respectively in different commodities.

Hu *et al*., (2013) found recoveries between 85.71 to 105.74 % in watermelon and soil spiked at 0.01, 0.1 and 0.5 mg/kg with RSDs of 0.90-6.34%.

In another experiment conducted by Sun *et al*., (2012) in Pakchoi and soil, the recovery ranges between 77.8-102.5% with RSDs of 1.6-8.9% when fortified @ 0.01, 0.05 and 0.1 mg/kg levels. Similarly, the recovery experiments performed by Dong *et al*., (2012) determined that when fortification was @ 10, 50 and 100 µg/kg levels in cucumber, tomato and soil, recoveries obtained were 74.7-96.2%.

5.3 Persistence and Dissipation of cyantraniliprole in cabbage heads and soil

The experimental data shown in Table-12 and 13 (Fig. 7 and 8) indicates that the application of cyantraniliprole (10.26% OD) at recommended (60 g a.i. ha^{-1}) and double of recommended dose (120 g a.i. ha^{-1}) on cabbage heads in field conditions shown an initial residue deposits in cabbage heads at the level of 0.449 mg kg^{-1} and 0.576 mg kg^{-1} respectively. The insecticide degrades faster just after its application in both the doses and fell abruptly to the level of 0.151 mg kg^{-1} on 3rd day for single dose and 0.101 mg kg^{-1} on 5th day for double dose respectively. Thereafter, occurred gradual degradation of cyantraniliprole residues till 7th day following first order degradation kinetics. It is evident from the table that on 1st day, the dissipation rate was slightly slower but goes on increasing day by day. Further it was noted that the residues reached below limit of quantification i.e. 0.05 mg kg^{-1} on 7th day and 10th day in single and double doses respectively. The half-life values were 1.91 and 2.29 days at single and double dose, respectively following first order degradation kinetics.

Residue level in soil under cabbage crop was found below limit of quantification (<LOQ) on 0 day and also on successive sampling days for both single and double doses.

Similar results were obtained by Lee *et al*., (2019) who scrutinized the dissipation of cyantraniliprole in IN-J9Z38 in proso millet using ultra-high-performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS) for multiple reaction monitoring of target compounds. Amount of initial residues in grains were 0.33, 0.23, 0.17 and 0.03 mg kg^{-1} and 0.98, 0.30, 0.17 and 0.11 mg kg^{-1} in straw in plot A, B, C and D respectively. Half-life values for cyantraniliprole was found to be 11.3 and 9.4 days for grain and straw respectively.

Our results find consistency with Malhat *et al*., (2018) who explored the persistence behaviour of cyantraniliprole in tomato following open field application. Results indicated that half-life of cyantraniliprole degradation was 2.6 days. After treatment at the recommended rate the pre-harvest interval (PHI) for cyantraniliprole in tomato was 3 days.

The results are in agreement with those of Hu *et al*., (2013) who studied residues of cyantraniliprole and its metabolite J9Z38 in watermelon and soil using UPLC/MS/MS. LOQs for cyantraniliprole were found to be 0.00021, 0.00015 mg kg^{-1} and for J9Z38 it was 0.0010 and 0.00090 mg/kg in watermelon and soil samples respectively. Half-lives of cyantraniliprole after application were 4.1 and 1.1 days in soil and watermelon in Zhejiang and 2.6 and 2.7 days in soil and watermelon in Hunan respectively. Cyantraniliprole and J9Z38 mean residue levels were all < 0.01 mg/kg within 14-day PHI.

Mei *et al.*, (2014) found residues and degradation of cyantraniliprole and its major metabolite in pepper and soil. Half-life ($T_{1/2}$) of cyantraniliprole ranged from 9.2-11.2 days in pepper and from 9.2 to 20.8 days in soil.

The results also agree with Sun *et al*., (2012) who inspected cyantraniliprole (10% SC) and its main metabolite residues in Pakchoi and soil using UPLC-MS/MS. Cyantraniliprole was applied @ 90 g a.i. ha^{-1} in Pakchoi and 120 g a.i. ha^{-1} in soil. LOQ for cyantraniliprole and J9Z38 was 0.01 mg/kg in both Pakchoi and soil. Half-life of cyantraniliprole ranges between 2.9-6.4 days in Pakchoi and 8.7-18.2 days in soil respectively. Cyantraniliprole final residues in Pakchoi and soil from Guangdong and Shanghai were > 0.20 and 0.10 mg/kg respectively.

Dong *et al.*, (2012) also reported cyantraniliprole and its main metabolite residues in vegetable and soil using UPLC/tandem mass spectrometry. LOQs for cyantraniliprole and J9Z38 determined to be 5 and 10 µg/kg in tomato, cucumber and soil matrices respectively. Residue concentrations in cucumbers degrades from 0.08 to 0.01 mg/kg and in tomatoes reduced from 0.07 to 0.01 mg/kg in Zhejiang after 7 days. Results showed half-life values of cyantraniliprole for soil, cucumber and tomato were 9.5, 2.2 and 2.8 days respectively. A ten days interval after treatment was reported safe before harvesting (< 0.01 mg/kg).

5.4 Impact of culinary processes on the dislodging of cyantraniliprole residues in cabbage heads

Effectiveness of any decontamination methods differ with type of pesticide, location and age of residues in fruit/vegetable. In many studies, peeling was found to be effective method in comparison with other methods, for those fruit/vegetable in which maximum residues are accumulated in topmost layer. Washing is also an effective method for decontamination of pesticide residues but its effectiveness depends upon many factors like water solubility, type of washing solution and temperature (Anita *et al*., 2018).

The results obtained from the present research studies are well described and discussed in the following section:

5.4.1 Impact of washing with tap water on the reduction of residues of cyantraniliprole in cabbage heads

The data related to washing under tap water have been represented in Tables-16 and 17 (Fig. 10 and 11). The cyantraniliprole residues diminished from 0.449 to 0.068 mg kg^{-1} on

0 day (1h), from 0.320 to 0.051 mg kg^{-1} on 1st day, thereby showing 84.86 and 84.06% reduction on the corresponding days at single dose.

For double dose, the residues diminished to level of 0.080, 0.065, 0.051 mg kg^{-1} after washing on 0, 1 and 3 day after application showing % reduction of 86.11, 85.06 and 82.47% on respective days.

The results observed were in accordance with those of Kar *et al*., (2012) who studied the decontamination of chlorantraniliprole in cabbage and cauliflower through household processing methods and concluded that tap water washing results into about 17-40% reduction in the amount of chlorantraniliprole residues.

The results are in accordance with Vijaysree *et al*., (2013) who observed the effect of various decontamination processes (vinegar 2%, baking soda 2%, turmeric 1%, tamarind 2%, common salt 2% solution and slaked lime 2%) in reduction of chlorantraniliprole residues in cowpea fruits. All these processing have reduced residues in the range of 44.56-91.70%. Washing with tap water removed the residues by 69.17% on the 0 day of spray.

Similar results were obtained by Kaushik *et al*., (2019) who studied impact of various culinary practices in reduction of residues of tetraniliprole in tomato crop in India. Tetraniliprole was sprayed on tomato crop @ 60 g a.i. ha^{-1} and 120 g a.i. ha^{-1}. Washing of tomato fruits with tap water declined the residues up to 37.63% and washing with lukewarm water reduces residues by 44.67%.

The results obtained are consistent with those of Vijaysree *et al*., (2015) who analysed the impact of various decontamination processes in reduction of chlorantraniliprole residues in brinjal and okra fruits. Percent decontamination found on washing with tap water was 86.38% and 66.74% in brinjal and okra fruits respectively on 0 day for single dose.

Reduction in chlorantraniliprole residues by washing was further supported by the research work of Ahlawat *et al*., (2019) who determined 53.79–50.80% reduction of residues in chilli by washing with tap water.

Chen *et al*., (2015) evaluated the impact of tap water washing and processing time on lowering of residues of chlorantraniliprole in cowpea fruits. Observed reduction rate was 12.79% and processing factor was 0.8721 in case of 2-min. washing. The initial deposit of 9.293 mg kg^{-1} reduced to 8.1048 mg kg^{-1} and decontamination level enhanced with increase in washing time. Washing for 15 min. caused 68.42% reduction in residues.

5.4.2 Impact of washing with 5% NaCl solution on the dislodging of residues of cyantraniliprole in cabbage heads

Washing with 5% NaCl solution reduced cyantraniliprole residues to the extent of 86.19% on 0 day (1h after spray) in case of single dose and on 1st day residue level reached below LOQ.

In case of double dose, washing with salt solution resulted in 86.28% and 85.98% reduction of residues on 0 and 1st day of spray respectively. At 1st day residue content reached < LOQ using this method.

Ahlawat *et al*., (2019) determined impact of various culinary processes in dislodging chlorantraniliprole residues in chilli and found that washing with 5% brine solution was most effective than washing with hot water and washing under tap water (53.79–50.80%) with 62.02% and 67.94% reduction of chlorantraniliprole residues irrespective of the dose on 0 day of spray.

Vijaysree *et al*., (2013) inspected the effect of various decontamination processes (vinegar 2%, baking soda 2%, turmeric 1%, tamarind 2%, NaCl 2% solution and slaked lime 2%) in reduction of chlorantraniliprole residues in cowpea fruits. Treatment with 2% common salt solution reduced the residues to the extent of 74.64%.

Comparable results were obtained by Kaushik *et al*., (2019) who evaluated impact of various culinary practices in reduction of tetraniliprole residues in tomato crop in India. Tetraniliprole was sprayed on tomato crop @ 60 g a.i. ha^{-1} and 120 g a.i. ha^{-1}. Washing with saline water removed the residues by 61.49%.

Reddy *et al.,* (2018) scrutinized cumulative effect of successive processing methods on removal of residues of different insecticides including chlorantraniliprole 20% SC @ 30 g a.i. ha^{-1} in field bean. Among these different decontamination methods treatment with formula 1 (4% acetic acid + 0.1% $NaHCO_3$ + 1 lemon) reduced residues of different insecticides to greater extent ranging from 67.60 □ 74.90 %. Next effective treatment found was soaking in 2% salt solution for 10 min. followed by tap water washing for (30 sec) brought 52.93% reduction in chlorantraniliprole residues.

Vijaysree *et al*., (2015) studied the effect of various decontamination processes (treatment with 2% slaked lime, 2% NaCl solution, 2% vinegar solution, 1% turmeric solution, 2% baking soda, 2% tamarind and scrubbing) on reduction of chlorantraniliprole residues in okra and brinjal fruits. Chlorantraniliprole residues declined to 0.051 and 0.068 mg kg^{-1} from initial residue level of 0.073 mg kg^{-1} after treatment with 2% slaked lime and 2% NaCl solution respectively showing >90% reduction in brinjal fruits after 2 h of spray.

5.4.3 Impact of washing followed by boiling in dislodging of residues of cyantraniliprole in cabbage heads

Washing followed by boiling found to be most significant and efficacious in complete (100%) removal of pesticide residues of cyantraniliprole in both single and double doses in cabbage samples.

Our results are consistent with Kar *et al*., (2012) who reviewed decontamination pattern of chlorantraniliprole in cabbage and cauliflower samples. They observed 100% removal of residues in both cabbage and cauliflower on washing + boiling and boiling alone

on 0 day (after 1 h) and 1day of spray. This indicated that boiling treatment was the most effective method for reducing chlorantraniliprole residues in these crops.

Ahlawat *et al*., (2019) determined dislodging of chlorantraniliprole residues in chilli and observed that washing with hot water leads to 59.40–58.54% reduction of chlorantraniliprole residues irrespective of the dose on 0 day of spray.

Reddy *et al.,* (2018) described the cumulative effect of various processing methods on reduction of residues of different insecticides including chlorantraniliprole 20% SC and found that cooking in pressure cooker for 10 min. followed by washing with tap water for 30 sec brought 41.5% reduction of residues in green pods of field bean.

Risk assessment of cyantraniliprole in cabbage:

For evaluation of risk assessment of cyantraniliprole in cabbage maximum residue level of cyantraniliprole at different interval of time (table-14) need to be compared with maximum residual limit (MRL) fixed in India by Food Safety and Standards Authority of India (FSSAI). Joint FAO/WHO Codex Alimentarius Commission fixed MRL value for cyantraniliprole in fruiting vegetables and cucurbits at 0.3 mg/kg. Its MRL value in tomato is established at 1 mg/kg by European Union (MRL-Cyantraniliprole-EU 2017) and 0.3 mg kg^{-1} by Joint FAO/WHO Codex Alimentarius Commission (Malhat' *et al*., 2018). In the present study the initial residue (0 day) level was 0.449 mg kg^{-1} for single dose (60 g a.i. ha^{-1}) spray and 0.576 mg kg^{-1} for double dose (120 g a.i. ha^{-1}) spray. The residue level reduced to 0.068 and 0.080 mg kg^{-1} after washing with tap water for single and double doses respectively in cabbage heads on 0 day (1h after spray) of insecticide application. At 3rd day residue concentration lowered to 0.151 mg kg^{-1} in case of single dose and 0.291 mg kg^{-1} for double dose which revealed that after a pre-harvest interval of 3 days for cyantraniliprole in cabbage, at the recommended dose (60 g a.i. ha^{-1}) it is safe for human health. Further residues of cyantraniliprole in cabbage heads after washing diminishes to the extent that residue concentration reached < LOQ on 0 day of application for both doses.

Level of residues in soil were found to be below LOQ (0.05 mg/kg) for both the doses on 0 day and also for successive sampling days which proves that it is safe for succeeding crops and soil health.

These results are in accordance with Malhat *et al*., (2018) who conducted experiment with tomato samples, sprayed cyantraniliprole @ 75 a.i. ha^{-1} in Egypt and broad Mediterranean under open field conditions. The initial deposits of cyantraniliprole in tomato at Menouf and Miet-Gamer were 0.751 and 0.841 mg kg^{-1}. The half-life ($T_{1/2}$) for cyantraniliprole degradation determined at 2.6 days and based on Codex MRL, the pre-harvest interval for cyantraniliprole on tomato was 3 days.

CHAPTER-VI

SUMMARY AND CONCLUSION

Research studies have been carried out to establish the persistence and dissipation behaviour of cyantraniliprole and the role of culinary processes (washing under tap water, washing with 5% NaCl solution and washing followed by boiling) on residue removal.

During current research, cyantraniliprole was applied as Benevia 10.26% OD @ 60 g a.i. ha^{-1} and 120 g a.i. ha^{-1} at recommended (T_1) and double of recommended dose (T_2) respectively by Central Insecticide Board (CIB) on cabbage heads at the fruit setting stage. The representative samples of cabbage heads and soil (under crop) were collected at 0 (1 hour after spray), 1, 3, 5, 7, 10 and 15 days after spray. These samples were analyzed for cyantraniliprole residues in combination with control of each set.

In cabbage heads dissipation and decontamination of cyantraniliprole taken from the treated plots was analysed by adopting modified QuEChERS method (Malhat *et al.*, 2018). For analysing the residues in soil column chromatography technique was used. Remake the final volume using acetonitrile (ACN). Recoveries of cyantraniliprole in cabbage and soil were in range 85.60–89.54% at 0.05, 0.50 and 1.00 mg kg^{-1} fortification levels. For analysing the residues in soil column chromatography technique was used. Accordingly, extraction and analysis technique used was quite satisfactory for both soil and cabbage heads and used as such without any correction factor.

The results of study on persistence, dissipation and dislodging of cyantraniliprole residues in cabbage heads (summary tables) and soil are summarised below:

1. The initial deposits of cyantraniliprole in cabbage heads at single and double doses were 0.449 and 0.576 mg kg^{-1} on 0 day which dissipated following first order kinetics with rate constant 0.363 day^{-1} and 0.303 day^{-1} respectively.
2. Cyantraniliprole residues in cabbage heads degrade more progressively for single dose (T_1) and reached the level of 0.320, 0.151 and 0.070 mg kg^{-1} on 1, 3 and 5 days after spray respectively, recording 28.73, 66.37 and 84.41 percent dissipation during this time with a half-life of 1.91 days. Residue amount reached below LOQ (0.05 mg kg-1) at 7^{th} day.
3. To examine the effect of culinary processes washing was done under tap water. The initial residues were reduced to 0.068 and 0.051 mg kg^{-1} resulting in 84.86 and 84.06 % reduction on 0 (1h) and 1 day after spray respectively. After 1^{st} day of spray washing declined the residue content < LOQ.

4. Reduction in the residue level of cyantraniliprole after washing with 5% NaCl solution was found to be more promising than washing alone and reduced the residues to 0.062 mg kg^{-1} resulting in 86.19% dissipation on 0 day (1h after spray) and residue level was below LOQ on 1st day for T_1.
5. Washing followed by boiling was found most effective in reduction of residues and residue content was found <LOQ even on same day (0 day) of spray.
6. In case of double dose (T_2) treatment, initial deposit of cyantraniliprole was 0.576 mg kg^{-1} which degraded to 0.435, 0.291 and 0.101 and 0.060 mg/kg on 1, 3, 5 and 7 days after spray with percent removal of 24.48, 49.48, 82.46 and 89.58 respectively following first order degradation kinetics with $T_{1/2}$ of 2.29 days.
7. For studying effect of culinary processes, cabbage samples collected from the field treated with double dose were washed under tap water. The residue study found 86.11, 85.06 and 82.47% reduction on 0 (1h), 1 and 3 days of treatment respectively. Thereafter, the residue content reached below LOQ.
8. Residues of cyantraniliprole after washing with 5% NaCl solution diminished to the extent of 86.28 and 85.98% after 0 (1 hr) and 1 day of treatment respectively. At 3rd day residue level was found below quantification level after washing with 5 % NaCl solution. The results showed that washing with 5 % NaCl solution was more effective than washing alone.
9. Cyantraniliprole residues after washing + boiling reached below LOQ even on 0 day (1h). Results indicate that washing + boiling is the most effective treatment among three methods for residue elimination.
10. Residues of cyantraniliprole dissipated following kinetics of first order degradation with a half-life of 1.91 and 2.29 days at single and double doses in cabbage heads. Residues in soil were found below LOQ for both single and double doses even on 0 day (1h) of spray and also on successive sampling days.
11. Maximum Residue Limit (MRL) established by Joint FAO/WHO Codex Alimentarius Commission for cyantraniliprole in fruity vegetables and cucumbers is 0.3 mg/kg.

CONCLUSIONS:

1. Insecticide dissipation met kinetics of first order degradation with a half-life ($T_{1/2}$) value of 1.91 and 2.29 days at single and double doses respectively.
2. Washing + Boiling was found to be most effective i.e. 100% in discharging cyantraniliprole residues than washing with 5% NaCl solution and tap water washing.
3. Cyantraniliprole residues were found < LOQ in soil on 0 day (after 1 h) of spray application which indicates that this insecticide is safe for succeeding crop and for soil health.

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ABSTRACT

a)	**Title of Thesis**	**:**	**Dissipation and decontamination studies of cyantraniliprole in cabbage**
b)	**Full name of the degree holder**	**:**	**Ms. Poonam Rani**
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g)	**Major subject**	**:**	**Chemistry**
h)	**Total number of pages in the thesis**	**:**	**51 + iv**
i)	**Number of words in the abstract**	**:**	**Approx. 225**

Keywords: Cyantraniliprole, Cabbage, Dissipation, Washing, Residue, Soil, Decontamination, Half – life.

Dissipation and decontamination studies of cyantraniliprole (Benevia 10.26% OD) in cabbage and soil was carried out following spray of cyantraniliprole at fruit setting stage on cabbage crop grown in plots of 5 x5 m size at recommended (60 g a.i. ha^{-1}) and double of recommended dose (120 g a.i. ha^{-1}) by Central Insecticide Board (CIB). Samples of cabbage were processed using modified QuEChERS method and analyzed using HPLC with retention time of 15.351 minutes. Recovery experiments were performed on cabbage heads and soil sample fortified @ 0.05, 0.50 and 1.00 mg kg^{-1} and the recoveries obtained were in the range of 86.11 – 89.54% and 85.60 – 88.05% respectively. Initial deposit of cyantraniliprole residue in cabbage heads was 0.449 mg kg^{-1} and 0.576 mg kg^{-1} for single (T_1) and double (T_2) dose respectively. These residues dissipated following first order degradation kinetics with half-life of 1.91 and 2.29 days respectively and reached below limit of quantification (LOQ i.e. 0.05 mg kg^{-1}) on 7^{th} and 10^{th} day for single (T_1) and double (T_2) dose respectively. Level of residues in soil were < LOQ for both single (T_1) and double dose (T_2) on 0 day of spray. Washing followed by boiling was found most effective (100%) in dislodging residues of cyantraniliprole than washing with 5% NaCl solution and washing alone.

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List of Publications: 1 Abstract and 1 Review Paper

- ✓ Poonam Rani, Sushil and Reena Chauhan (2019). Dissipation dynamics of cyantraniliprole in cabbage and soil (abstract). In: 25th International Conference on "Physical and Biological Sciences at Cross-roads: Interdisciplinary Explorations and Exciting Challenges", December 29-31,2019, Guru Jambheshwar University, Hisar, India.
- ✓ Poonam Rani, Sushil and Reena Chauhan (2020). Biosurfactants Production and Applications (Review) In: 1st International Conference of Indian Science Congress Association-Rohtak Chapter "Science & Technology: Rural Development", March 4-5, 2020, Maharshi Dayanand University, Rohtak.

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- ✓ Participated in Quiz, National Seminar cum Exhibition, February 28,2020 at CBLU, Bhiwani.

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I, **Poonam Rani**, Admn. No. 2018BS09M undertake that I give copyright to the CCS HAU, Hisar of my thesis entitled, **"Dissipation and decontamination studies of cyantraniliprole in cabbage and soil".**

I also undertake that patent, if any, arising out of the research work conducted during the programme shall be filed by me only with due permission of the competent authority of CCS HAU, Hisar.

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