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Article

Development of analytical method for fipronil determination using Gas Chromatography Triple Quadrupole Mass Spectrometry

Mohammad Dalower Hossain Prodhan*

Pesticide Analytical Laboratory, Pesticide Research & Environmental Toxicology Section, Entomology Division, Bangladesh Agricultural Research Institute, Gazipur-1701, Bangladesh

*Corresponding author: Dr. Mohammad Dalower Hossain Prodhan, Pesticide Research & Environmental Toxicology Section, Entomology Division, Bangladesh Agricultural Research Institute, Gazipur-1701, Bangladesh. Phone: +88-01819849657; E-mail: mdhprodhan@gmail.com

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Abstract: The application of fipronil for the control of insect pests of different crops is increasing day by day. As a result, it is inevitable to remain residues of fipronil in the treated crops. Therefore, it is an urgent need to develop analytical method for the quantification of fipronil. Keeping this view, this study was initiated to develop and optimize an analytical method for the quantification of fipronil residue using Gas Chromatography triple quadrupole Mass Spectrometry (GC-MS/MS). A number of experiments has been conducted to select the parent ion and precusor ion and based on these findings, the analytical method for the determination of fipronil residue using GC-MS/MS was developed. The linearity of the developed analytical method was very good and it was 0.999. The optimization of MS/MS parameters has been done properly through direct injection of 100 ug/L standard solution of fipronil.

Keywords: fipronil residue; method development; gas chromatography; mass spectrometry

1. Introduction

Fipronil is a broad-spectrum insecticide and belongs to the phenylpyrazole chemical group. The use of fipronil in Bangladesh is increasing day by day. Fipronil disrupts the insect central nervous system by blocking the ligand-gated ion channel of the γ-Amino Butyric Acid (GABA) receptor and glutamate-gated chloride (GluCl) channels. This causes hyper excitation of contaminated insects nerves and muscles. The specificity of this insecticide towards insects is believed to be due to its greater binding affinity to the GABA receptors of insects, than to those of mammals, and to its action on GluCl channels, which do not exist in mammals. Because of its effectiveness on various pests, fipronil is used as the active ingredient in flea control products for pets and home roach traps as well as field pest control for corn, and commercial turf. The widespread use makes its specific effects of considerable attention (Valerie et al., 2005). This includes ongoing observations on possible off-target harm to humans or ecosystems as well as the monitoring of resistance development.

To determine pesticide residues in fruits, vegetables and other matrices Gas Chromatography (GC), Gas Chromatography associated with Mass Spectrometry (GCMS), High Performance Liquid Chromatography (HPLC), and Liquid Chromatography associated with Mass Spectrometry (LC-MS) are the most commonly used techniques. In recent years, GC-MS/MS has been used for the determination of pesticide residues in the extracts of fruits and vegetables as it is an excellent technique which generally reduces the excessive cleanup

steps, exhibits little chance of false-positive findings, and reduces the analysis time and cost (Hiemstra and Kok, 2007).

GC-MS is a powerful technique that has very high sensitivity, making it useful in many applications. Different mass analyzers are used in GC/MS, including single quadrupole, triple quadrupole, ion trap, time of flightmass spectrometry (TOF-MS). In Bangladesh, for the analysis of pesticide residues researchers mostly depends on Gas Chromatography (Hoque et al., 2022; Hossain *et al.*, 2014; Islam *et al.*, 2021; 2019; 2019a; 2019b; Nahar*et al.*, 2020; Rahman *et al.*, 2021; Prodhan *et al.*, 2018; 2017; 2010; 2009), and in a very few cases the researchers using LC-MS/MS for the analysis of pesticide residues (Prodhan *et al*., 2022; 2022a), however, in many of the countries, they are now using LC-MS/MS for the quantification of pesticide residues (Prodhan *et al*., 2015; 2015a; 2016; 2016a). On the other hand, a lot of research work has been conducted with mostly organophosphorus pesticides (Tasnim et al., 2022; 2022a; Parven et al., 2021; Parvin *et al.*, 2021; Hasan *et al.*,2017; 2021; Habib *et al.*, 2021; Prodhan *et al.*, 2018a) and synthetic pyrethroid pesticides (Islam *et al.*, 2014; Prodhan *et al.*,2023; 2021; 2021a).There are also several research work was found with organochlorine pesticides (Prodhan *et al*., 2018b) and carbamate pesticide residues (Kabir *et al.*,2007; 2008; 2008a). To the best of our knowledge in Bangladesh, none of the research work has been conducted with fipronil determination using GC-MS/MS. Therefore, the present study was initiated to develop an analytical method for the determination of fipronil using Gas Chromatography Triple Quadrupole Mass spectrometry (GC-MS/MS).

2. Materials and Methods

2.1. Ethical approval

This study did not require ethical approval.

2.2. Chemicals and reagents

Reference standard of fipronil was obtained from Sigma-Aldrich (St Louis, MO, USA). GC-MS grade methanol, MS grade acetonitrile, and chromatography grade water were from Merck (Darmstadt, Germany). Sodium chloride (NaCl) was purchased from Chem-Lab (Zedelgem, Belgium), anhydrous magnesium sulphate ($MgSO_4$) was from Panreac (Barcelona, Spain), and Primary Secondary Amine (PSA) was from Agilent (Santa Clara, CA, USA). Formic acid, ammonium acetate and ammonium formate of mass spectrometry grade was purchased from Fluka (Buchs, Switzerland) via SAF scientific, Bangladesh Limited.

2.3. Preparation of pesticide standard solution

Pesticide standard stock solution of fipronil was prepared separately in acetonitrile at a concentration of 1000 mg/L and stored at -20^{0}C until use. A standard solution of 50 mg/L in acetonitrile containing fipronil was prepared by adding the appropriate volume in a 50 ml volumetric flask and made to volume by addition of acetonitrile. An intermediate standard solution of 10 mg/L in acetonitrile was prepared from the standard solution of 50 mg/L. Then working standard solutions of 0.01, 0.05, 0.1, 0.2, 0.4, 0.8, 1.0, 2.0, 3.0, 4.0, and 5.0 mg/L in acetonitrile were prepared by transferring the appropriate amount from 10 mg/L intermediate standard solution into eleven separate 10-ml volumetric flasks. All the standard solutions were kept in a freezer at -20^{0}C until use.

2.4. Instrumental analysis

The development of analytical method was accomplished using a Shimadju Gas Chromatography-Tandem Mass Spectrometry (GCMS-TQ8040). A specific method was developed for the determination of fipronil. The determination of the analyte was achieved by a Rxi-5Sil MS column (30 m long, 0.25 mm i.d). The injection volume was 1µL, and the total run time was 41.86 min. The data acquisition was performed using Multiple Reaction Monitoring (MRM) Mode.

3. Results and Discussion

3.1. Optimization of GC-MS/MS operating conditions

Direct injection was used to obtain the mass spectra and the optimum collision energy for fipronil using a standard solution of 100 ug/L in MS grade acetonitrile. Several experiments in positive mode and in different solvents were performed to optimize these parameters for fipronil. Finally, the following parameters were selected for proper quantification of fipronil (Tables 1 and 2).

Table 1. The instrument parameters for GC-MS/MS.

Instruments	Conditions
GC Condition	Injection Temperature: 250°C; Injection mode: splitless; flow control mode: linear velocity.
	Column oven temperature: Programmed
MS Condition	Interface temperature :250°C Ion Source temperature :230°C

Table 2. Conditions for column oven temperature for fipronil determination.

Column oven	Incremental Rate (°C/min.)	Temperature (°C)	Hold time (min)
Initial temperature: 70°C	-	70	2
	25	150	0
	3	200	0
	8	280	10

3.2. Method development and GC–MS/MS parameters optimization

Direct injection of fipronil standard solution (100 ug/L in acetonitrile) was accomplished to obtain the precursor ion, product ions, and the collision energy (CE). The chromatogram of fipronil is illustrated in Figure 1. The chromatogram of fipronil mentioning the parent ion, quantification and confirmation ions are presented in Figure 2. The calibration curves prepared with different concentrations are presented in Figure 3. The parent ion, quantification ion, confirmation ion, collision energy (CE) along with the retention time of fipronil are presented in Table 3.

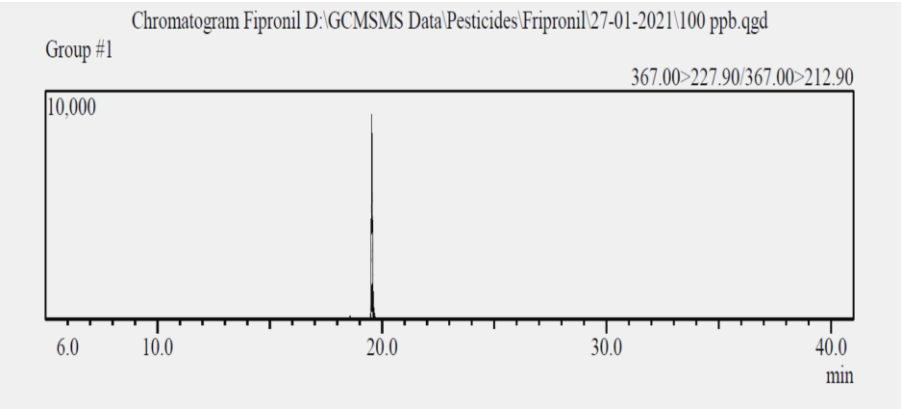

Figure 1. Chromatogram of fipronil with a concentration of 100 ug/L.

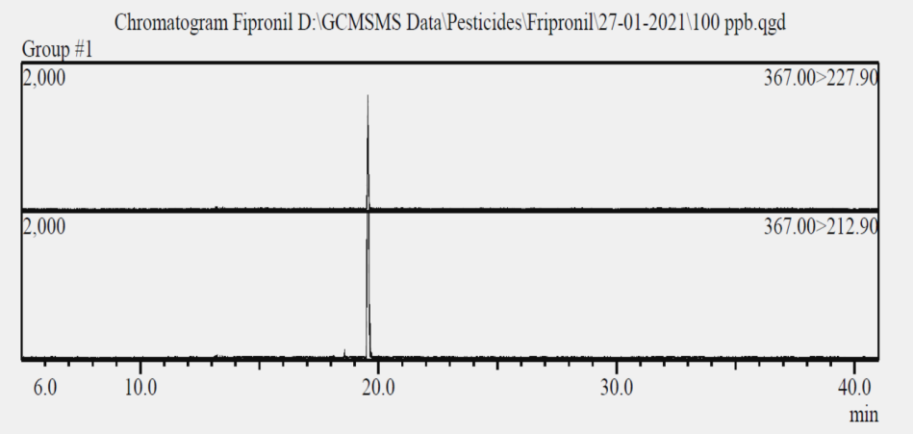

Figure 2. Chromatogram of fipronil showing parent ion and precursor ion with a concentration of 100 ug/L.

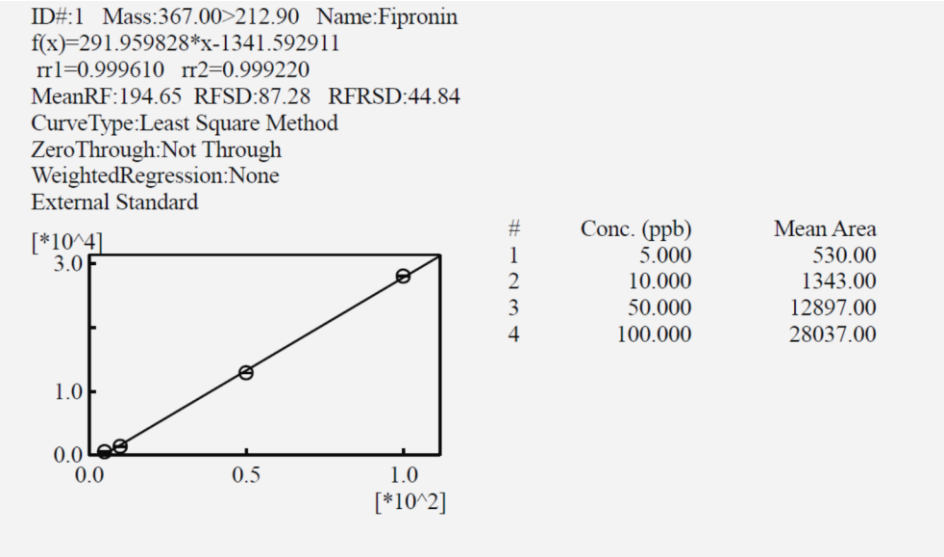

Figure 3. Calibration curve of fipronil made with different concentrations ranging from 5 ug/L to 100 ug/L.

Table 3. GC-MS/MS parameters for fipronil determination.

Pesticides	RT (min.)	Parent ion (m/z)	Quantification ion (m/z)	CE (V)	Confirmation ion (m/z)	CE (V)
Fipronil	19.82	367.00	227.90	26	212.90	26

3.3. Preparation of calibration curve

The standard solutions of different concentrations of fipronil was prepared and injected with the suitable instrument parameters. The samples were calibrated (retention time, peak area, etc.) against four pointed calibration curve of standard solution of fipronil. Each peak was characterized by its retention time. The calibration curves prepared with different concentrations ranging from 5 ug/L to 100 ug/L are presented in Figure 3. The linearity of the calibration curve for fipronil was very good and the co-efficient of determination was ≥ 0.999.

Now-a-days, food safety is a major concern for the consumers. To ensure the safe food for the consumers, it is necessary to quantify pesticide residues with an accurate analytical method using GC, GC-MS, GC-MS/MS, HPLC and LC-MS/MS. However, in case of conventional GC and HPLC, there is a change to produce false positive and false negative findings. But, using GC-MS/MS and LC-MS/MS, it is possible to minimize both false positive and false negative findings (Hernández *et al*., 2013; Prodhan *et al*., 2017). On the other hand, it is difficult to quantify residues at a very trace levels using GC or HPLC. To minimize these problems, GC-MS/MS and LC-MS/MS is the best option (Hernández *et al*., 2013; Prodhan *et al*., 2018b; Hiemstra and Kok, 2007).

However, in order to analyze pesticide residues researchers mostly depends on Gas Chromatography especially in the developing countries including Bangladesh (Alam *et al*., 2023; 2022; Aktar et al., 2017), and in a very few cases the researchers using LC-MS/MS for the analysis of pesticide residues (Prodhan *et al*., 2022). Therefore, a precise quantification method was developed in this study using GC-MS/MS. The linear range was 5 ug/L to 100 ug/L and the linearity of the method was also very good (≥ 0.999). The findings of the present study are in a good agreement with Nasiri *et al*., 2016, where they developed a multi residue analytical method with GC-MS and found that the linearity of the selected pesticides ranged from 0.996-0.999.

4. Conclusions

In this study, an accurate and efficient analytical method was developed for fipronil residue determination using GC-MS/MS. The linearity of the developed analytical method was very good and it was ≥ 0.999. The developed method can be used successfully for the quantification of fipronil residues in different agricultural commodities. The developed analytical method will assist the analysts to quantify fipronil residues accurately and precisely.

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Data availability
The tables, figures and texts in this article contain the data that support the findings of this study.

Conflict of interest
None to declare.

Authors' contribution
Conceptualization, Methodology, Formal analysis and investigation, Writing - original draft preparation, review and editing: Mohammad Dalower Hossain Prodhan. All authors have read and approved the final manuscript.

References
Aktar MA, R Khatun and MDH Prodhan, 2017. Determination of pesticide residues in eggplant using modified QuEChERS Extraction and Gas chromatography. Int. J. Agron. Agri. Res., 11: 22-31.

Alam MM, D Sultana, SMM Rahman and MDH Prodhan, 2023. Monitoring of pesticide residues in yard long bean collected from Mymensingh district of Bangladesh. Asian Australas. J. Biosci. Biotechnol., 8: 8-16.

Alam MM, R Hasan, SMM Rahman, MAR Choudhury and MDH Prodhan, 2022.Analysis of pesticide residues in vegetables purchased from local markets of Mymensingh district of Bangladesh based on QuEChERS Extraction and Gas Chromatography. Asian Australas. J. Food Saf. Secur., 6: 10-17.

Habib M, A Kaium, MSI Khan, MDH Prodhan, N Begum, MTI Chowdhury and MA Islam, 2021. Residue level and health risk assessment of organophosphorus pesticides in eggplant and cauliflower collected from Dhaka city, Bangladesh. Food Res., 5: 369-377.

Hasan R, MM Alam, SMM Rahman, D Sultana, MDH Prodhan, 2021. Monitoring of pesticide residues in vegetables collected from retail markets of Dhaka district of Bangladesh using QuEChERS Extraction and Gas Chromatography. Asian Australas. J. Food Saf. Secur., 5: 63-70.

Hasan R, MDH Prodhan, SMM Rahman, R Khanom and AUllah, 2017. Determination of organophosphorus insecticide residues in country bean collected from different markets of Dhaka. J. Env. Anal.Toxicol.,7: 489.

Heimstra M and Kok A de, 2007. Comprehensive multi-residue method for the target analysis of pesticides in crops using liquid chromatography–tandem mass spectrometry. J. Chromatogr. A., 1154: 3–25.

Hernández F, MI Cervera, T Portolés, J Beltrán and E Pitarch, 2013. The role of GC-MS/MS with triple quadrupole in pesticide residue analysis in food and the environment. Anal. Methods., 5: 5875-5894.

Hoque MS, F Tamanna, MM Hasan, MHA Banna, P Mondal, MDH Prodhan, MZ Rahman and MLV Brakel, 2022. Probabilistic public health risks associated with pesticides and heavy metal exposure through consumption of common dried fish in coastal regions of Bangladesh. Environ Sci. Pollut. Res., 29: 20112-20127.

Hossain MS, MM Rahman, KH Kabir, MRU Miah and MDH Prodhan, 2014. Determination of Pre Harvest Interval (PHI) for cypermethrin and acephate in yard-long bean under supervised field trial. Bangladesh J. Entomol., 24: 101-115.

Islam MS, MR Rahman, MDH Prodhan, D Sarker, MM Rahman and MK Uddin, 2021. Human health risk assessment of pesticide residues in pointed gourd collected from retail markets of Dhaka city, Bangladesh. Accredit. Qual Assur., 26: 201–210.

Islam MA, A Ullah, M Habib, MTI Chowdhury, MSI Khan, A Kaium and MDH Prodhan, 2019. Determination of major organophosphate pesticide residues in cabbage collected from different markets of Dhaka. Asia Pac. Environ. Occup. Health J., 5: 30-35.

Islam MS, MDH Prodhan and MK Uddin, 2019a. Analysis of the pesticide residues in bitter gourd using modified QuEChERS extraction coupled with Gas Chromatography. Asia Pac. Environ. Occup. Health J., 5: 6-15.

Islam MS, MDH Prodhan and MK Uddin, 2019b.Determination of major organophosphorus pesticide residues in eggplant using QuEChERS Extraction and Gas Chromatography.Int. J. Innov. Sci. Res. Technol. 4: 212-219.

Islam MW, KMG Dastogeer, I Hamim, MDH Prodhan and M Ashrafuzzaman, 2014. Detection and quantification of pesticide residues in selected vegetables of Bangladesh. J. Phytopathol. Pest Manag., 1: 17-30.

Kabir KH, MA Rahman, MS Ahmed, MDH Prodhanand MW Akon, 2008. Determination of residue of diazinon and carbosulfan in brinjal and quinalphos in yard long bean under supervised field trial. Bangladesh J. Agril. Res., 33: 503-513.

Kabir KH, MA Rahman, MS Ahmed, MDH Prodhan and MW Akon, 2008a. Quantitative analysis of some commonly used insecticides in vegetables. Bangladesh J. Agriculturist, 1: 259-264.

Kabir KH, M Abdullah, MDH Prodhan, MS Ahmed and MN Alam, 2007. Determination of carbofuran residue in the samples ofsugarcane and soil of sugarcane field. The Agriculturist, 5: 61-66.

Nahar KM, MSI Khan, M Habib, SM Hossain, MDH Prodhan and MA Islam, 2020. Health risk assessment of pesticide residues in vegetables collected from northern part of Bangladesh. Food Res., 4: 2281-2288.

Nasiri A, M Amirahmadi, Z Mousavi, S Shoeibi, A Khajeamiri and F Kobarfard, 2016. A multi residue GC-MS method for determination of 12 pesticides in cucumber. Iran J. Pharm. Res., 15: 809-816.

Parven A, MSI Khan, MDH Prodhan, K Venkateswarlu, M Mallavarapu and IM Meftaul, 2021. Human health risk assessment through quantitative screening of insecticide residues in two green beans to ensure food safety. J. Food Compos. Anal., 103: 104121.

Parvin R, AAA Al-Subeihi, MMC Mahmud, MTI Chowdhury, MDH Prodhan and MA Islam, 2021. Determination of pesticide residues and health risk assessment in cucumber and eggplant sold in northern part of Bangladesh. Poll Res., 40: 1180-1187.

Prodhan MDH, M Afroze, A Begum, MS Ahmed and D Sarker, 2023. Optimization of a QuEChERS based analytical method for the determination of organophosphorus and synthetic pyrethroid pesticide residues in betel Leaf. Intern. J. Environ. Anal.Chem., 103: 1292-1303.

Prodhan MDH, M Afroze, A Begum, MS Ahmed, NK Dutta and D Sarker, 2022. Optimization of an analytical method for carbaryl and chlorpyrifos residues determination by LC-MS/MS. Asian Australas. J. Biosci. Biotechnol., 7: 107-113.

Prodhan MDH, A Begum, M Afroze, MS Ahmed, NK Dutta and D Sarker, 2022a. Development of analytical method for pesticide residue determination using LC-MS/MS. Asian Australas. J. Food Saf. Secur., 6: 65-72.

Prodhan MDH, M Afroze, A Begum and D Sarker, 2021. Determination of organophosphorus and synthetic pyrethroid pesticide residues and their variability in large size fruit crops. J. Sci. Food. Agric., 101: 4847–4854.

Prodhan MDH, MS Ahmed, NK Dutta, D Sarker and SN Alam, 2021a. Determination of organochlorine and synthetic pyrethroid pesticide residues in water samples collected from different locations of Bangladesh. J. Biophys. Chem., 12: 11-21.

Prodhan MDH, MW Akon and SN Alam, 2018. Determination of pre-harvest interval for quinalphos, malathion, diazinon and cypermethrin in major vegetables. J. Environ. Anal.Toxicol., 8: 553.

Prodhan MDH, MW Akon and SN Alam, 2018a. Decontamination of organophosphorus insecticide residues from eggplant and yard long bean. Int. J. Expt. Agric., 8: 6-9.

Prodhan MDH and SN Alam, 2018b. Determination of multiple organochlorine pesticide residues in shrimp using modified QuEChERS extraction and gas chromatography. SAARC J. Agri., 16: 81-93.

Prodhan MDH, EN Papadakis and E Papadopoulou-Mourkidou, 2018b. Variability of pesticide residues in eggplant units collected from a field trial and marketplaces in Greece. J. Sci. Food. Agric., 98: 2277–2284.

Prodhan MDH, SN Alam, MJ Uddin, 2017. Analytical methods in measuring pesticides in foods. Pesticides residues in foods: Sources, Management and Control, 135-145.

Prodhan MDH, EN Papadakis and E Papadopoulou-Mourkidou, 2016. Variability of pesticide residues in cauliflower units collected from a field trial, and market places in Greece. J. Environ. Sci. Health, Part B., 51: 644-653.

Prodhan MDH, EN Papadakis and E Papadopoulou-Mourkidou, 2016a.Analysis of pesticide residues and their variability in cabbage using QuEChERS Extraction in combination with LC-MS/MS. Food Anal. Methods, 9: 3470-3478.

Prodhan MDH, EN Papadakis and E Papadopoulou-Mourkidou, 2015. Determination of multiple pesticide residues in eggplant with liquid chromatography-mass spectrometry. Food Anal. Methods, 8: 229-235.

Prodhan MDH, EN Papadakis and E Papadopoulou-Mourkidou, 2015a. Analysis of pesticide residues in melon using QuEChERS extraction and liquid chromatography triple quadruple mass spectrometry. Int. J. Env. Anal. Chem., 95: 1219-1229.

Prodhan MDH, MA Rahman, MS Ahmed and KH Kabir, 2010. Pesticide residues in fish samples collected from different fish cultivation regions of Bangladesh. SAARC J. Agri., 8: 53-64.

Prodhan MDH, MA Rahman, MS Ahmed and KH Kabir, 2009. Quantification of organophosphorus and organochlorine insecticide residues from fish samples using smiple GC technique. Bangladesh J. Agriculturist, 2: 197-204.

Rahman A, A Kaium, MSI Khan, MA Islam, N Begum, MDH Prodhan, A Hossain, SSB Mustafij, MTI Chowdhury, 2021. Residue level and health risk assessment of organophosphorus pesticides in country bean and bitter gourd collected from Cumilla, Bangladesh. Food Res., 5: 238-246.

Tasnim N, MN Millat, S Sultana, SMM Rahman, and MDH Prodhan, 2022. Multiple pesticide residue determination in major vegetables purchased from Gazipur district of Bangladesh.Asian Australas. J. Food Saf. Secur., 6: 57-64.

Tasnim N, MN Millat, S Sultana, SMM Rahman, and MDH Prodhan, 2022a. Analysis of organophosphorus pesticide residues in selected vegetables purchased from Narsingdidistrict of Bangladesh using QuEChERS Extraction. Asian Australas. J. Biosci. Biotechnol., 7: 114-121.

Valérie RD, M Kazuhiko, BM Sattelle, JJ Rauh, and DB Sattelle, 2005. Ion channels: molecular targets of neuroactive insecticides. Invert Neurosci., 5: 119–133.