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Probabilistic exposure assessment, a risk-based sampling plan and food safety performance evaluation of common vegetables (tomato and brinjal) in Bangladesh

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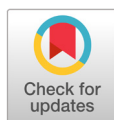
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Abstract

Along with the widespread use of pesticides in the world, concerns over human health impacts are rapidly growing. There is a large body of evidence on the relationship between the exposure to pesticides and the elevated rate of chronic diseases such as different types of cancers, diabetes, neurodegenerative disorders like Parkinson, Alzheimer, and amyotrophic lateral sclerosis (ALS), birth defects, and reproductive disorders. This research assessed the health risk of pesticide residues by the dietary intake of vegetables collected from the agro-based markets of Dhaka, Bangladesh. As some of the banned pesticides were also found in vegetable samples, they may pose a higher risk because of cheaper availability and hence the government of Bangladesh should take strong measures to control these banned pesticides. Five organo phosphorus (chlorpyrifos, parathion, ethion, acephate, fenthion) and two carbamate (carbaryl and carbofuran) pesticide residues were identified in twenty four samples of two common vegetables (tomato and brinjal). The pesticide residues ranged from below a detectable limit (< 0.01) to 0.36 mg·kg⁻¹. Acephate, chlorpyrifos, ethion, and carbaryl were detected in only one sample, while co-occurrence occurred twice for parathion. Continuous monitoring and strict regulation should be enforced regarding the control of pesticide residues in fresh vegetables and other food commodities in Bangladesh.

Keywords: diagnostic tools, food safety, maximum residue limits (MRLs), pesticide residues, @Risk palisade

Introduction

Pesticides are considered as one of the main factors involved in environmental contamination of today's world. These chemicals are on purpose designed to be toxic to pest and vectors of diseases. These compounds are among more than 1,000 active ingredients that are marketed as insecticide, herbicide and fungicide (Bempah et al., 2011). Over the past couple of decades, a rapid increase in the quantity and use of different pesticides in the agricultural sector has been observed and this growth trend is expected to continue for the next decades due to several socio-economic and technological developments (Bempah et al., 2011). However, pesticide use has also been associated with several concerns, including the potential risks to human health from both occupational and non-occupational exposures, the death of farm animals and alteration of the local environment (Mansour et al., 2009). Many of these compounds can cause moderate to severe respiratory and neurological damage or act as genotoxic, carcinogenic and mutagenic agents, endocrine disruption *etc.*, through routes that include consumption of dietary residues (Hayat et al., 2011).

Very common effects of pesticide residues in the human body include nausea, vomiting, blurred vision, coma, difficulty in breathing, deficit hyper activity disorder, a disorder in fetuses and children, etc. (Rauh et al., 2006). Many pesticides and their residues are also known to be contributory factors in several diseases such as cancer, heart diseases, Alzheimer's and Parkinsonism (Khaniki, 2007). The WHO estimated an annual thirteen million people under the age 70 died from these harmful diseases worldwide in 2016 (WHO, 2018). The majority of these cases of poisoning and deaths occur in developing countries, although far greater quantities of pesticides are used in the developed countries represents in Fig. 1 (Bhanti et al., 2004). Bangladesh is an agro-dominant country where about 62% of the population is involved directly or indirectly in the agricultural sector. The most alarming concern is indiscriminate use of various types of pesticides (Fig. 1) due to their easy availability, relatively cheap cost and easy to application (Chowdhury et al., 2012).

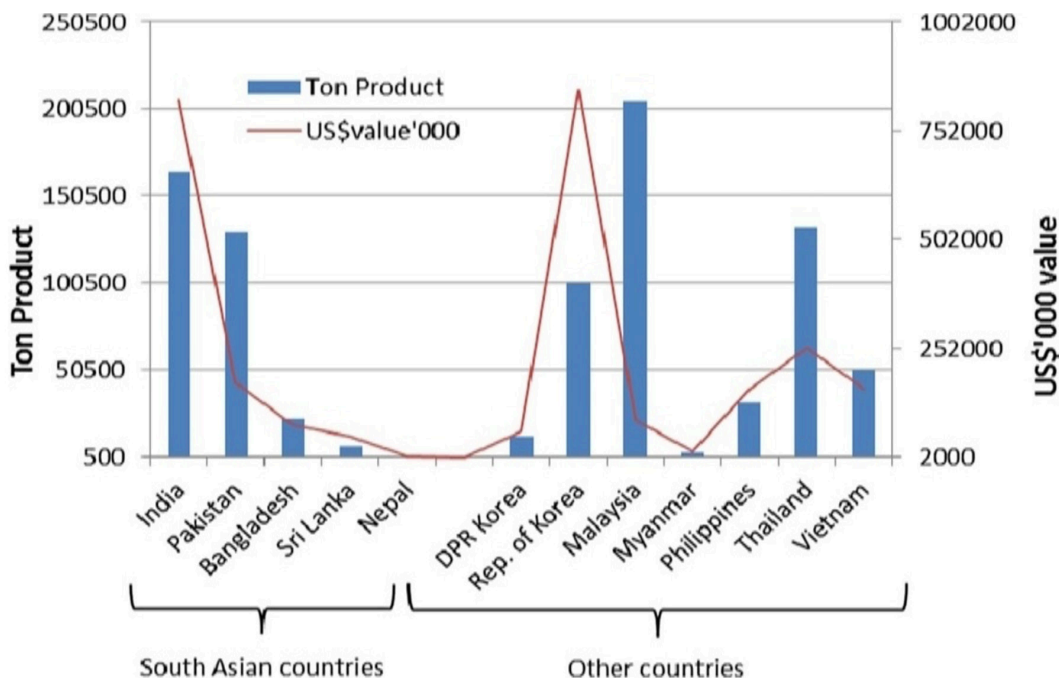


Fig. 1. South Asian annual pesticide consumption (tons) and associated cost (US\$) in comparison with other countries (Abhilash and Singh, 2009; Ali et al., 2014).

Actually, pesticide residues in food and crops are direct results of an application of pesticides to the agricultural field and to a lesser extent from pesticide residues contaminating the soil (Businell et al., 1992). Moreover, sometimes few farmers do not wait long enough for the residues to wash off after spraying before harvesting because of their high demand for farm products and low perception of the toxic effects of pesticide residues in food (Amoah et al., 2006). Thus, increased use of pesticides in agriculture has resulted in the occurrence of residues in food commodities that has always been a matter of serious concern especially when these commodities are consumed fresh (Solecki et al., 2005). In the present study health risk of a total of five organophosphorus (chlorpyrifos, parathion, ethion, acephate, fenthion), two carbamate (carbaryl and carbofuran) pesticide residues were assessed via dietary intake of two common vegetables (tomato and brinjal) collected from some top agro-based markets of Dhaka. Every risk assessment process begins with a quantitative characterization of hazards that need an effective sample size judgment as a key prerequisite in the design of sound surveillance systems. The need for efficient and cost-effective surveillance systems is stimulating the use of a risk-based selection of hazards and population strata and risk-based sample size calculation.

The global trend of using a systematic approach in governing food safety demands the obligation for food processing companies to develop a food safety management system based upon good agricultural practices (GAP) and Hazard Analysis and Critical Control Point (HACCP). In addition to this, a proactive measure of adopting self-checking and evaluation system through appropriate diagnostic tool can improve the performance of an operational food safety management system and its overall food safety output (Jacxsens et al., 2010; Jacxsens, 2011). One recently designed tool is the Food Safety Management Systems Diagnostic Instrument (FSMS-DI) which is a self-assessment tool premeditated to assess the control and assurance activities in the food safety management system (FSMS) of a company. With this assessment it is possible to explore the weak and strong points of the FSMS and/or context situation of a company (Jacxsens et al., 2015).

The general objectives of this study were to determine the risk levels of different pesticide residues in two common vegetables in Bangladesh using @Risk software (Palisade Corp., Ithaca, NY, USA) with Monte Carlo simulation and self-assessment by FSMS-DI, assurance activities in the FSMS of vegetable farms in Bangladesh.

Materials and Methods

Experimental section

Twenty four samples of two vegetables viz., brinjal (*Lycopersicon esculentum* L.) and tomato (*Solanum melongena* L.) were collected during the winter season (October-November) of 2015 at random basis from four (Hatirpool, RajaBazar, Karwon Bazar and Mahammudpur) vegetable markets of Dhaka City and these are top agro-based markets of Dhaka from where most of the vegetables are supplied to the city dwellers. Sampling was conducted according to the international standard guideline (SAC, 2008). Samples were taken among commodities considering high consumption rate and relatively cheap to buy. The high-performance liquid chromatography with photo diode array detector (HPLC-PDA) were used to determine the concentration of the pesticide residues present in the vegetables (tomato and brinjal) and then health risk index (HRI) were calculated from the obtained results and other statistics (Donkor et al., 2015). The chromatographic system consisted of a chromatographic separation for organophosphorus and organocarbamate residues were performed with a capillary column with VF-5 ms and carrier and make-up gases were nitrogen at a flow rate of 1.0 and 29 mL·min⁻¹

respectively, while that of the organophosphorus were performed with capillary column coated with VF-1701 ms. The carrier was nitrogen at a flow rate of 2.0 mL·min⁻¹ with Air 1, Air 2 and hydrogen flow rates of 17, 10 and 14 mL·min⁻¹ respectively and the organocarbamate were detected with ⁶³Ni electron capture detector (ECD) (Chowdhury et al., 2012). The instrumental control based on the results obtained from the calibration of each pesticides residues of the collected vegetables of tomato and brinjal.

Fitting distribution for pesticides concentration data

For those pesticides whose maximum and minimum values of concentration for Brinjal and Tomato in Bangladesh were available in the literature (WHO/FAO, 1997), they were directly fitted with uniform distribution using @Risk software (Palisade Corp., Ithaca, NY, USA). But for those are not available, some adjustments were done on the available raw data before applying any appropriate distribution (Fig. 2). The prescribed maximum residue value was used (as in Bangladesh, in case of food safety concern we used the European Union Maximum Residue Level due to facilitate the exporting of fresh fruits and vegetables in EU) to adjust the data for the worst-case prediction. If the available datum for pesticide detection was above the maximum residue limits (MRLs), it was taken as a maximum value and the corresponding MRL was taken as the minimum value. On the other hand, if the data is not available then level of detection (LOD) is taken as the maximum value and half of LOD is taken as the minimum value for worst case scenario. The final table for pesticide residues concentration data fitted with uniform distribution in @Risk software (Palisade Corp., Ithaca, NY, USA) has been shown below in Table 1.

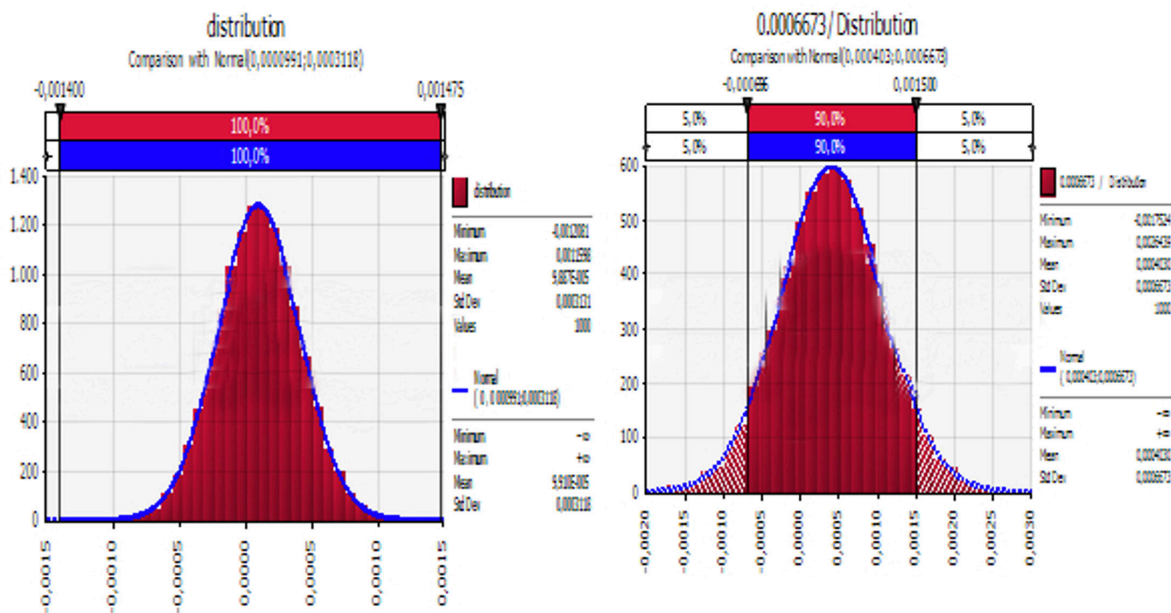


Fig. 2. Distribution fitted (maximum, minimum, mean & std dev) for tomato and brinjal consumption data.

Table 1. Pesticide residues detected in brinjal and tomato samples collected from Dhaka City.

| Sample | Pesticide | MRLs | No. of sample detected | Min. value | Max. value |
|---------|--------------|------|------------------------|------------|-------------------|
| Brinjal | Acephate | 0.02 | 1/5 | 0.01* | 0.02 ^z |
| | Chlorpyrifos | 0.5 | 1/5 | 0.01* | 1.03 |
| | Malathion | 0.02 | 1/5 | 0.01* | 0.29 |
| | Parathion | 0.05 | 1/5 | 0.01* | 0.17 |
| | Ethion | 0.01 | 0/5 | 0.005** | 0.01 ^z |
| | Carbaryl | 0.05 | 1/5 | 0.01* | 0.82 |
| | Carbofuran | 0.02 | 1/5 | 0.01* | 0.61 |
| Tomato | Acephate | 0.02 | 0/5 | 0.01* | 0.02 ^z |
| | Chlorpyrifos | 0.5 | 1/5 | 0.01* | 0.5 ^z |
| | Malathion | 0.02 | 1/5 | 0.01* | 0.33 |
| | Parathion | 0.05 | 1/5 | 0.01* | 0.31 |
| | Ethion | 0.01 | 0/5 | 0.005** | 0.01 ^z |
| | Carbaryl | 0.5 | 1/5 | 0.01* | 1.6 |
| | Carbofuran | 0.02 | 0/5 | 0.01* | 0.02 ^z |

Min., minimum; Max., maximum.

^z Represents the EU maximum residue limits (MRLs) used to assign range of concentration for the corresponding pesticides for distribution fitting at worst case scenario.

*: LOD (level of detection) (Below detection limit [BDL] 0.01 mg·kg⁻¹), **: 1/2 LOD.

Consumption data and body weight

There are a number of methods for assessing dietary consumption of pesticides through vegetables (brinjal and tomato) which includes food consumption, surveys at an individual or household level or, less accurately, from food production statistics. Consumption can be recorded by one or more prospective diaries, retrospective dietary recall, and food frequency questionnaires. The average national consumption of those two popular vegetables is 0.4030 gm·kg⁻¹ bw·day⁻¹ with std. dev. of 0.6673 (WHO, 2015) for Brinjal of an adult woman which can be assumed same for an adult man also and 0.0991 gm·kg⁻¹ bw·day⁻¹ with std. dev. of 0.3118 (WHO, 2013) for Tomato in Bangladesh. Consumption rates were expressed in kg vegetables per kg body weight per day considering the average body weight for an adult Bangladeshi person as 60 kg. The final data was fitted with normal distribution in the @Risk software (Palisade Corp., Ithaca, NY, USA) and presented in (Thompson, 1999) Table 2.

Table 2. Distribution fitted for vegetable consumption in Bangladesh.

| Vegetable | Characters | Vegetable (kg·kg ⁻¹ bw·day ⁻¹) |
|-----------|------------|---|
| Brinjal | Mean | 0.000403 |
| | Std. Dev. | 0.000667 |
| Tomato | Mean | 0.0000991 |
| | Std. Dev. | 0.0003118 |

Std. Dev., standard deviation.

Source: WHO, 2015.

Risk-based sampling plan of brinjal and tomato

Pesticide is not distributed homogeneously in all vegetables but all vegetable in a lot have an equal chance of being

selected in the sample. Correct sampling method plays a very important role in representing the lot. For risk-based sampling, the total number of vegetable samples to be monitored was calculated by the method developed by the food safety scholars of Ghent University, Belgium (Jacxsens et al., 2010) which is as follows;

$$n = [1 - (1 - \alpha)1/D] \times [N - (D - 1)/2] \tag{1}$$

Where n, α, D, and N are the number of samples to be taken, confidence interval, the expected number of non-compliance results, and the size of a population where a sample is taken, and where N is estimated at 10,000 units (Table 3).

Table 3. Calculation of risk based sampling methods and sample size for different vegetables (brinjal, tomato) of Bangladesh (Jacxsens et al., 2010).

| Criteria | Score (1 - 4) | Remarks for score | Total score = criteria 1+ (criteria 2 × criteria 3) | Confidence interval (α) | D | Number of samples (n) |
|--|---------------|---|---|-------------------------|---------------------------|-----------------------|
| Criteria 1: Harmfulness | 2 | Harmful | | | | |
| Criteria 2: Prevalence | 1 | Less, below standard limit | | | | |
| Criteria 3: Share/contribution to the population | 3 | Good contributor (> 10% of vegetable consumption) | 2 + (1 × 3) = 5 | 90% | D = 5% for N 10,000 = 500 | 45 |

Statistical methods

For probabilistic exposure assessment, the concentration distribution for each residue type was first multiplied with consumption distribution and then Monte Carlo simulation was performed (Keramati et al., 2018). The simulation generated exposure risk at mean; std. deviation and 99th percentile was then assessed. The Monte Carlo simulations with 1,000 iterations were performed using @Risk software (Palisade Corp., Ithaca, NY, USA) (Thompson, 1999) (Fig. 2). This exposure assessment assumes that for all the detected samples were far from the MRLs, ADI and acute references dose (ARfD) values of those pesticides. It shows that all pesticides exposure limits are far from 1 which indicates population are almost safe for all pesticides.

Results and Discussion

Probabilistic exposure and risk assessment of pesticide residues

Out of twenty-four sample pesticide residues ranged from below detectable limit (< 0.01) to 0.36 mg·kg⁻¹ and acephate, chlorpyrifos, ethion, carbaryl were detected in only one sample, while co-occurrence occurred twice for parathion. Probabilistic estimation of exposure was performed to evaluate the content of pesticides in vegetables which are the main part of dietary of Bangladeshi population. For those pesticides whose reference dose (RfD) values were not available, the corresponding acceptable daily intake (ADI) values were used as RfD cannot be less than the prescribed ADI. The descriptive statistics of the exposure distribution, such as the mean, std. deviation and 99th percentile value for exposure was calculated and the latter was compared to the ADI and RfD (EFSA, 2013; JMPR, ECCO & WHO Pesticide Database) values for each pesticide type in order to estimate the real exposure for chronic and acute risk, respectively (Table 4). The probabilistic estimation of exposure at 99th percentile showed that none of the studied pesticide residues may cause a significant health

Table 4. Exposure assessment of different pesticides in Brinjal and Tomato in Bangladesh.

| Vegetable | Pesticide | Mean exposure (mg · kg ⁻¹ bw · day ⁻¹) | **Standard deviation | 90% | 99% | % below ADI ^z | % below ARfD ^z | % below MRL ^z |
|-----------|--------------|---|----------------------|------------|------------|--------------------------|---------------------------|--------------------------|
| Brinjal | Acephate | 6.04E - 06 | 1.04E - 05 | 1.96E - 05 | 3.20E - 05 | 100 | 100 | 100 |
| | Chlorpyrifos | 0.0002 | 0.000 412 | 0.0007 | 0.0013 | 100 | 100 | 100 |
| | Malathion | 5.99E - 05 | 0.0001 | 0.0002 | 0.0004 | 100 | 100 | 100 |
| | Parathion | 3.70E - 05 | 7.09E - 05 | 0.0001 | 0.0002 | 100 | 100 | 100 |
| | Ethion | 2.95E - 06 | 5.13E - 06 | 9.48E - 06 | 1.48E - 05 | 100 | 100 | 100 |
| | Carbaryl | 0.0001 | 0.0003 | 0.0006 | 0.001 | 100 | 100 | 100 |
| | Carbofuran | 0.00012164 | 0.0002 | 0.0004 | 0.0008 | 100 | 100 | 100 |
| Tomato | Acephate | 1.45E - 06 | 4.79E - 06 | 7.31E - 06 | 1.27E - 05 | 100 | 100 | 100 |
| | Chlorpyrifos | 2.52E - 05 | 9.45E - 05 | 0.0001 | 0.0003 | 100 | 100 | 100 |
| | Malathion | 1.55E - 05 | 6.34E - 05 | 9.6E - 05 | 0.0002 | 100 | 100 | 100 |
| | Parathion | 1.68E - 05 | 5.75E - 05 | 9.09E - 05 | 0.0001 | 100 | 100 | 100 |
| | Ethion | 7.44E - 07 | 2.40E - 06 | 3.76E - 06 | 6.71E - 06 | 100 | 100 | 100 |
| | Carbaryl | 7.32E - 05 | 0.0002 | 0.0004 | 0.0009 | 100 | 100 | 100 |
| | Carbofuran | 1.52E - 06 | 4.83E - 06 | 7.57E -06 | 1.41E -05 | 100 | 100 | 100 |

ADI, acceptable daily intake; ARfD, acute reference dose; MRLs, maximum residue limits.

^z Source: European pesticide database (EFSA, JMPR, ECCO) & WHO pesticide database. 2020.

risk (highest hazard quotient [HQ] = 0.25 < 1) both at the chronic and acute level for vegetable consumption in Bangladeshi population. Since these values are still less than 1, this implies that the synergistic risk associated with the exposure form all the seven detected pesticide residues is also not of concern. Sensitivity analysis was performed using *@Risk* software (Palisade Corp., Ithaca, NY, USA) with Monte Carlo simulation to determine the extent of the influence of each exposure input variable on the total exposure (Fig. 3).

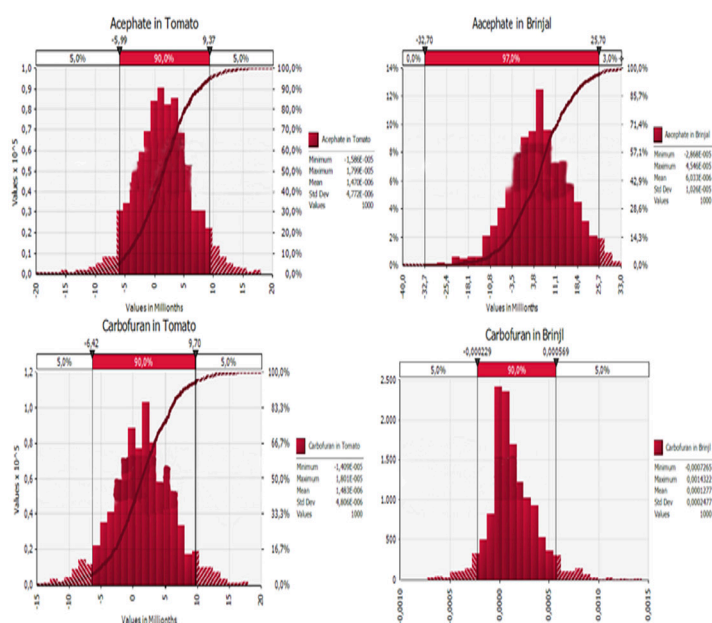


Fig. 3. Probability density function and cumulative distribution for probabilistic exposure of different pesticides in Tomato and Brinjal of Bangladesh using *@Risk* Palisade software.

Food safety management systems diagnostic instrument (FSMS-DI)

Risk assessment of pesticide residue in vegetables was conducted in the previous part of the study and a risk-based sampling method was used to recommend the appropriate sample size for monitoring pesticide residue in Bangladesh. In order to have further deep insight into the actual problem scenario of pesticide residue contamination in vegetables, a more proactive approach would be to characterize and evaluate the food safety management system of that farm which is producing vegetables in Bangladesh. A FSMS diagnostic instrument developed by the scholars of Ghent University and Wageningen University was used to gain an insight into the performance of food safety management systems in vegetable production farms in Bangladesh view of their typical context, control, assurance and performance characteristics. The diagnostic instrument explored the four contextual factors related to a primary produce farm (product, process, organization and chain characters) and at the same time assessed the performance of a food safety management system (Fig. 4) within the boundary of the core control and assurance activities. Each indicator had a grid including three/four situational descriptions that correspond with respectively a low (score 0), basic (score 1), average (score 2), and advanced (score 3) performance level in order to enable a differentiated assessment (Luning et al., 2008; Jacxsens, 2011).

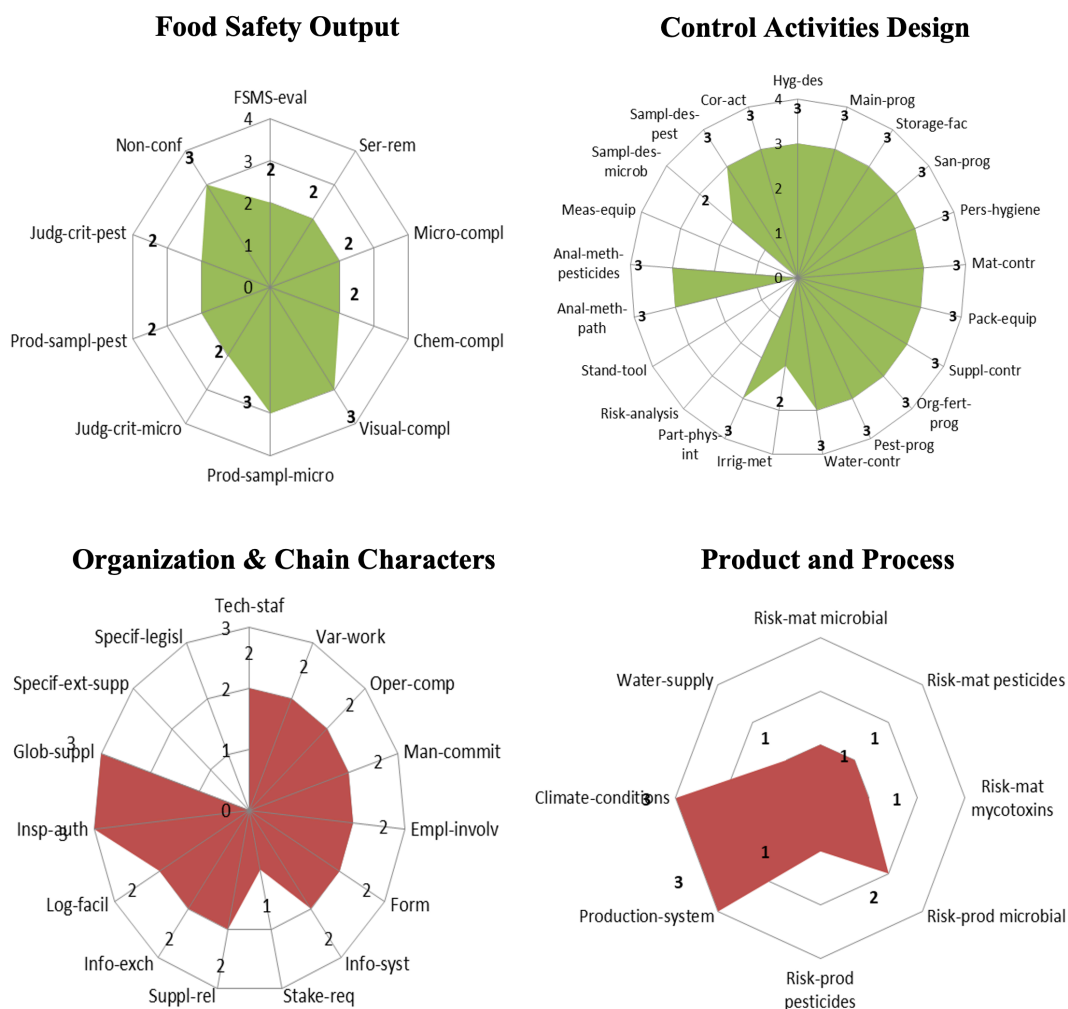


Fig. 4. Diagnostic tools of spider web (Jacxsens et al., 2010) for primary product (vegetables) in Bangladesh.

For contextual factors, a higher score indicates a higher risk, whereas for FSMS activities a higher score indicates a more satisfactory food safety status. This instrument was generated the following spider web diagrams while self-checking the general scenario of food safety performance of a typical vegetable farm in Bangladesh. The generated spider web diagram (Fig. 4) demonstrated that the vegetable production farm in Bangladesh was operating at the moderate risk level and hence appropriate intervention like pesticide residue management in the production process must be done in order to minimize the existing risk from contextual factors. On the other side, it was revealed that the vegetable production in Bangladesh had a weak performance on food safety management system at the design and operation control levels. This suggests the need for effective intervention strategies such as the acceptance of raw materials from trusted source and application of rapid pesticide screening kits, simple analytical methods, manpower training, and calibrated instruments. Furthermore, the diagnostic tool also revealed that a typical vegetable production farm in Bangladesh had food safety assurance activities only at the basic level and hence appropriate validation and verification regarding monitoring of pesticide contaminants in vegetable production chain are suggested for adoption. In the area of food safety output, the diagnostic tool demonstrated that the vegetable production in Bangladesh was operating at a moderate level.

Conclusion

Pesticides that are chosen for application on vegetable farming should be biologically effective, user-friendly and environmentally safe. Since only a limited number of pesticides, samples and vegetables were taken into consideration, a further elaborated study needs to be done to concretely estimate the exposure from all possible pesticides in vegetables. As some of the banned pesticides were also found in vegetable samples, they may pose a higher risk because of cheaper availability and hence the government of Bangladesh should take strong measures to control these banned pesticides. One important and effective measures should be taken is the use of pheromone trap to control pest in the case of vegetable production in Bangladesh which reduces the use of pesticide drastically, which is also safe and friendly to the environment also for the users and consumers. Another possible solution to prevent the use of banned pesticides along with the optimizing use of authorized pesticides is to promote the farmers' association in establishing local pesticide co-operatives from where vegetable farmers can be controlled and trained in using the pesticides inappropriate way. On the other hand, there is also important for the farmers' concern about the application of pesticide before harvesting, proper washing just after harvest and marketing.

There is huge responsibility should belong to the Agricultural Extension Department (AED), concern scientists, BADC (Bangladesh Agriculture Development Corporation) personnel of Bangladesh to ensure the proper marketing and uses of pesticides by proper manners during vegetable production. As consumers and retailers are demanding lower and lower number of residues, the government of Bangladesh should allocate appropriate resources in the sector of monitoring, product control, training, and capacity building of inspectors. Food safety authority should adopt a risk-based sampling plan for developing its annual pesticide monitoring plan. Application of risk-based sampling plan in this study revealed that for efficient monitoring of pesticide residues in vegetables. Risk-based surveillance systems are useful to support both strategic and operational decision making. Furthermore, the government of Bangladesh must encourage vegetable farms to adopt voluntary standards like HACCP and ISO 22000 by supporting with appropriate incentives. Monitoring studies are imperative to know the actual status of contamination due to toxic pesticide residues for future policies as well as to strengthen the confidence of consumers in quality of food. Therefore, it is suggested that such studies may be extended to

other vegetables grown indifferent agro-climatic regions of Bangladesh. Besides this, the application of good agricultural practices (GAP), integrated pest management system (IPM), integrated crop management system (ICM), use supply chain integration and traceability, as well as higher investments in research programs are crucial measures that must be set in priority.

Conflict of Interests

No potential conflict of interest relevant to this article was reported.

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