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COMMENTARY ARTICLE

PROBLEMS AND CHALLENGES TO DETERMINE PESTICIDE RESIDUES IN NEPAL

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Background

Nepal is predominately an agrarian country where still the majority of the total population rely on agriculture (Adhikari, 2018). The productivity of agriculture should be augmented to fulfill the increasing demand for food. Despite countless efforts, the agriculture sector could not come across to accomplish the global market for foodstuffs due to climate change. By which, plants will be attacked by various insects, funguses, and microorganisms. To tackle such problems, pesticides are being widely used to defend and block agriculture products from harmful organisms. But due to lack of public awareness, large numbers of farmers have been using pesticides as medicine - a conviction implanted in their mind (Pingali et al., 1995).

For the first time in 1952, the pesticides were instigated in Nepal, which was taken from the USA. Chemicals such as 3-(1-methyl pyrrolidine-2-yl), pyridine (nicotine sulfate) and Lindane (Gammexane) was cast-off for the malaria control program (Aryal, 2015). Furthermore, the use of Dichloro Diphenyl Trichloroethane (DDT) in Nepal was started from the 1950s for the control of vector-diseases such as malaria (Giri et al., 2006) and yellow fever (Dhital et al., 2015). The Government of Nepal had started to import pesticides for farming purposes from the mid-1950s (Gurung & Azad, 2013). Steadily, new kinds of insecticides corresponding to organochlorine (chemical group: endosulfan), organophosphate (dichlorvos, malathion, dimethoate, chlorpyrifos), carbamate (carbofuran), synthetic pyrethroids (cypermethrin) persuaded in Nepal from the 1950s to 1980s, respectively in ten-year gaps (Shrestha et al., 2010).

Main imported pesticides in Nepal enclosed 22.2% insecticides, 48.3% fungicides, 15.4% herbicides, 2.3% Rodenticides, and 10% bio-pesticides (Sharma, 2015). The use of pesticides in Nepal has been increasing rapidly to improve the crops yield (Adhikari, 2018). The use of pesticides rises by 10-20% per year in Nepal. In Terai, mid-hill, and mountain, the consumption of pesticides for vegetable cultivation is 25%, 9%, and 7%, respectively (Nepali et al., 2018). In early days, organochlorine was widely used in Nepal, but in contemporary days, organophosphate has been more pronounced (GC, 2015). Plant Quarantine and Pesticide Management Center, Nepal has registered 3035 trade (commercial) products of pesticides grouped under 170 common names; 60 insecticides, six acaricides, 42 fungicides, one bactericide, 30 herbicides, two rodenticides, one molluscicide, 14 bio-pesticides, 13 herbals and one nematicide (PQPMC, 2019b).

Most pesticides are broad-spectrum and linked with a wide range of severe illnesses and diseases from respirational disorders to malignant growth (Giri et al., 2006). Pesticides have shown harmful effects on the nervous system, reproductive system, and endocrine system. They are also considered as the cause of some cancer and many other diseases like congenital disabilities, Parkinson's disease, acute and chronic neurotoxicity (GC & Ghimire, 2018). It also adulterates land-dwelling ecosystem, poison human food, marine food resources, fisheries, and hydroponics (Carvalho, 2017). When pesticides are applied to crops, they may be exposed to the surface by various factors such as wind, rainfall, and sun rays. Pesticides will be absorbed by the root surface and transport to planting (Keikotlhaile & Spanoghe, 2011). Reliance on pesticides has been increased in developing countries like Nepal. Farmers exposed to pesticides have died because of unsafe handling (Devi et al., 2017). To overcome the problem, Nepal Government has imposed ban on 21 pesticides namely chlordane, DDT, dieldrin, endrin, aldrin, heptachlor, mirex toxafen, lindane, BHC, phosphamidon, organomercury chloride, methyl parathion, monocrotophos, endosulfan, phorate, carbofuran, carbaryl, dichlorvos, triazophos, and benomyl up to 2019 (PQPMC, 2019b). Unfortunately, banned pesticides are illegally traded due to open Nepal-India border, and they are still in use in Nepal. Because of that reason, it is now crucial to tag pesticides rather than using keywords, sayings, codes, and icons (WHO and FAO).

In Nepal, Central Agriculture Laboratory opened its central facility in the name of Rapid Bioassay of Pesticide Residues (RBPR) laboratory in Kalimati, Kathmandu in 2014. The laboratory is solely based on a bioassay kit developed by Taiwan Agriculture Research Institute (TARI). This kit is used to crisscross the residue of organophosphate and carbamate by RBPR, followed by UV spectrophotometer (Bhandari et al., 2019).

Farmers in Nepal use pesticides in a dangerous fashion to account harvests for high yield. Unwarranted use of insect repellent destroy crops yields and diminish the immunity power of the field (Palikhe, 2002). Farmers use pesticides throughout the pre-harvest period and submerged before marketing due to unlettered (Gyawali, 2018). The use of pesticides can be lessened by taking the plants and animals' extracts as an alternative. Use of such bio-pesticides kills or repels insects, thus protecting the crops from such pest. For example, Neem (*Azadirachta indica*) tree and Garlic (*Allium sativum*) produce oil that alters the hormones of bugs so that they cannot fly, breed or eat. Tite-pati (*Artemisia vulgarius*) and Timur (*Zanthoxylum armatum*) are used for grain storage (Giri et al., 2006).

To determine pesticides residues, Gas Chromatography-Mass Spectroscopy (GC/MS) is widely used to know parent compounds along with their metabolites. GC cannot straightly detect the sample, so extraction should be done (Nshimiyimana et al., 2014). In GC/MS technique, retention time and specific ions are recognized by Selected Ion Monitoring (SIM) to detect pesticide residues. Sufficient quantification is provided by SIM mode at low levels as required for the observing process. Higher selectivity and little finding limit can be enlarged by subsiding the matrix effects (Patel et al., 2005). GC-MS/MS with triple quadrupole ion can be used for the oily diet pesticide residues analysis. Numerous pesticide residues from the food matrix can be explored by using acquisition mode, Selected Reaction Mode (SRM) and Multiple Reaction Mode (MRM), respectively (Frenich et al., 2006).

High-Performance Liquid Chromatography (HPLC) is another technique used to determine pesticide residues. HPLC is used primarily in the production of curative, living, finding, parting of the constituents, medicinal segment for identifying Vitamin D levels in blood serum, and so on. In the same way, HPLC has enlarged its attractiveness for the transparency of pesticides. It is used for the investigation of individual pesticide residues in diverse food matrices (Panhwar & Sheikh, 2013).

As pesticide monitoring programs were criticized for high costs and complicated methods, scientists have developed Quick Easy Cheap Effective Rugged Safe (QuEChERS) method quantification of pesticide residues in fruits and vegetables (Anastassiades et al., 2003). Pesticide residues in these days can be resolute by Liquid Chromatography-Mass Spectroscopy (LC-MS/MS) in extracts of fruits, vegetables, etc. This technique is a more apposite and powerful technique as it shrinks analysis phase, charge, and displays a little chance of false-positive discoveries and its sensitiveness (Hiemstra & Kok, 2007).

Different mass analyzers are used in LC/MS, which includes single quadrupole, triple quadrupole, Time of Flight Mass Spectroscopy (TOF-MS), ion trap, and so on. Both GC/MS and LC/MS are used to examine pesticide residues, but LC/MS encompass over with much possibility and goals than GC/MS (Mol et al., 2008). Numerous residues of a varied diversity of matrices can be evaluated by LC-MS/MS with Electrospray Ionization (ESI) and Atmospheric Pressure Chemical Ionization (APCI) (Stachniuk & Fornal, 2016). In tandem MS, two stages of mass analysis are done; one to preselect the ion and second to analyze induced fragments (Banerjee et al., 2007).

Experimental challenges

For monitoring pesticide residues, there are limited tools and techniques used in Nepal. The country lacks accredited laboratories for pesticides testing. An analytical instrument such as gas chromatography, liquid chromatography, and mass spectrometry are necessary for chemical analysis of pesticides, which are not available on Central Agriculture Laboratory.

In addition to RBPR lab in Kalimati, six more laboratories are established as wings of Central Agriculture Laboratory in Jhapa (Birtamod), Sarlahi (Nawalpur), Kaski (Pokhara), Rupandehi (Butwal), Banke (Nepalgunj) and Kailali (Attaria). But none of them have an analytical instrument for pesticides testing. RBPR is a low-cost method of analysis to get quick test results for pesticide residues present in fruits and vegetables through the enzymatic reaction of the AChE which was isolated from the head of the housefly which catalyzes the hydrolysis of acetylthiocholine to form acetic acid and thiocholine. The hydrolyzed product reacted with 5, 5'-dithiobis-(-nitrobenzoic acid) (DTNB) produces a yellow color compound, 2-nitro-5-sulfanylbenzoic acid whose absorbance was measured at 412 nm (Ellman et al., 1961), (Chiu et al., 1991). Some metabolites present on plant materials may also inhibit this acetylthiocholine (Wang et al., 2016). This enzymatic assay could be influenced by numbers of factor such as the concentration of extract made from fruits and vegetables, pH of water, and reaction scale. Besides, UV spectrophotometer should be periodically calibrated.

Other various extraction methods such as electrochemical techniques, chemiluminescence, fluorescence methods, and biochemical assays have been established for the examination of pesticide residues (Sharma et al., 2010) but Nepal is not ready to adopt such techniques due to lack of modern laboratory facility.

Current situation

In between 2018 to 2019, RBPR laboratories examined organophosphate and carbamate groups in 4,782 samples by using AChE based kit and found that AChE inhibition of less than 35% in 4,646 items, between 35% - 45% in 103 items and 33 items showed more than 45% inhibition. Only 0.69% of the total samples analyzed was found to be necessarily disposed of (PQPMC, 2075a).

Samples with percentage inhibition below 35% are edible, inhibition below 35-45% needs quarantine for a few days, and washing and inhibition more than 45% are non-edible and need to be disposed of (Adhikari, 2018), (PQPMC, 2075a). If samples contain more pesticide residues, then it would inhibit AChE action resulting in a decrease in absorbance value and gives more inhibition percentage and vice versa.

Of 86 vegetable samples, pesticide residues were analyzed in 32 tomatoes, 27 eggplants, and 27 chilies by LC-MS/MS and GC-MS/MS. Among the tested samples, 93% of eggplant samples and all of chilies and tomatoes samples were found to contain pesticide residues. 56% of eggplants, 96% of chilies, and all of the tomato samples showed multiple residues. The residues of carbendazim, triazophos, chlorpyrifos in a few vegetable samples exceeded the Maximum Residue Levels (MRL) set by the European Union (EU). Chlorpyrifos residues in 25% of tomato samples and 4% of chilies samples were found to contain more than as indicated in Nepalese foodstuff MRL (Bhandari et al., 2019).

The survey in Rupandehi district of Nepal found cypermethrin as the most commonly used pesticides in vegetable farming followed by profenofos, mancozeb, chlorpyrifos, dimethoate, emamectin, alphamethrin, carbendazim, quinalphos, and so on. The average seasonal application of pesticides in vegetable growing was ranged from 0.27 to 7.78 kg a.i/ha. Farmers did not adopt safety measures while handling pesticides. Headache (73.8%), skin irritation (62.3%), eye irritation (32.8%), weakness (22.4%), and muscle pain (19.1%) were some of the common self-reported toxicity symptoms of pesticides by them (Bhandari et al., 2018). Similarly, a study carried out in Duhabi-Bhaluwa municipality of Sunsari district showed that dizziness, headache, skin irritation, nausea, paraesthesia, restlessness, eye pain, and vomiting were common health problems among farmers within 48 hours of pesticides application. Farmers were little aware of the negative impacts of pesticides on human health and environment than retailers (Lamichhane et al., 2019).

Various research carried in Nepal showed that farmers lack awareness on the correct use of pesticides that would otherwise impart negative impacts on health and the environment (Giri et al., 2014). Due to greed of farmers in using suitable pesticides, results in several problems such as resistance development of pests towards pesticides, toxic residues in food, water, soil and air disrupting ecosystems (Atreya et al., 2012). Other countries have developed many techniques for monitoring pesticide residues in food items, but Nepal is an infant in this regard. Nepal has only RBPR monitoring methods for detecting pesticide residues in vegetables and fruits, which only monitor organophosphate and carbamate groups. Laboratories report showed little pesticide residues as they only test carbamate and organophosphate groups of pesticides. Food items might contain other pesticides that have not been tested in our country. Nepal still lacks well-equipped laboratories with trained human resources.

Laboratories in Nepal still do not have standard samples of all registered pesticides. Farmers are unaware of the pre-harvesting waiting period and recommended a dose of the pesticides for different crops. Trans-boundary movement, illegal importation of banned pesticides, indiscriminate use, overdose, use of broad-spectrum of pesticides, lack of precautionary measures, wrong disposal, lack of implementation of rules are some common reasons behind the problems that country is facing today. Free border and lose security may be responsible for the availability of banned and illegal pesticides in Nepal.

The market monitoring programs should be done strictly in Nepal. Additionally, test centers with a well-equipped instrument and trained workforce should be accounted in all provinces, including border areas. For this, the government can hold hand in hand with donor agencies, funding agents, governmental and non-governmental organizations (R Palikhe, 2002). For inquiry, Pesticides Inspectors should be authorized. To control the use of banned pesticides, the detection of the samples should be done in dealers sample (FAO). Residues of tricyclazole, more than EU MRI, is found in Indian Basmati rice while examined in Europe. Thus, Nepal should also test fungicide residues on rice and vegetables.

Conclusion

Although Nepal has formulated rules and regulation on pesticides (Pesticide Act 1991 and Pesticide Rules 1993), its implementation is not practical. The course content should be expressed in such a way that they can create awareness among the society about the pesticides. Farmers should be aware of the use of harmless and eco-friendly bio-pesticides to control pests instead of chemical pesticides. The government should focus on integrated pest management and should establish laboratories with proper infrastructure and skilled human resources. The conduction of awareness programs about appropriate use, handling, and pre-harvest or waiting periods of pesticides could make a significant impact in overcoming the present situation. Regular seminars, talk shows, and documentaries should be organized throughout the country. Not only fruits and vegetables, but all food items should also be examined to know the level of pesticide residues. Research and development on the use of bio-pesticides instead of chemical pesticides are highly recommended.

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