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Article

Quantification of purity of some frequently used insecticides in vegetables insect pests

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Abstract: The study was carried out to quantify the purity of different marketed brands of three insecticide namely diazinon, acephate and cypermethrin using suitable protocols GC-FID and GC-ECD. Nineteen marketed brands of these insecticides collected from retailers of Jessore, Gazipur and Rangpur region were analyzed and estimated their purity in two seasons of 2006-2007 and 2007-2008. Among six marketed brands of diazinon, only one brand (RSN) had 96.71-100% purity in both the seasons in all locations which considered to be standard product, but four brands (DZN, SBN, HZN, DNN) in other regions, contained <95% pure which were substandard product. The remaining 2 brands (DNN, AZN) contained 33.71-51.94% purity in 2006-07 and the other two brands (SBN, DZN) had very small amount of active ingredient (0.16-0.84% purity) in 2007-08 and all these were impure in quality. All five tested brands of acephate had 57.14-88.59% purity in 2006-07 which were substandard but in 2007-08, three brands (SNT, BNS, ATF) showed >90% purity and the remaining 2 had <80% purity which were less AI than required. Of eight tested brands of cypermethrin, three brands were almost to have >95% pure in 2006-07 in all locations. The other two brands (CPR, AMT) contained >90% purity. In 2007-08, two brands of cypermethrin (RCD, SCR) contained >95% pure, another two brand (CRN, RLT) had 88.77-91.15% purity. The brands UTD was standard in purity in 2006-07 but this brand was below standard in quality in 2007-08 in all locations. Most of the tested brands of cypermethrin were found standard and sub standard level of purity in comparison to diazinon and acephate.

Keywords: quantification; purity; insecticides; diazinon; acephate; cypermethrin

1. Introduction

Pesticides are an integral component in agriculture and used for the better protection of field crops and stored grains losses caused by insect and diseases. Their use decrease the infestation of insects and extent of vector borne diseases which resulted at improving both quality and quantity of food. Pesticide usage for agriculture in developing counties is constantly increasing about 34-40% of the world total in 1975 (Alabaster, 1981). Pesticide consumption of Bangladesh has increased every year, to over 758 metric ton in 1960, 3028 metric ton in 1980, 19000 metric ton in 2000, 37712 metric ton in 2007 and 37781 metric ton in 2013 (Hasanuzzoha, 2004; Anonymous, 2007 & 2013). The rate of pesticide consumption in a period of 24 years shows an average of 9% annual increase (Ali, 2004). It was reported that the growth rate of pesticide consumption is likely to increase by the year 2020, especially in the developing countries (Yudelman *et al.,* 1998). Several survey conducted (Kabir *et al*., 1996; Anonymous, 2001 and Ahmed *et al*., 2005) at different region of Bangladesh, the farmers spray pesticide every day or in alternate day on vegetables. Insufficient knowledge and non-availability of sustainable alternatives to pesticide farmers of Bangladesh become dependent on pesticide for crop production. Unjustified and excessive use of pesticide may cause severe harmful effect on human health, environmental pollution and destruction of agricultural ecosystem and emergence of resistance in insect pest, pathogens and

weeds (Handa and Walia, 1996). It was noted in the country report produced by FAO (2011) the regulatory scheme for pesticide registration is systematic. But in practice, there are gaps between policies and implementation. Lack of trained manpower and lab facilities does not allow proper monitoring. In most of the cases specification and prescription of marketed pesticides may differ from those registered (Aziz, 2006). So, concern on the purity in respect of active ingredient of the marketed brands of pesticides is therefore likely key factor for repeated use of pesticides in vegetables. It is reported that less amount of active ingredient in the formulated pesticides, they do not work against insect pests and the farmers use more pesticide for better result (Kabir *et al.,* 2008). It was assumed that impurity or adulteration of pesticide might be one of the major causes of extensive use of pesticide in Bangladesh. In this perspective, it has become important to analyze the marketed brands of insecticide for their purity quantification and to ensure the actual amount of active ingredient that required.

2. Materials and Methods

The research works were carried out on testing purity of three commonly used insecticides in Pesticide Analytical Laboratory, Division of Entomology, Bangladesh Agricultural Research Institute (BARI), Gazipur. The samples were collected from markets of Jessore, Rangpur and Gazipur districts during 2006-07 and 2007-08 seasons.

2.1. Materials used in insecticide analysis

Tested insecticides

There were three insecticides like diazinon, acephate and cypermethrin, the first two insecticides belonging to organophosphorus class and the last one of pyrethroid class, all being EC formulation except acephate of soluble powder (SP), showing below their baseline information.

Diazinon 60EC

Common name: Diazinon (Anonymous, 2000)
Chemical abstract name: *O,O*-diethyl *O*-[6-methyl-2-(1-methylethyl)-4-pyrimidinyl] phosphorothioate)
Molecular formula: $C_{12}H_{21}N_2O_3PS$, Mol. wt.304.3
Available tested brands: AZN, RSN, HZN, DZN, DNN, SBN.
Mode of action: Non-systemic insecticide and acaricide with contact, stomach and respiratory action.

Acephate 75SP

Common name: Acephate (Anonymous, 2000)
Chemical abstract name: *N*-[methoxy (methylthion) phosphinoyl]acetamide)
Molecular formula: $C_4H_{10}NO_3PS$, Mol. wt. 183.2
Available tested brands: ATF, TDT, SNT, BNS, LNR.
Mode of action: Systemic insecticide. Of moderate persistence, with residual activity lasting 10-21 days.

Cypermethrin 10EC

Common name: Cypermethrin (Anonymous, 2000)
Chemical abstract name: cyano(3-phenoxyphenyl) methyl 3-(2,2-dichloroethenyl)-2,2 dimethyl=cyclopropanecarboxylate)
Molecular formula: $C_{22}H_{19}Cl_2NO_3$, Mol. wt. 416.3
Available tested brands: CMB, RCD, CMR, UTD, RLT, CRN, SCR, AMT, CPR.
Mode of action: Non-systemic insecticide with contact and stomach action. Also exhibits anti feeding action. Good residual activity on treated plants.

Chemicals: Methanol, Acetone, n-hexane, Acetonitrile and Insecticide standard.
Glass wares: Conical flask, Beaker, Pipette, Syringe and Vials with septum.
Others: Scissors, Spatula, Knife, Chopping board, PTFE filter, Forceps, Zipper bag, Zip Stick, Teflon stopcock, Aluminium foil, Para film, etc.

2.2. Analytical device used in insecticide analysis

Gas Chromatograph-2010, Auto injector AOC 20i, Ultrasonic bath, Refrigerator (-20^0C), Ultra pure water distillation with deionizer and reservoir, Vortex mixture, Solvent dispenser, Micro pipette, Digital balance and Computer.

2.3. Testing purity of marketed insecticides

The tested brands of three different insecticides were selected on the basis of frequency of insecticide use and demand among the farmers from survey and research reports of Jessore, Rangpur and Gazipur regions of Bangladesh (Kabir *et al*., 1996; Anonymous, 2001; Ahmed *et al*., 2005). Each formulated product either of powder or liquid was being dissolved in the respective solvent. The solvents were selected on the basis of the criteria described by Lehotay and Mastovska (2004). The brands of diazinon and cypermethrin were varied in two years but the brands of acephate were same in both the years with at least five brands in each insecticide were tested. There were nineteen brands of three insecticides showing individual batch number and expiry date but not mentioning manufacture date in all brands on the label. The purity tests were done before the expiry date of each brand of the insecticides. The solutions of different brands of marketed insecticides were prepared following the procedure compatible with the respective equipment. In case of color less liquid or powder insecticide, the known concentrations of the solutions were prepared directly. Thus known and similar concentrated solutions of each of the standard and formulated insecticides were prepared. Methods for testing of different brands with GC-FID and GC-ECD were developed by setting the instrument parameters suitable for analyzing concerned insecticide selected on the basis of peak sharpness of the chromatogram and retention time for respective compound. The carrier and makeup gas used in the instrument for analysis was helium during 2006-2007 and nitrogen was used in 2007-2008 depending upon the availability of gases. The instrument parameters of Gas Chromatography set for analysis of each group of insecticide are listed in Tables 1 and 2.

Table 1. The instrument parameters of GC-2010 set for analysis of different groups of insecticide during 2006-2007.

Pesticide group	Detector	Solvent	Temperature	Carrier gas	Make up gas	Injector	Inj. vol.
Diazinon	FID	Hexane	Column-170°C Injection port-200°C Detector-240°C	Helium	Helium	Auto	1 µl
Acephate	FID	Hexane	Column-180°C Injection port-200°C Detector-250°C	Helium	Helium	Auto	1 µl
Cypermethrin	ECD	Hexane	Column-160°C(1min)-190°C (10°C /min)- 240°C (2°C /min) Injection port-250°C Detector-280°C	Helium	Helium	Auto	1 µl

Table 2. The instrument parameters of GC-2010 set for analysis of different groups of insecticide during 2007-2008.

Insecticide group	Detector	Solvent	Temperature	Carrier gas	Make up gas	Injector	Inj. vol.
Diazinon	FID	Hexane	Column-170°C Injection port-200°C Detector-240°C	Nitrogen	Nitrogen	Auto	1 µl
Acephate	FID	Hexane	Column-140°C Injection port-180°C Detector-220°C	Nitrogen	Nitrogen	Auto	1 µl
Cypermethrin	ECD	Hexane	Column-160°C(1min)-190(10°C /min) 270°C (2°C/min) Injection port-280°C Detector-300°C	Nitrogen	Nitrogen	Auto	1 µl

FID: Flame Ionization Detector; ECD: Electron Capture Detector.

After injection, data were acquired and processed by the instrument of GC-2010. Each peak of the chromatogram for formulated products was characterized by the retention time of the concerned standard solution. The similar retention time of the obtained peak of standard solution and the tested brands solution assured the presence of AI (active ingredient) in the tested brands. The actual amount of AI present in different

marketed brands and the purity percentage was determined by comparing it with the amount of AI actually required in the concerned insecticide using the following formula.

$$\text{Purity (\%)} = \frac{\text{Actual amount of AI present in the insecticide}}{\text{Amount of AI recommended/required}} \times 100$$

3. Results

The results of this investigation were the purity test of three prevalently used insecticides from local markets of three regions viz., Jessore, Rangpur and Gazipur under different brands. The results were obtained on the chromatograms in tabular form based on the quantification of active ingredient (AI) of the insecticides. Only one chromatogram of standard solution of tested insecticides and one chromatogram of marketed brand of each insecticide are shown in Figure 1 to Figure 6. In this way the results of other marketed brands were also made by in-built GC-2010 software. The lowest detection limit of diazinon and acephate was 0.01 $mgkg^{-1}$ and 0.02 $mgkg^{-1}$ in GC-FID and cypermethrin, it was 0.02 $mgkg^{-1}$ in GC-ECD.

3.1. Diazinon

Five different brands of diazinon from each location as Jessore, Rangpur and Gazipur region were tested with GC-FID to estimate their purity during 2006-07 and 2007-08 seasons. The standard of diazinon and the marketed brands showed similar retention time in Figure 1 and 2. The purity percentages of different marketed brands of diazinon are presented in the Tables 3 and 4.

Table 3. The percentages of active ingredient and purity of marketed brands of diazinon 60EC collected from different locations during 2006-07.

Diazinon brands (Code no.)	Amount of AI present (%) at different locations			Purity (%) at different locations		
	Gazipur	Jessore	Rangpur	Gazipur	Jessore	Rangpur
RSN	58.141	58.052	58.062	96.862	96.714	96.731
DZN	41.577	41.754	41.507	69.267	69.562	69.150
SBN	36.072	36.189	35.858	60.095	60.290	59.739
AZN	30.757	31.011	31.179	51.056	51.664	51.944
DNN	20.235	20.458	20.404	33.711	34.083	33.993

Table 4. The percentages of active ingredient and purity of marketed brands of diazinon 60EC collected from different locations during 2007-08.

Diazinon brands (Code no.)	Amount of AI present (%) at different locations			Purity (%) at different locations		
	Gazipur	Jessore	Rangpur	Gazipur	Jessore	Rangpur
RSN	60.00	59.301	59.779	100.00	98.795	99.591
HZN	56.921	57.601	56.360	94.830	95.963	93.895
DNN	54.247	54.522	54.571	90.375	90.833	90.915
DZN	0.502	0.475	0.468	0.836	0.791	0.779
SBN	0.095	0.100	0.098	0.158	0.166	0.163

Among 5 tasted brands of diazinon only RSN had acceptable level of purity as found >96% in all the locations in 2006-07 (Table 3). In the remaining brands the level of purity was 69.150-69.562% for DZN, 59.739-60.290% for SBN, 51.056-51.944% for AZN and 33.711-34.083% for DNN. All these four brands were considered impure and substandard. During 2007-08, the purity level of first three brands of diazinon was higher (Table 4) than in 2006-07. Purity of two brands (RSN and HZN) ranged 93.895-100%. This could be considered as acceptable level. The brand DNN showed 90.375-90.915% purity which was substandard. The brands DZN and SBN had almost no active material counting only 0.095-0.502% AI. Their purity ranged from 0.158 to 0.836%. These results are unacceptable in contest of required AI presence.

3.2. Acephate

Five marketed brands of acephate from each location viz., Gazipur, Rangpur and Jessore region were tested with GC-FID to estimate their purity during 2006-07 and 2007-08 seasons. The retention time (RT) for the standard

of acephate was 2.09 min (Figure 3). The formulated brands also showed similar RT proving the presence of acephate (Figure 4). The purity percentages of the formulated brands of acephate are presented in the Tables 5 and 6.

Table 5. The percentages of active ingredient and purity of marketed brands of acephate 75SP collected from different locations during 2006-07.

Acephate brands (Code no.)	Amount of AI present (%) at different locations			Purity (%) at different locations		
	Gazipur	Jessore	Rangpur	Gazipur	Jessore	Rangpur
ATF	66.449	66.409	66.461	88.598	88.523	88.592
BNS	46.564	42.935	45.263	62.069	57.232	60.335
LNR	43.329	45.061	42.866	57.772	60.066	57.140
SNT	51.616	49.628	49.966	68.804	66.154	66.604
TDT	46.564	45.983	46.928	62.069	61.295	62.555

Table 6. The percentages of active ingredient and purity of marketed brands of acephate 75SP collected from different locations during 2007-08.

Acephate brands (Code no.)	Amount of AI present (%) at different locations			Purity (%) at different locations		
	Gazipur	Jessore	Rangpur	Gazipur	Jessore	Rangpur
ATF	74.996	73.272	72.221	100.00	97.671	96.270
BNS	71.276	70.041	69.602	95.010	93.364	92.779
LNR	48.419	46.849	48.094	64.542	62.449	64.109
SNT	67.913	68.545	67.913	90.428	91.370	90.528
TDT	55.142	56.196	53.495	73.504	74.909	71.308

In 2006-07, five marketed brands of acephate were tested of which only one brand (ATF) showed >80% purity (Table 5). The purity of remaining four brands (BNS, LNR, SNT and TDT) showed <70% purity in all locations. These were poor in purity and unacceptable. During 2007-08, the purity level of 5 tested brands of acephate was higher than in 2006-07. The purity of three brands (ATF, BNS and SNT) ranged from 90.428-100% (Table 6). Among the three brands, BNS of Gazipur had 95.010% purity and ATF had also ≥ 96% purity in all locations which was acceptable. The brand BNS of two other locations had 92.779-93.364% purity while the brand of SNT had the purity ranged from 90.428-91.370%. This level of purity could be considered as substandard. The brand TDT had lower purity ranging 71.308-74.909% and LNR had purity below the purity of TDT. These two brands were contained little amount of AI that required which were also below standard.

3.3. Cypermethrin

Cypermethrin brand were not available in common in the period of 2006-07 and 2007-08. Only RCD and UTD were analyzed in both the years. The three remaining brands in each year were different. The RT for the standard and formulated cypermethrin was the same as 12.58 min (Figure 5 and Figure 6). The purity percentages of the formulated brands of cypermethrin are presented in the Tables 7 and 8.

Table 7. The percentages of active ingredient and purity of marketed brands of cypermethrin 10EC collected from different locations during 2006-07.

Cypermethrin brands (Code no.)	Amount of AI present (%) at different locations			Purity (%) at different locations		
	Gazipur	Jessore	Rangpur	Gazipur	Jessore	Rangpur
RCD	9.800	9.887	9.654	98.00	98.870	96.540
CMB	9.764	9.877	9.672	97.640	98.770	96.720
UTD	9.438	9.733	9.783	94.380	97.330	97.830
CPR	9.304	9.399	9.430	93.040	93.990	94.300
AMT	9.157	9.166	9.536	91.570	91.660	95.360

Table 8. The percentages of active ingredient and purity of marketed brands of cypermethrin 10EC collected from different locations during 2007-08.

Cypermethrin brands (Code no.)	Amount of AI present (%) at different locations			Purity (%) at different locations		
	Gazipur	Jessore	Rangpur	Gazipur	Jessore	Rangpur
RCD	10.00	9.976	10.00	100.00	99.760	100.00
SCR	9.780	9.709	9.666	97.800	97.090	96.660
CRN	9.026	9.115	9.072	90.260	91.150	90.720
RLT	8.877	9.004	9.039	88.770	90.040	90.390
UTD	7.734	7.839	7.853	77.340	78.390	78.530

Two brands (RCD, CMB) showed is greater than 96% purity in all locations in 2006-07 and the same brand RCD and a new brand SCR also showed similar purity in 2007-08 (Tables 7 and 8). The brand RCD was reaching 100% purity in the second year in all locations. UTD also showed over ≥97% purity except one in Gazipur in the first year but this brand was having less AI and 77.34-78.53% purity in the second year which were sub-standard. Although the brand AMT of Rangpur was found to be 95.36% pure but the similar brand of two other locations had less purity. The brand CPR showed <95% purity. The brands CRN and RLT had the purity ≥ 90% excepting RLT which was collected form Gazipur having 88.77% purity in 2007-08. These two brands were also considered as substandard and impure in quality.

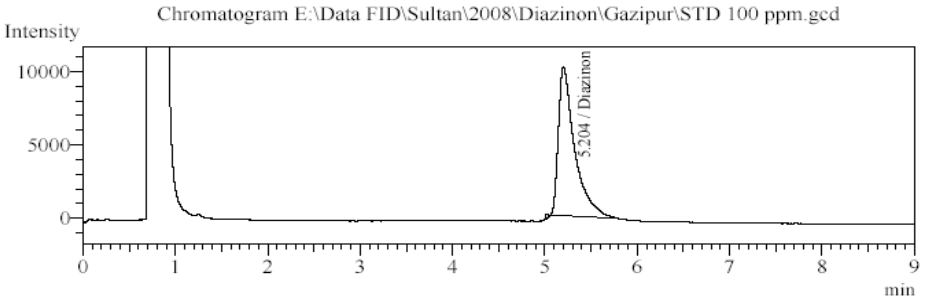

Figure 1. Chromatogram of diazinon standard solution.

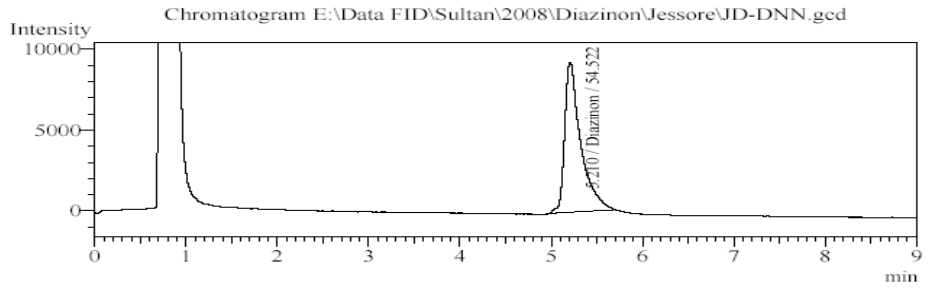

Figure 2. Chromatogram of diazinon obtained from the marketed brand of JD-DNN.

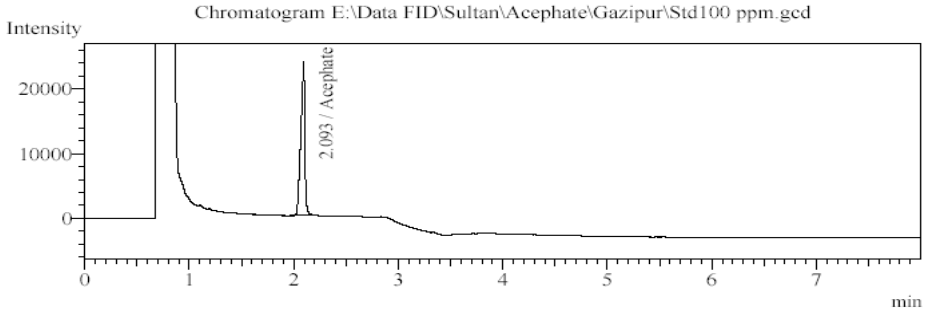

Figure 3. Chromatogram of acephate standard solution.

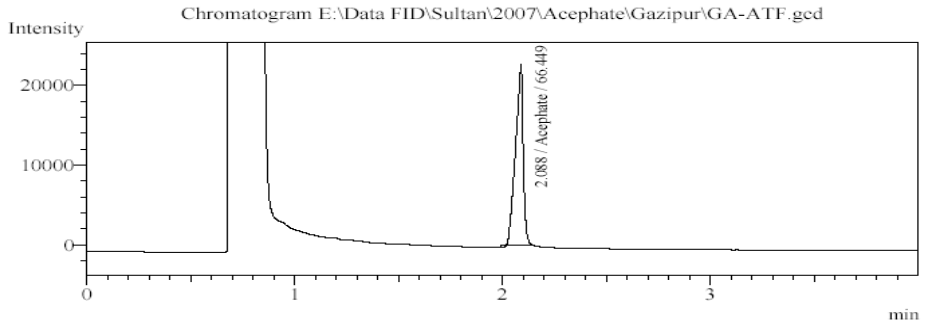

Figure 4. Chromatogram of acephate obtained from the marketed brand of GA-ATF.

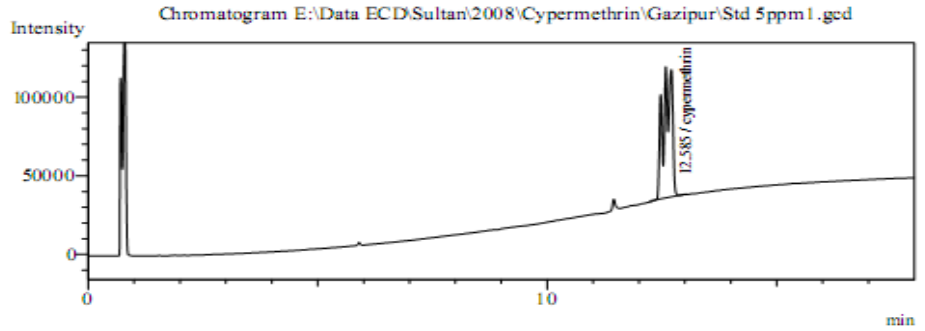

Figure 5. Chromatogram of cypermethrin standard solution.

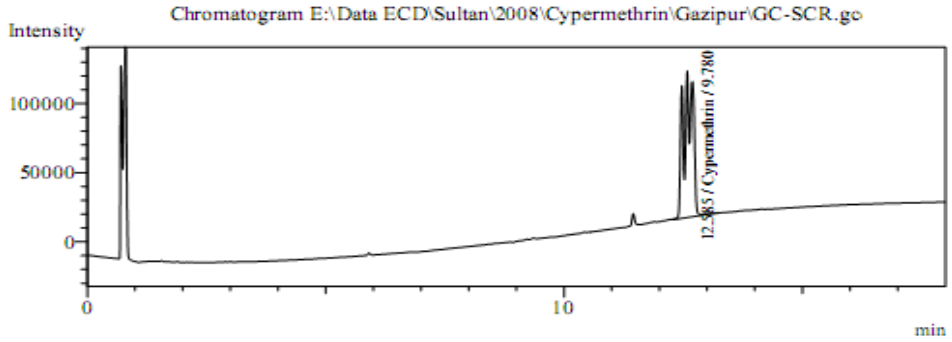

Figure 6. Chromatogram of cypermethrin obtained from the marketed brand of GC-SCR.

4. Discussion

The purity of different brands of tested insecticides were classified in to three categories viz., standard or acceptable level (>95%), substandard (<95-80%) and lower level or little amount (<80%) of active ingredient (AI) present in the formulated product. During 2006-07 among 5 tested brands of diazinon only RSN had acceptable level of purity (>96%) whereas the remaining four brands (DZN, SBN, AZN and DNN) had 33.71-69.56% purity and were not acceptable. In 2007-08 the purity level of various brand of diazinon was found to be better than 2006-07. Only RSN was found ≥ 98% purity in all locations and remained acceptable level of purity. The brands HZN and DNN showed <95% purity which were as substandard. The brands DZN and SBN were very poor in purity ranging from 0.15 to 0.83% and these two products are absolutely unacceptable. The purity of acephate was at a level of almost 100% in its ATF brand particularly in 2007-08. But this brand was substandard in 2006-07. The purity of other four brands BNS, LNR, SNT and TDT were lost in all locations in both the seasons. It is possible that the wettable powder formulation of this insecticide might show more impurity for the unknown reason. However, there was possibility of making impurity with this insecticide by easily mixing other materials. In cypermethrin the brand RCD was more pure with almost required (100%) active ingredient in all locations in both the seasons. The brands UTD and CMB were standard in purity in 2006-07 but the former was below standard in quality in 2007-08. The brands CPR, AMT, CRN and RLT recorded below standard. Cypermethrin was emulsifiable concentration in formulation with more brands

showing standard and substandard level of purity as compared to other brands of insecticides of wettable powder formulation such as in acephate insecticide. Cypermethrin was more pure in quality in comparison to diazinon with emulsifiable concentration (EC) in formulation. Ahmed *et al.*, (2016) found purity ranged from 22-100% in malathion, 74.43-100% in fenitrothion and 59.32-100% in quinalphos with EC formulation which were collected from Gazipur, Rangpur and Jessore region. Kabir *et al.*, (2008) reported that three of four tested brands of cypermethrin 10EC were 100% pure and the one brand was found to have below standard with 65% purity and among five tested brands of diazinon 60EC, 3 had more than 80% purity and the rest two were <70% pure. Begum *et al.*, (2016) found that six of eight tested brands of diazinon 60EC were 100% pure and one contained 65% active ingredient and the remaining one had no AI. They also found only one of eleven tested brands of cypermethrin 10EC was pure, two had >90% purity and the remaining others had below 90% purity except one brand which contained no active ingredient. The results of the present works were similar with the works of the mentioned authors in Bangladesh. So, adulteration, transportation and inadequate storage facilities (presence of light, temperature, humidity, etc.) might be the probable cause of different degrees of purity of marketed insecticides.

5. Conclusions

The results of three different groups of insecticides with nineteen marketed brands found variations in purity. Five of eight tested brands of cypermethrin, two of five tested brands of acephate and one of six tested brands of diazinon were showed standard level (>90%) of purity. Most of the brands of diazinon and acephate were found at substandard level (< 90%) of purity. Some brands of diazinon had lower level of purity which contained less than 50% purity. These levels are below standard and quite unacceptable.

Conflict of interest

None to declare.

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