ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

RESEARCH ARTICLE

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

Determination of Pesticide Residues in Pulses Seed and Evaluation of their Phytotoxicity in Term of Germination and Early Seedling Growth by Hydroponic Culture

Satyajeet Rai, PurushottamTrivedi, Abhishek K Singh, Ritul kamal, Mohd. Haris Siddiqui! Sarika Malik and L P Srivastava*

Pesticide Toxicology Laboratory, CSIR-Indian Institute of Toxicology Research, Lucknow

! Department of Bioengineering Integral University, Lucknow

Manuscript Info *Abstract*

Manuscript History:

Received: 15 September 2014
Final Accepted: 22 October 2014
Published Online: November 2014

Key words:

Pesticide Residues, Pulses seed, Germination and Early Seedling Growth

Corresponding Author

L P Srivastava

Residue of 37 pesticides was analyzed in pulse seeds (chick pea brown, green gram, black gram and soya bean) and effect of pesticide residues on germination and early seedling growth was evaluated to know the residual phytotoxicity. Out of 37 analyzed pesticides only five pesticides (Σ-HCH, malathion, chlorpyriphos, ethion, Σ-cypermethrin) were detected in pulse seeds, in which chlorpyriphos and cypermethrin were frequently encountered in 39 % of total analyze (120) samples. Mean lowest residual level of chlorpyriphos and cypermethrin found in analysis was taken as lower treatment concentration (0.3 mg L^{-1}), middle (3.0 mg L^{-1}) and higher (30.0 mg L^{-1}) with three exposure regimes continuous exposure during germination, short-term initial exposure of 4h and exposure after pre-germination. Phytotoxicity of pesticides treatments were evaluated by % shoot, root elongation and germination index. Finding of the study suggested that increase in concentration and exposure regimes reduced the % shoot, root elongation and germination index. Higher concentration and exposure regimes increase the residual load of pesticides which may be attributed to this effect. Continuous exposure of chlorpyriphos and cypermethrin at middle (3.0 mg L^{-1}) and higher (30.0 mg L^{-1}) concentration of treatment adversely affect the germination and early seedling growth of pulse seeds. Treatment of pre-germinated pulse seeds only show adverse effect on higher concentration treatment. Variation of reduction in germination and early seedling growth was reported because of pesticides uptake and sensitivity to pesticides treatment differs among the pulse seeds. Soyabean was found to be most sensitive to treatment of these pesticides. In green gram residual uptake was nearly same as in soyabean and greater than black gram, chick pea brown but reduction in germination and early seedling growth reasonably less to other pulse seeds due their less sensitivity to these treatments. Lower treatment concentration of pesticides with all exposure regimes does not affect the germination and early seedling growth.

Introduction

Agriculture forms the backbone of third world economics. The increase of population and demand results in the need for great efficiency in agriculture production and storage of agricultural products. The use of synthetic pesticides as crop protection chemical has become the most accepted weapon to assured crop production and storage (Groot, 2004; Parweenet al.2012). Seed treatments are one of the most important tools used for many crops to control a variety of pests during

storage and to ensure uniform stand establishment by protecting against soil born pathogen and insect to ensure the productivity. Various pesticides including bactericides fungicides, and insecticides were used in seed treatment (Paulsrud et al. 2001).

Seed treatment delivers insecticides and fungicides to the root zone of the growing plant, allowing chemical inputs into the environment to be reduced (Stevens et al. 2002). Although seed treatments have important benefit, they also pose certain risks if application rate and dose of these chemical are not carefully controlled like, reduction in the self life of seed, residual problem of pesticides and phytotoxicity to plant i.e lower germination rate or stunting and reduction in length of sprout, hence affecting the choice of planting depth. (Shaheed and Ahmed 2006; Martin and Ronco 2006; Stevens et al 2008; Akoto and et al 2013). The risk of phytotoxicity response to seed treatment is affected not just by the active chemical being applied but also by factors such as, exposure period, conditions and crop species.

In most of the agro based economies, injudicious application of pesticides irrespective of concentration and the crop phenology, results in lower yields and incurring of losses to those associated with agriculture sector. Hence it is important to determine the present status of pesticide residues in various seeds available at seed store of Lucknow, Uttar Pradesh, India. In view of the above consideration four varieties of pulse seeds Chick pea brown (*Cicerarietinum*L.), Green gram (*Vignaradiata*), Black gram (*vigna green gramo L*) Soyabean (*Glycine max* L) were selected for determination 37 pesticide residues (12 organochlorine, 10 organophosphate, 11 Synthetic pyerthroid, 4 Herbicide). Selected pulses are most commonly cultivated in India and made important contribution to agricultural economy (Economic survey of India, 2012). These pulses are an important source of protein having high protein content (20-22%), richer in fibers, minerals (phosphorus, calcium, magnesium, iron and zinc) and β-carotene. These Pulses are infested by variety of insects and pests during storage and early seedling growth like *seed beetle, pod-feeding Lepidoptera, pod-sucking Hemiptera, Helicoverpa spp. and seed-feeding Diptera, pod-borer and Hymenoptera, Aphids, Semilooper, Termites, bruchid,* and reduced the productivity (Dhaliwal et al. 2010, Nadeem et al. 2010). Seed treatment is a cause of exposure of pesticides during germination and early seedling growth which are often most sensitive stage of plant development. The objectives of present study were to the determination of pesticides residues in pulses. Most frequent detected pesticides were selected to evaluate whether differences in concentration and exposure regimes caused any phytotoxicity in term of germination and early seedling growth by Hydroponic Culture.

2 Materials and Methods

2.1 Chemicals

All solvents like n-hexane, acetone and ethyl acetate (HPLC grade) were purchased from Sisco Research Laboratories Pvt. Ltd. India. Sodium chloride (NaCl) and anhydrous magnesium sulphate ($MgSO_4$) procured from Sigma aldrich Pvt. Ltd. India. Primary secondary amine (PSA) bondasil 40 μm part 12213024 of Varian was used for sample preparation.

2.2 Pesticide Standards:37 Pesticides Standards including 12 Organochlorine (α- HCH, β-HCH, Lindane, δ-HCH, Alpha Endosulfan, Beta Endosulfan, p,p′ -DDT, p,p′ DDD, p,p′ DDE, o,p′ DDE, o,p′ DDT, Heptachlor) 10 Organophosphate (Dichlorvos, 4-Bromo,2-Chlorophenol, Phosphomidan, Dimethoate, Chlorpyriphos, Malathion, Profenophos, Ethion, Edifenphos, Triazophos), 11 Synthetic pyerthroids (Bifentrin, Lamda-Cyhalothrin, fenpropathrin, β-cyfluthrin-I, β-cyfluthrin-II, Cypermethrin-I,Cypermethrin-II, Fenvalerate-I, Fenvalarate-II, Taufluvalenate, Delta-methrin) and 4 Herbicides (Atrazine, Alachlor, Butachlor, Pendimethylene) were procured from Supelco Sigma Aldrich USA, Fluka Sigma-Aldrich Schweis and Rankem Pvt. Ltd. New Delhi, India. Their purity ranged from 97-99 %.

2.3 Sample collection: A total of 120 samples of four verities of pulse seeds Chick pea brown (*Cicerarietinum*L.), Green gram (*Vignaradiata*), Black gram (*vigna green gramo L*), Soyabean (*Glycine max* L) were collected from three different locations of Lucknow, Uttar Pradesh, India were analyzed. Samples were brought to the laboratory, stored in dry place (at 27˚C±2) and analyzed with in 24 h from the time of their collection for the presence of pesticide residues.

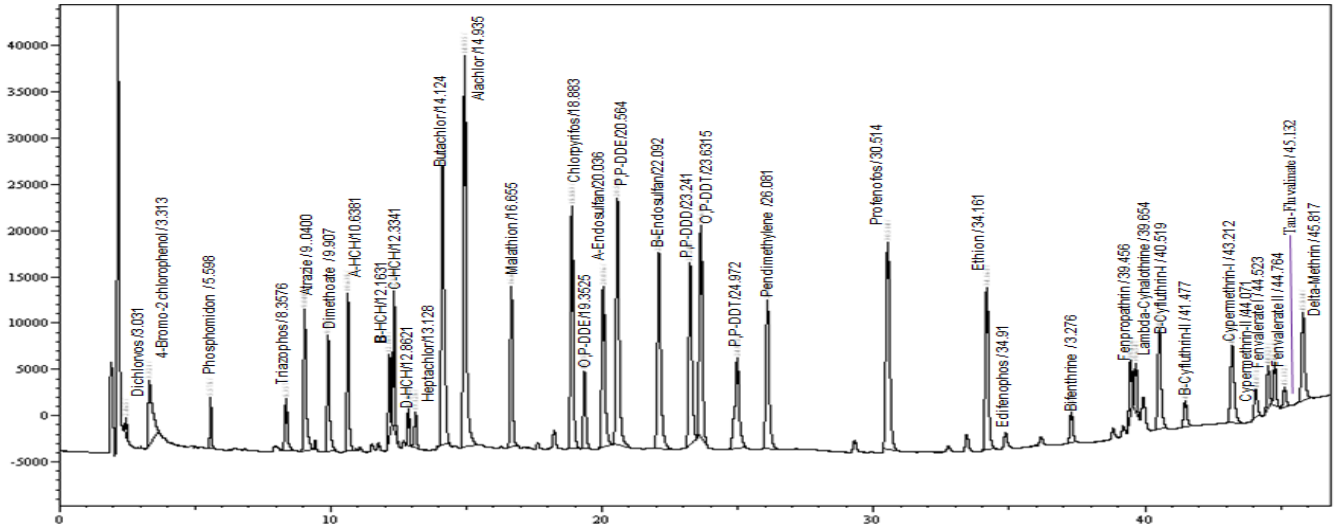
2.4 QuEChERS Sample Preparations

The pulse seeds samples (100 g) of each variety were grinded in warring blander. 10 g powdered sample of each verities of pulse seeds in triplicate was extracted and cleaned by **Qu**ick, **E**asy, **Ch**eap, **E**ffective, **R**ugged and **S**afe, (QuEChERS) method (Lehotay 2007; Aysal et al. 2007). Powdered samples (10 g) was taken in 50 ml centrifuge test tube and mixed with 10 ml of double distilled water and allowed to macerate for 10 min then 10 ml of ethyl acetate, 4 g of activated anhydrous $MgSO_4$, 1.0 g activated NaCl were added and shaken for 10 min. at 50 rpm on rotospin. The extract was centrifuged for 10 min at 8,000 rpm. 1 ml aliquot of extract was cleaned with the mixture of 100 mg PSA, 150 mg anhydrous $MgSO_4$ and 10 mg activated charcoal. The extract was again shaken for 10 min. at 50 rpm on rotospin and centrifuged for 10 min at 8,000 rpm. The supernatant was collected in 2 ml GC vial and mixed with 5μl acidified ethyl acetate. 1μl clean extract was injected in gas chromatography equipped with Electron capture detector (ECD) for the analysis of pesticide residues and further confirmed by GC-MS

2.5 GC Analytical conditions

Residues were analyzed on Shimadzu GC-2010 equipped with fused silica capillary column, DB-1 (30 mt. × 0.25mm. id) coated with 100% di-methylpolysiloxane (0.25 µm film thickness) using electron capture detector (ECD). General operating conditions were as follows; Injector port temperature: 280^0C; detector temperature 300; using carrier gas Nitrogen (N_2); Total flow 7.7ml/min, Column 0.79 ml/min, purge flow 3.0 ml/min, makeup flow 30 ml/min column temperature program: initially 165^0C for 1.50 min, increase at 2.70^0C/min to 210^0C hold for 2.70 min, then increase to 265 at 2.70^0C/min and hold for 1.90 min; injection volume: 1µl split ratio 1:5. The total run time was 46.83 min and Shimadzu, GC Solution software was used for instrument control and data analysis. Quantification of the pesticides was done by peak area using the external standard method and further confirmed by GC-MS.

Fig-1 Standardized GC-ECD Chromatogram of OCs, OP, SPs, HERB



3. Germination of Seeds and Hydroponic Culture

3.1 Seeds

Pesticides free seed of Chick pea brown (*Cicerarietinum*L.), Green gram (*Vignaradiata*), Black gram (*vigna green gramo L*), Soyabean (*Glycine max* L) were kept in a dry place in the dark under room temperature. Prior to use, seeds of nearly equal size were selected and disinfected by soaking in a solution of 1% sodium hypochlorite for 20 min.

3.2 Treatment of Pulse seeds with Pesticides

On the basis of pesticides residues frequently encountered in study. Mean lowest Residual concentration of the pesticides found in analysis was selected as lower treatment concentration, middle treatment concentration was (10 X lower treatment concentration) and higher (100 X lower treatment concentration). Pesticides standard of technical grade with 99 % purity were used for treatment. Stock solution of 4000 mg L^{-1} were made in di-methyl polysiloxane DMSO and diluted with double distilled water up to lower, middle and higher treatment concentration.

3.2.1 Treatment Experiment-1

In this experiment effect of continuous exposure of pesticides during seed germination was evaluated. Samples of each pulse seeds (100 seed) were placed in glass beakers and 50 ml solution of each treatment concentration of pesticides was added and shaken several and maintained at room temperature (23-25 °C) for 10 h . After 10 h remaining unabsorbed solution was removed. Seeds were transferred to glass petri dishes containing a Whatman No.1filter paper, and maintained at (23-25°C) in darkness for 4 day prior to assessment.

3.2.2 Treatment Experiment-2

In this experiment effect of short term initial 4h exposure of pesticides was evaluated using the above same basic protocol for germination. Seeds were transferred to 95 mm diameter perspex tubes with wire gauze bottoms after the completion of exposure duration. The tubes were mounted under a hose outlet and continuously flushed with tap water for 15 min for removing the pesticides.

3.2.3 Treatment Experiment-3

This experiment involved the evaluation of effect of pesticides treatment on pre-germinated pulse seeds. After 24h of pre-germination process, seeds were treated with 50 ml of pesticides solution and transfer to glass petri dishes containing a Whatman No.1filter paper, and maintained at (23-25°C) in darkness for the completion of germination process. Each experiment was carried out in triplicate. Germination was considered to be impaired if radicle was ≤ 2mm in length. In order to maintain an adequate hydration level, seeds were sprayed with distilled water at 24h interval.

3.4 Hydroponic Culture

After the completion of germination by each treatment experiment, ten seedlings of equal length were transferred to polyethylene pots containing 100mL Hoagland solution (Dhoke et al. 2013). The pH of the nutrient solution was adjusted to 6.8. The pots were kept in diffuse sunlight mediated room with 10 h light/14 h dark cycle at 28-30°C and 58-60 % humidity. The Hoagland solution was replaced every two day interval with maintained p^H. The experiment was carried out for 12 days.

3.5 Phytotoxicity Evaluation of Pesticides Treatment

Phytotoxicity evaluation of pesticides treatment was done by studying the germination index, % shoot and root elongation and was calculated according to (Selim et al. 2012) as follows:

$$\% \text{ Shoot Elongation} = \frac{\text{Mean shoot length in treated pulse seed}}{\text{Mean shoot length in control pulse seed}} \text{ X } 100$$

$$\% \text{ Root Elongation} = \frac{\text{Mean shoot length in treated pulse seed}}{\text{Mean shoot length in control pulse seed}} \text{ X } 100$$

$$\text{Germination Index (GI)} = \frac{\% \text{ Seed Germination X \% Root Elongation}}{100}$$

4 RESULTS AND DISCUSSIONS

4.1Pesticides residues in Pulse seeds

The QuEChERS sample preparation applied in the present study fulfils the established criteria for confident identification of pesticide residues at low level in matrix like pulse seeds. The overall recovery ranged from 72.60 to 95.01 %. The Limit of detection (LOD) and Limit of quantification (LOQ) ranged from (0.002-0.017) and (0.008-0.054) mg kg^{-1} respectively (Table-1). Precision of the method was evaluated through the relative standard deviation (% RSD) associated with pesticide measurement during recovery. RSD below 10 % represent the satisfactory repeatability of the method for all pesticides (Barakatet al.2007; European Commission 2007). The analytical results of the method showed lower LOD and LOQ and good precision for recovery estimation (Anastassiades et al. 2003; Paya et al. 2007).

Table-1 The percent recoveries and LOD and LOQ of fortified Pulse Seeds samples

Pesticides	Classes	Fortification Level (mg kg^{-1})	% Recovery	*LOD (mg kg^{-1})	* LOQ (mg kg^{-1})	% *RSD
α- HCH	OC	0.10	94.10	0.002	0.008	10.245
β-HCH	OC	0.10	89.33	0.005	0.017	1.954
Lindane	OC	0.10	87.07	0.007	0.023	2.802
δ-HCH	OC	0.10	92.89	0.008	0.026	2.981
Alpha Endosulfane	OC	0.10	90.93	0.005	0.018	2.032
Beta Endosulfane	OC	0.10	92.94	0.004	0.014	1.609
p,p′ –DDT	OC	0.10	82.90	0.008	0.027	3.356
p,p′ DDD	OC	0.10	87.69	0.012	0.037	2.097
p,p′ DDE	OC	0.10	90.85	0.006	0.019	4.332
o,p’ DDE	OC	0.10	81.00	0.008	0.026	3.266
o,p’ DDT	OC	0.10	92.00	0.006	0.019	2.173
Heptachlor	OC	0.10	77.99	0.004	0.014	1.871
Dichlorvos	OP	0.50	95.00	0.009	0.030	1.431
Phosphomidan	OP	0.50	77.40	0.010	0.031	0.834
Dimethoate	OP	0.50	72.60	0.011	0.035	0.993
4-Bromo,2-Chlorophenol	OP	0.50	76.00	0.017	0.054	1.44
Chlorpyriphos	OP	0.50	90.80	0.012	0.038	0.804
Malathion	OP	0.50	81.90	0.011	0.036	0.956
Profenophos,	OP	0.50	84.60	0.006	0.020	0.491
Ethion	OP	0.50	83.00	0.009	0.029	0.722
Edifenphos	OP	0.50	73.05	0.007	0.023	0.646
Triazophos	OP	0.50	78.21	0.003	0.010	0.268
Bifenthrin	SP	0.10	84.55	0.015	0.049	1.189
Lamda-Cyhalothrin	SP	0.10	80.30	0.006	0.020	2.591

Fenpropathrin	SP	0.10	78.16	0.003	0.012	1.596
β-cyfluthrin-I	SP	0.10	86.77	0.008	0.026	3.140
β-cyfluthrin-II	SP	0.10	84.76	0.003	0.011	1.371
Cypermethrin-I	SP	0.10	87.67	0.002	0.008	1.026
Cypermethrin-II	SP	0.10	88.66	0.002	0.007	0.801
Fenvalerate-I	SP	0.10	95.01	0.010	0.032	3.481
Fenvalarate-II	SP	0.10	93.66	0.003	0.009	1.078
Taufluvalenate	SP	0.10	92.88	0.006	0.020	2.186
δ –methrin	SP	0.10	93.66	0.003	0.011	1.297
Atrazine	H	0.10	93.08	0.005	0.016	1.766
Alachlor	H	0.10	93.01	0.005	0.015	1.738
Butachlor	H	0.10	84.68	0.010	0.034	4.130
Pendimethylene	H	0.10	92.46	0.006	0.019	2.171

*LOD -Limit of Detection *LOQ - Limit of Quantification *RSD Relative Standard Deviation

The residues of each pesticide was identified by matching the retention time of the sample with standard on GC-ECD and further confirmed by GC-MS. Out of 37 analyzed pesticides only five pesticides Σ-HCH, malathion, chlorpyriphos, ethion, Σ-cypermethrin, were detected in pulse seeds, their residual level were showed in Table-2.

Chlorpyriphos and cypermethrin were detected in forty seven samples. The presence of pesticide residues in agricultural products has become a global phenomenon, their injudicious use during cultivation, storage and avoidance of waiting period prior to use are mainly responsible for this. Various authors reported the residues of OCs, OPs, SP, HERB and neo-nicotinoids in different agricultural products (Shahi et al. 2005; Kumari et al. 2006; Kumariand Kathpal 2008). Pesticides residues in agricultural products may cause adverse health hazard to consumer population. On the basis of pesticide residues determination chlorpyriphos and cypermethrin were found to be frequently encountered pesticides and selected for further studies on germination and early seedling growth by above discussed protocols. Lower concentration of treatment for chlorpyriphos and cypermethrin was 0.3 mg L^{-1}, middle treatment concentration 3 mg L^{-1} and higher treatment concentration 30 mg L^{-1}.

Table-2 Level of pesticide residues (mg kg^{-1}) in Pulse Seeds sample

Pulse Seeds	Pesticides	Number of Sample		Mean (residues range; mg kg−1)	No of samples >MRL	
		Analyzed	Detected		CODEX *	PFA*
Chick Pea Brown	Σ-HCH	30	3	0.064 (0.016-0.121)	NA	0
	Σ-Cypermethrin	30	7	0.294 (0.027-0.75)	3	3
Soya Bean	Σ-HCH	30	2	0.038 (0.021-0.056)	NA	0
	Σ-Cypermethrin	30	9	1.060 (0.029-3.051)	5	NA
	Chlorpyriphos	30	12	0.584 (0.016-3.451)	3	5
Black Gram	Σ-Cypermethrin	30	5	1.207 (0.028-3.152)	3	NA
	Malathion	30	2	1.411 (1.236-1.587)	2	0
Green Gram	Σ-Cypermethrin	30	6	1.309 (0.036-4.231)	NA	2
	Chlorpyriphos	30	8	0.313 (0.022-1.23)	NA	2
	Ethion	30	3	0.331 (0.015-0.951)	NA	2

NA- Not available in codex and PFA MRL list

4.2 Residual level of pesticides after different treatment durations

First of all it is important to discuss the residual level of pesticides in seeds after treatment. 5 g of treated pulse seed (as a whole with radicle) was taken for estimation of residual uptake by discussed QuEChERS sample preparation and GC-ECD analysis. Table 3 shows the residual level of pesticides in treated seeds with different concentration and exposure regimes. Residual data of pesticides treatment showed that chlorpyriphos has more penetration through seed coat as compare to cypermethrin. Seed coat morphology and anatomy play a key role in water uptake. As pesticides were delivered through emulsion in water, water uptake plays a key role in pesticide uptake. In four varieties of pulse seed thickness of macrosclereid cells layer (macrosclereid cells layer is a outer layer in the seed coat and are responsible for preventing water uptake) is in order of chick pea brown > black gram > green gram > soya bean. Residual uptake of pesticide was in order of soya bean>green gram > black gram >chick pea brown, showed that macrosclereid cell layer thickness play (lesser thickness more uptake) a regulatory role in pesticide uptake during seed treatment.

Table-3 Level of pesticide residues (mg kg^{-1}) in pulse seeds after different treatment experiments

	Chick Pea Brown			Soya Bean			Black Gram			Green gram		
A	**Ex 1**	**Ex 2**	**Ex 3**	**Ex 1**	**Ex 2**	**Ex 3**	**Ex 1**	**Ex 2**	**Ex 3**	**Ex 1**	**Ex 2**	**Ex 3**
0.3	0.186	0.041	0.126	0.279	0.081	0.143	0.202	0.090	0.285	0.252	0.069	0.158
3	1.184	0.096	0.786	2.381	0.635	1.748	1.909	0.102	1.265	2.232	1.623	1.435
30	16.365	5.952	12.23	19.693	6.859	9.958	17.992	5.236	10.539	17.632	6.326	15.629
B	**Ex 1**	**Ex 2**	**Ex 3**	**Ex 1**	**Ex 2**	**Ex 3**	**Ex 1**	**Ex 2**	**Ex 3**	**Ex 1**	**Ex 2**	**Ex 3**
0.3	0.096	0.024	0.042	2.149	0.141	1.53	0.164	0.090	0.102	0.102	0.034	0.075
3	0.954	0.072	0.482	2.634	0.986	1.625	2.736	0.445	1.161	1.523	0.713	1.263
30	10.125	3.266	7.494	16.422	5.339	8.225	14.421	5.316	9.246	15.725	7.569	12.623

A- Chlorpyriphos Treatment s mg L^{-1} **B-**Cypermethrin Treatments mg L^{-1}, Ex- Experiment

4.3 Phytotoxicity of pesticides treatment on germination and early seedling growth:

To alleviate insect pest problems in storage, synthetic pesticides are recommended, but their use may create toxicity to non target organisms, development of pest resistance and residues in treated products (Talukder 2006). Germination is a key process in plants' phenological cycles. Germination and early seedling growth are most sensitive stage of plant development. Presence of chemical stress may cause consequences like reduction in germination and growth. Accelerating this process could lead to improvement of the seedling growth as well as the cultivation efficiency. Insecticides like chlorpyriphos and cypermethrin are well known neurotoxin with different mode of action (Ecobichon 1996) which make their suitability for large number of insect and they are frequently used in storage and in seed treatment (Paulsrud et al. 2001; Groot 2014).

Phytotoxicity of chlorpyriphos and cypermethrin treatment was evaluated by % shoot, root elongation and germination index (Table-4). % shoot and root elongation was calculated in comparison with control. Continuous exposure of pesticides during seed germination was resulted in pronounced effect on germination and early seedling growth. Treatment of chlorpyriphos and cypermethrin with 0.3 mg L^{-1} had (92-100), (79-100) and (67-100) % shoot, root elongation and germination index respectively. Treatment of 3 mg L^{-1} of chlorpyriphos and cypermethrin had % shoot elongation (67-94), % root elongation (67-100) and GI (31-91). Treatment of 30 mg L^{-1} of chlorpyriphos and cypermethrin had pronounced affect on germination and early seedling growth as compare to lower and middle treatment concentration. % Shoot, root elongation and GI was recorded respectively in the range of (38-88), (43-89) and (24-78). Trend of reduction in % shoot, root elongation and germination index was Soyabean> Chick pea brown> Black Gram> Green Gram.

Short-term initial exposure of 4h had not any significant effect on germination and growth at 0.3 mg L^{-1} and 3.0 mg L^{-1}, only in case of soyabean GI was reduced to 88 and 86 due to reduction in germination. Short-term initial exposure of 4h had only significant effect on germination and growth at 30.0 mg L^{-1}. % Shoot, root elongation and GI was found respectively in the range of (63-98), (67-100) and (46-93). Trend of reduction in % shoot, root elongation and germination index was same as in continuous exposure of pesticides.

In case treatment of pre-germinated pulse seeds GI was not calculated as they are germinated at the time of treatment. Treatment of pre-germinated pulse seeds with pesticides revealed that at lowest concentration 0.3 mg L^{-1} only growth of soya bean and chick pea brown was affected. % Shoot, and root elongation was respectively found in the range of (86-100) and (83-100). Treatment of 3.0 mg L^{-1} of pesticides had % shoot and root elongation respectively in the range of (74-100), (76-100). Treatment of 30.0 mg L^{-1} of pesticides had % shoot and root elongation respectively in the range of (46-96) and (56-90). Trend of reduction in % shoot, root elongation and GI was same as in experiment 1 and experiment 2.

Data published on the effect of insecticides (chlorpyriphos and cypermethrin) seed treatments on pulse seeds germination and growth are relatively limited, but the available information of other insecticides suggested that plant responses are complex and strongly influenced by treatment protocols. Studies found that all the studied parameter such as plant height, number of branches, leaves per plant, total leaf area, plant biomass and photosynthetic pigments viz. Chlorophyll (Chl) *a*, Chl *b*, Total Chl, and Carotenoid (Car) content decreases as the chlorpyriphos concentration regimes increases in green gram (*Vigna radiata*) (Parween et al. 2013).Treatment of other insecticides with different varieties of seeds showed that as the concentration and exposure regimes increase negative effect observed on germination and growth. In a study it has been found that imidacloprid applied to seed that was not subsequently dried before germination suppressed

various growth parameters including percentage germination, vigour index and plant weight (Murthy and Rajesh 2004). Liu et al. 2009 found that cypermethrin could strongly inhibit the germination of *Pakchoi* in solution. In the single-factor experiments and joint effect tests of cypermethrin and copper on the seedling growth, it was found that there were significant liner relationships between concentrations of pollutants and root elongation and the toxicological effect on seed germination. Shoot and root elongation was observed in the following order: root elongation > shoot elongation > germination rate. The severity of suppression increased in response to longer exposure periods and with higher imidacloprid treatment concentrations in rice, which together would be expected to result in increased levels of chemical uptake (Stevens et al. 2002). To overcome this assumption authors also evaluate the residual level in pulse seeds after treatment and found that residual level increased as the treatment concentration and exposure regimes was increased. Increased residual level of chlorpyriphos and cypermethrin in soya bean and black gram reduced the germinability and % elongation of shoot and root, whereas in chick pea brown reduction in germination and shoot, root growth occurred with lower residual level as compared to soya bean and black gram. In green gram residual levels were higher but reduction in germination and shoot, root growth was lower as compared to other pulses seeds. These observations demonstrated that chick pea brown, soya bean and black gram were the most sensitive in term of phytotoxicity to cypermethrin and chlorpyriphos treatments whereas green gram was less sensitive to these treatments. In comparison of chlorpyriphos and cypermethrin treatment, chlorpyriphos was found more phytotoxic due to their higher penetration.

Table-4 % Shoot, root elongation and Germination Index at different treatment experiments

				Chlorpyriphos Treatment			Cypermethrin Treatment		
		Pulse Seeds		% Shoot Elongation	% Root Elongation	GI	% Shoot Elongation	% Root Elongation	GI
Experiment -1	Treatment Concentration mg L^{-1}	0.3	C	96	83	72	100	87	75
			S	92	81	67	75	79	81
			B	95	100	100	92	100	100
			G	97	88	95	95	100	98
		3	C	86	68	49	87	70	48
			S	76	64	31	67	71	71
			B	72	67	62	86	98	82
			G	87	80	87	94	100	91
		30	C	48	49	38	58	57	27
			S	38	43	24	41	53	46
			B	44	60	40	50	84	43
			G	85	81	72	88	89	78
Experiment -2	Treatment Concentration mg L^{-1}	0.3	C	95	100	95	100	100	100
			S	92	95	87	90	99	97
			B	94	100	94	97	100	100
			G	100	100	100	100	100	100
		3	C	94	90	94	100	100	91
			S	90	91	86	93	97	88
			B	91	96	92	100	100	100
			G	100	98	96	100	100	100
		30	C	89	72	47	88	90	72
			S	80	67	46	63	82	67
			B	67	91	66	89	84	81
			G	93	80	85	98	100	93
Experiment -3	Treatment Concentration mg L^{-1}	0.3	C	92	90	-	100	92	-
			S	93	83	-	86	88	-
			B	99	100	-	100	100	-
			G	95	93	-	100	100	-
		3	C	78	76	-	96	75	-
			S	74	79	-	82	84	-
			B	84	88	-	95	100	-

			G	80	86	-	100	100	-
		30	**C**	59	64	-	58	67	-
			S	46	56	-	50	58	-
			B	47	78	-	66	89	-
			G	73	84	-	96	90	-

C- Chick Pea Brown, S- Soyabean, B- Black Gram, G- Green Gram, GI- Germination Index

5. Conclusions

Results of pesticide residues determination revealed that only in 47 samples of pulse seeds chlorpyriphos and cypermethrin residues were detected and it was above MRL (PFA and Codex) in twenty samples, representing there indiscriminate and injudicious uses during storage. Treatment of pulse seeds with varied concentration of pesticides conclude that continuous exposure of pesticides during seed germination at middle 3.0 mg L^{-1} and higher 30.0 mg L^{-1} adversely affect the germination and early seedling growth. Treatment of pre-germinated pulse seeds only show adverse effect on higher concentration treatment. Variation in reduction in germination and early seedling growth was reported, it may be due to pesticides uptake and sensitivity to pesticides treatments. Soyabean was found to be most sensitive to treatment of these pesticides.

Treatment of these two pesticides with higher concentration and exposure regimes should be avoided otherwise it would cause reduction in production efficiency and increase in residual load of pesticides which may pretence adverse toxicological effect in early seedling growth.

Acknowledgments

The authors are grateful to the Director, CSIR-Indian Institute of Toxicology Research (IITR), Lucknow for his keen interest and providing research facilities. The financial assistance of All India network project on Monitoring of Pesticides Residues at National level (MPRNL) Gov. India is also acknowledged.

REFRENCES

Akoto O., Andoh H., Darko G., Eshun K.andOsei-Fosu P. 2013. Health risk assessment of pesticides residue in maize and cowpea from Ejura, Ghana. *Chemosphere* (92): 67-73.

Anastassiades M., Lehotay SJ., Stajnbaher D. and Schenck FJ. 2003. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and ''dispersive solid phase extraction'' for the determination of pesticides residues in produce. *Journal AOAC International.*(86): 412–431.

Aysal P., Ambrus AR., Lehotay SJ. and Andrew C. 2007. Validation of an efficient method for the determination of pesticide residues in fruits and vegetables using ethyl acetate for extraction. *Journal of Environment Science and HealthPart B*(42): 481–490.

Barakat A.A., Badaway HMA., Salama E., Attallah E. and Maatook G. 2007.Simple and rapid method of analysis for determination of pesticide residues in honey using dispersive solid phase extraction and GC determination. *J Food Agriculture & Environment* (5): 97-100

DhaliwalGS., Jindal V. and Dhawan AK. 2010. Insect Pest Problems and Crop Losses: Changing Trends. *Indian Journal Ecolology*(37): 1-7.

Dhoke K.S., Mahajan P., Kamble R. and Khanna A. 2013.Effect of nanoparticles suspension on the growth of green gram (Vignaradiata) seedlings by foliar spray method. *Nanotechnology Development* (3): e1 (2013).

Ecobichon DJ. 1996. Toxic effect of pesticides In: Klaassen CD (ed) casarett & Daull's toxicology pp-643. The basic science of poison *McGraw hill* New York.

Economic survey of India, 2012.

European Commission, 2007. Method validation and quality control procedures for pesticide residues analysis in food and feed, Document No. SANCO/2007/3131. Available from:,www.ec.europa.eu/plant/prtection/resources/qualcontrol_en.pdf.

Food and Agriculture Organization: 2002 FAO. Submission and evaluation of pesticide residues data for the estimation of maximum residues levels in food and feed (Ist 347 edition). Rome: Food and Agriculture 348 Organization.

Food and Agriculture Organization/World Health Organization Food standards programme 2003. Codex Alimentarius Commission, Twenty-Seventh Session, Geneva, Switzerland

Groot-Inge de, 2004. Agrodok 18 Protection of stored grains and pulses. Agromisa Foundation, Wageningen ISBN (90): 77073-49-3 NUGI 835.

Kumari B., Madan VK. andKathpal TS. 2006. Monitoring of pesticide residues in fruits. *Environmental Monitoring and Assessment* (123): 407–41.

Kumari B. and Kathpal TS. 2008. Monitoring of pesticide residues in vegetarian diet. *Environ Environmental Monitoring and Assessment* doi:10.1007/s10661-008-0210-0.

Lehotay SJ. 2007. Determination of pesticide residues in foods by acetonitrile extraction and partitioning with magnesium sulphate: Collaborative study. *Journal of AOAC International* (90): 485–520.

Liu TF.,Wang T., Sun C. and Wang YM. 2009. Single and joint toxicity of cypermethrin and copper on Chinese cabbage (Pakchoi) seeds. *J Hazardous material* (163): 344-348.

Martin ML. and Ronco AE. 2006. Effect of Mixtures of Pesticides Used in the Direct Seeding Technique on Nontarget Plant Seeds. *Bulletin of Environmental Contamination and Toxicology* (77): 228–236.

Murthy GP. and Rajesh N. 2004. Individual and combined effects of Krilaxyl and Gaucho on chloroplast pigments, relative water content and early growth attributes in rice seedlings, during germination under wet treatment. *Journal of Phytological Research* (17): 7–11.

Nadeem S., Shafique M., Hamed M., Atta BM. and Shah TM. 2010. Evaluation of advanced chickpea genotypes for resistance to pod borer, helicoverpaarmigera(hubner)(lepidoptera: noctuidae). *Pakistan Journal Agriculture Science* (47): 132-13.

Parween T., Jan S. and Fatma-Mahmooduzzafar T. 2012.Evaluation of oxidative stress in Vignaradiata L. in response to chlorpyrifos. *International Journal Science and Technology* (9): 605-612 (2012).

Parween T., Jan S. and Fatma-Mahmooduzzafar T. 2013.Differential response of vignaradiata L. to varied doses of chlorpyrifos. *International Journal Science and Technology* DOI: 10.1080/01904167.2013.799185.

Payá P., Anastassiades M., Mack D., Sigalova I., Tasdelen B. and Oliva J. 2007.Analysis of pesticide residues using the Quick Easy Cheap Effective Rugged and Safe (QuEChERS) pesticide multiresidue method in combination with gas and liquid chromatography and tandem mass-spectrometric detection. *Analytical and Bio analytical Chemistry*(389): 1697–1714.

Paulsrud Bruce E., Martin D., Babadoost M., Malvick D., Weinzierl R. and Lindholm Craig D *et al.* 2001. Oregon pesticide applicator training manual seed treatments Department of Crop Sciences University of Illinois.

PFA (1954) Prevention of Food Adulteration Act 1954. Act No. 37 with Prevention of Food Adulteration Rules 1955 and Notification and Commodity Index (16thed.). Lucknow: Eastern Book

Prasad ER., Gupta Dutta A. and Padmasree K. 2010. Insecticidal potential of Bowman–Birk proteinase inhibitors from red gram (Cajanuscajan) and black gram (Vigna green gramo) against lepidopteran insect pests. *Pesticide Biochemistry and Physiology* (98): 80–88a.

Shahi DK., Nisha K. and Sharma A. 2005. Monitoring of pesticide residues in market vegetable at Ranchi, Jharkhand (India). *Journal of Environmental Science and Engineering* **(**47): 322–325.

Siddiqui-Shaheed Z. and Ahmed S. 2006.Combined effects of pesticide on growth and nutritive composition of soybean plants. *Pakistan Journal of Botany* (38): 721-733.

Stevens MM.,Pimental D. and Dekker M. 2002. Seed treatments Encyclopedia of Pest Management. New York, NY, USA, 754–756..

Stevens MM., Reinke-Russell F., Coombes-Neil E., Helliwell S. and Jianhua M. 2008. Influence of imidacloprid seed treatments on rice germination and early seedling growth. *Pest Management Science* (64): 215–222.

Talukder- Farid A. 2006. Plant products as potential stored-product insect management agents – A mini review *Emirates Journal Food and Agriculture Science* (18): 17-32.

Wang L., Yongchao L. and Xin J. 2008. Analysis of eight organphosphorus pesticide residues in fresh vegetables retailed in agrictural product market of Nanjing, China. *Bulletin of Environmental Contamination and Toxicology* (81): 377–382.