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In silico study by using ProTox-II webserver for oral acute toxicity, organ toxicity, immunotoxicity, genetic toxicity endpoints, nuclear receptor signalling and stress response pathways of synthetic pyrethroids

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ABSTRACT

Till date, it is well-known that synthetic pyrethroids are safe to mammal but toxic to non-mammals. The present objective was an *in silico* study to detect oral acute toxicity, organ toxicity, immunotoxicity, genetic toxicity endpoints, nuclear receptor signalling, and stress response pathways of common synthetic pyrethroids by using ProTox-II webserver. The chemical compounds especially different synthetic pyrethroids such as pyrethrin I, Cinerin I and Jasmolin I (esters of Chrysanthemic acid), Pyrethrin-II, Cinerin II and Jasmolin II (Esters of Pyrethric acid), type I pyrethroids (esters without alpha-cyano group) such as allethrin, resmethrin, permethrin and bifenthrin and type II pyrethroids (esters with alpha-cyano group) such as fenvalerate, cyhalothrin, cypermethrin and deltamethrinwere selected from available literature. ProTox-II webserver was used for toxicological assessment in organism, organs, cell and gene level along with molecular mechanisms of toxicity. The predictive

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results for the toxicity of common synthetic pyrethroids compounds, Deltamethrin showed highly toxic compound among 14 compounds as fatal if swallowed as class II followed by Cypermethrin, Cyhalothrin, Bifenthrin, Resmethrin, Fenvalerate and Permethrin but hepatotoxic potential was only Deltamethrin and Fenvalerate while immunotoxic was obtained Permethrin. On the other hand, none of the compounds were obtained cytotoxic and carcinogenic but 9 compounds viz. Pyrethrin I, II Cinerin I, II, Jasmolin I, II, Allethrin, Resmethrin and Permethrin were observed mutagenic active. In case of NR signalling pathways, all compounds were inactive but eight compounds such as Pyrethrin I, II, Cinerin I, II, Jasmolin I, II, Allethrin and Resmethrin were obtained nrf2/ARE and HSE active while MMP active compounds were obtained Fenvalerate, Cyhalothrin and Deltamethrin respectively. For p53 and ATAD5 parameters, all fourteen compounds such as were obtained inactive. In conclusion, the present predictive results are suitable for academician, researchers, industries, etc. those who are making drugs and environmental chemicals. This web server helps faster screening of large numbers of compounds within short duration and no animal testing. This present *in silico* study easily detects toxin(s), which can be validated in future through *in vitro* and *in vivo* experimental assay.

Keywords**:** *In silico* study, Synthetic pyrethroids, Predictive toxicology, Molecular mechanism of toxicity, Nuclear receptor signalling and stress response pathways

1. INTRODUCTION

The use of pesticides encompasses to eradicate pests from crops, vegetables, etc. Among several types of pesticides such as organochlorine, organophosphate and carbamate, the synthetic pyrethroids are safe, and less toxic to the non-target organisms as described earlier (Adelsbach et al., 2003; Rehman et al., 2014; Patel and Patil, 2016). Pyrethroids are synthetic organic compounds and known as new class of insecticides synthesized from chrysanthemum flowers, which are used extensively as household and commercial insecticides (Elliott and Janes, 1978). Rehman et al. (2014) mentioned that two pyrethrins are most prominent, pyrethrin-I and pyrethrin-II. These pyrethrins have four types of active ingredients viz. cinerin I, II, jasmolin I and II. Beside these, type I pyrethroids (esters without alpha-cyano group) such as allethrin, resmethrin, permethrin and bifenthrin and type II pyrethroids (esters with alpha-cyano group) such as fenvalerate, cyhalothrin, cypermethrin and deltamethrin are well-established by researchers (Roy, 2002; Soderlund et al., 2002; Rehman et al., 2014). However, these pesticides have observed toxic effects on bees, freshwater fish, and other aquatic organisms (Werner et al., 2002; de Assis et al., 2009; Kaviraj and Gupta, 2014). But in case of mammal these showed low toxicity as per researchers (Adelsbach et al., 2003; Rehman et al., 2014).

In present days, an alteration of experimental toxicity study, the predictive study through computational simulation is showing interest in the researchers to prevent cost, less duration and no animal harming. The ProTox-II is a webserver (http://tox.charite.de/protox_II/) to predict toxicity and multiple toxicological endpoints for several chemical compounds developed by Drwal et al. (2014) and Banerjee et al. (2018). According to Banerjee et al. (2018), the ProTox-II platform is classified into a five different steps such as oral acute toxicity prediction model as per six different toxicity classes; organ toxicity model especially liver toxicity prediction; (3) toxicological (immunotoxicity model) and genotoxicological (cytotoxicity, mutagenicity and carcinogenicity model) endpoints; (4) toxicological pathways such as nuclear receptor signalling pathways is classified seven target-pathway based models

viz. aryl hydrogen receptor (AhR), androgen receptor (AR), androgen receptor ligand binding domain (AR-LBD), aromatase, estrogen receptor alpha (ER), estrogen receptor ligand binding domain (ER-LBD), and peroxisome proliferator activated receptor gamma (PPARGamma) as well as stress response pathways is classified five target-pathway based models such as nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (ARE), heat shock factor response element (HSE), mitochondrial membrane potential (MMP), phosphoprotein tumor suppressor (p53), and ATPase family AAA domain-containing protein 5 (ATAD5) and toxicity targets model of 14 nos. All the predictive models for toxicology pathways have been implemented as toxicology in the 21st Century (Tox21), which is a federal collaboration among United States Environmental Protection Agency (EPA), National Institute of Health (NIH), including National Center for Advancing Translational Sciences, and the National Toxicology Program at the National Institute of Environmental Health Sciences, and the Food and Drug Administration (Banerjee et al., 2016).

Present *in silico* study was to detect oral acute toxicity, organ toxicity, immunotoxicity, genetic toxicity endpoints, nuclear receptor signalling, and stress response pathways of synthetic pyrethroids by using ProTox-II webserver.

2. MATERIALS AND METHODS

2. 1. Selection of compounds

The chemical compounds especially different synthetic pyrethroids were selected from available literature studied by several researchers (Miyamato, 1976; Kaviraj and Gupta, 2012; Rehman et al., 2014; Patel and Patil, 2016). Detail list of compounds is tabulated in Table 1.

Table 1. Different types of synthetic Pyrethroids and related compounds.

Sl. No.	Synthetic Pyrethroids type	Compounds name
1.	Esters of Chrysanthemic acid	Pyrethrin-I
2.		Cinerin-I
3.		Jasmolin-I
4.	Esters of Pyrethric acid	Pyrethrin-II
5.		Cinerin-II
6.		Jasmolin-II
7.	Type I (Esters without alpha-cyano group)	Allethrin
8.		Resmethrin
9.		Permethrin
10.		Bifenthrin

11.	Type II (Esters with alpha-cyano group	Fenvalerate
12.		Cyhalothrin
13.		Cypermethrin
14.		Deltamethrin

2. 2. *In silico* study of studied compounds

In silico study was done by using ProTox-II webserver developed by Drwal et al. (2014) and the parameters such as rat oral acute toxicity with special reference to median lethal dose (LD_{50}) as mg/Kg, organ toxicity especially hepatotoxicity, immunotoxicity, genetic toxicity endpoints especially cytotoxicity, mutagenicity and carcinogenicity, nuclear receptor signalling (AhR, AR, AR-LBD, ER, ER-LBD and PPARGamma), and stress response pathways (nrf2/ARE, HSE, MMP, p53 and ATAD5) were predicted for established synthetic pyrethroids of 14 nos. as per protocol followed of Banerjee et al. (2018).

3. RESULTS AND DISCUSSION

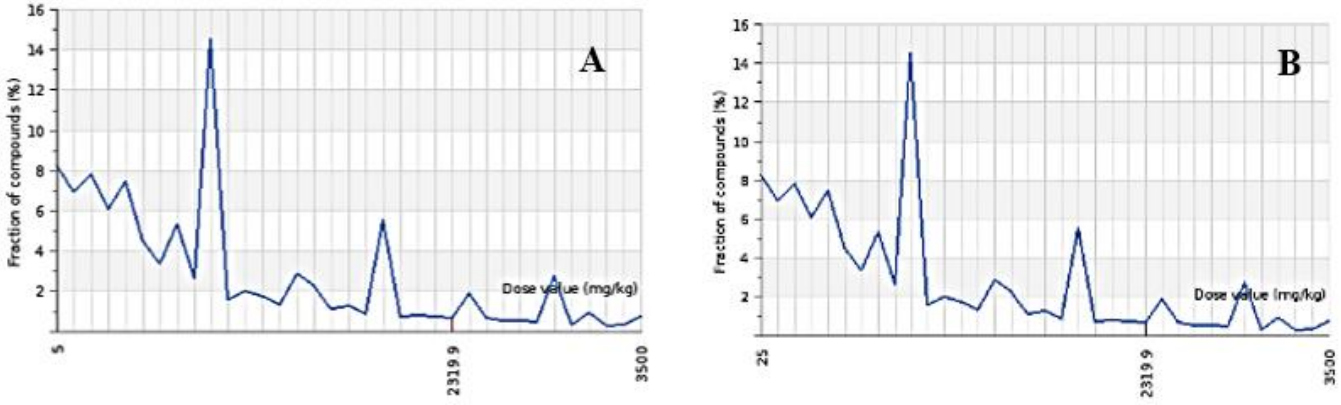
Table 2 indicates the rat oral acute toxicity (LD_{50}) as mg/Kg, predicted different toxicity classes (I–VI) and prediction accuracy in % for different synthetic pyrethroids. Among 14 compounds, Deltamethrin obtained the highest toxicity (LD_{50} = 5.0 mg/Kg) as class II i.e. prescribed as death after swallowing ($5 < LD_{50} \leq 50$) with 100% prediction accuracy (Fig. 1A) followed by Cypermethrin, Cyhalothrin, Bifenthrin, Resmethrin, Fenvalerate and Permethrin as LD_{50} value of 25.0, 50.0, 55.0, 63.0, 70.0 and 85.0 respectively (Fig 1B-G) and former two compounds showed class II i.e. prescribed also as death after swallowing ($5< LD_{50} \leq 50$) and rest four compounds were obtained class III i.e. prescribed toxic after swallowing ($50 < LD_{50} \leq 300$) with 100% prediction accuracy. Same LD_{50} value (210.0 mg/Kg) was obtained for three compounds such as Allethrin, Cinerin I and Jasmolin I (Fig 1H) while two compounds Pyrethrin I and II were obtained LD_{50} values of 260.0 and 130.0 mg/Kg (Fig. 1I-J) as class III with 100% prediction accuracy in Allethrin, Pyrethrin I and II while 72.9% prediction accuracy was in Cinerin I and Jasmolin I. The compounds Cinerin II and Jasmolin II (Fig. 1K) were obtained same LD_{50} value (1410.0 mg/Kg) as class IV i.e. prescribed may be harmful after swallowing ($2000 < LD_{50} \leq 5000$). These toxicity classes have been prescribed by Drwal et al. (2014) in ProTox-II webserver.

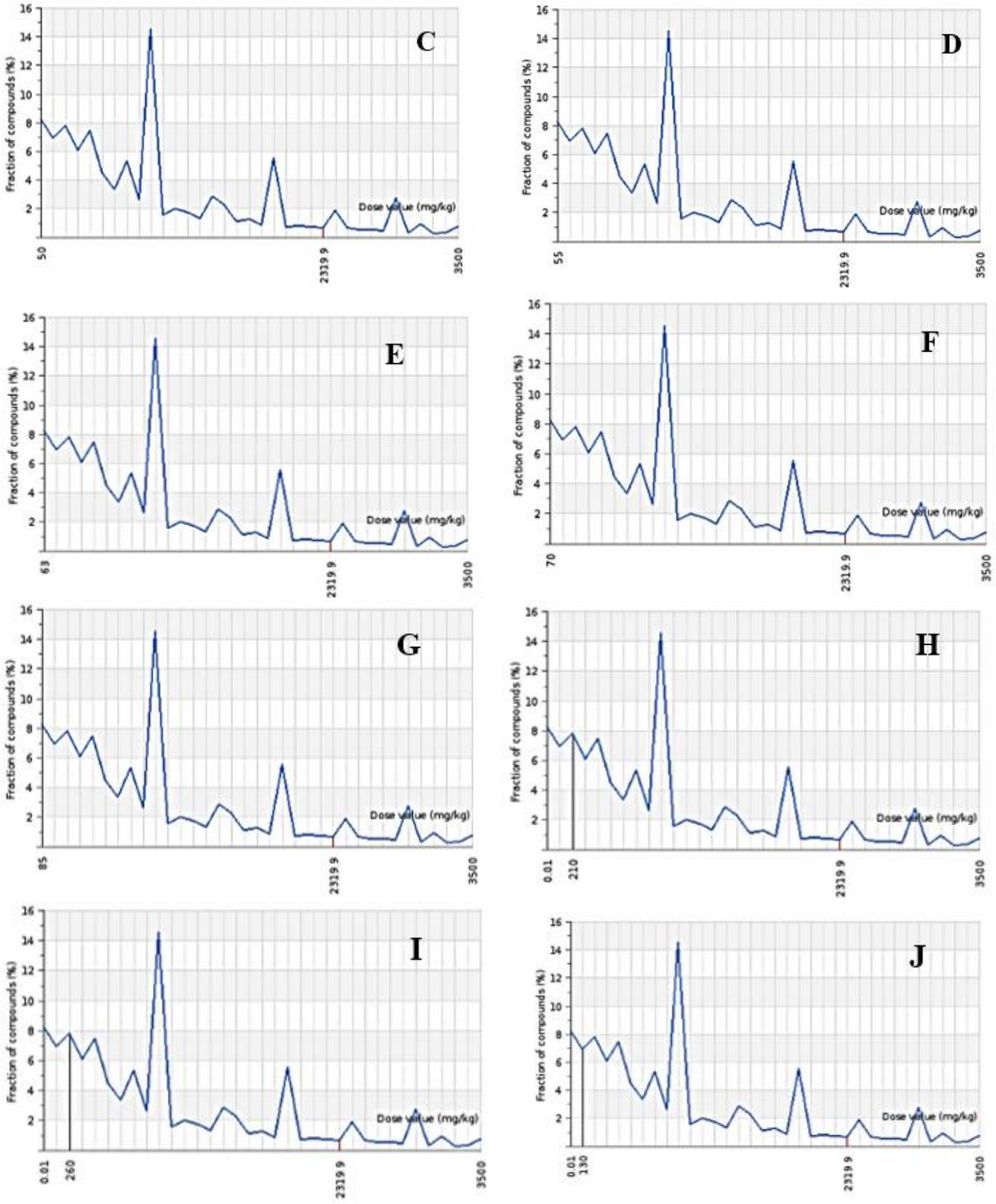
Table 2. Prediction of oral acute toxicity, class and accuracy of studied compounds.

Sl. No.	Compounds name	Oral LD_{50} value (mg/Kg)	Predicted toxicity class	Prediction accuracy (%)
1.	Pyrethrin I	260.0	III	100

2.	Cinerin I	210.0	III	72.9
3.	Jasmolin I	210.0	III	72.9
4.	Pyrethrin II	130.0	III	100
5.	Cinerin II	1410.0	IV	72.9
6.	Jasmolin II	1410.0	IV	72.9
7.	Allethrin	210.0	III	100
8.	Resmethrin	63.0	III	100
9.	Permethrin	85.0	III	100
10.	Bifenthrin	55.0	III	100
11.	Fenvalerate	70.0	III	100
12.	Cyhalothrin	50.0	II	100
13.	Cypermethrin	25.0	II	100
14.	Deltamethrin	5.0	II	100

As per Drwal et al. (2014), Class I: death after swallowing ($LD_{50} \leq 5$); Class II: death after swallowing ($5< LD_{50} \leq 50$); Class III: toxic after swallowing ($50< LD_{50} \leq 300$); Class IV: harmful after swallowing ($300<LD_{50} \leq 2000$); Class V: may be harmful after swallowing ($2000<LD_{50} \leq 5000$) and Class VI: non-toxic ($LD_{50}>5000$)




C
Fraction of compounds (%)
Dose value (mg/kg)
50
2319.9
3500
D
Fraction of compounds (%)
Dose value (mg/kg)
55
2319.9
3500
E
Fraction of compounds (%)
Dose value (mg/kg)
63
2319.9
3500
F
Fraction of compounds (%)
Dose value (mg/kg)
70
2319.9
3500
G
Fraction of compounds (%)
Dose value (mg/kg)
85
2319.9
3500
H
Fraction of compounds (%)
Dose value (mg/kg)
0.01
210
2319.9
3500
I
Fraction of compounds (%)
Dose value (mg/kg)
0.01
260
2319.9
3500
J
Fraction of compounds (%)
Dose value (mg/kg)
0.01
130
2319.9
3500

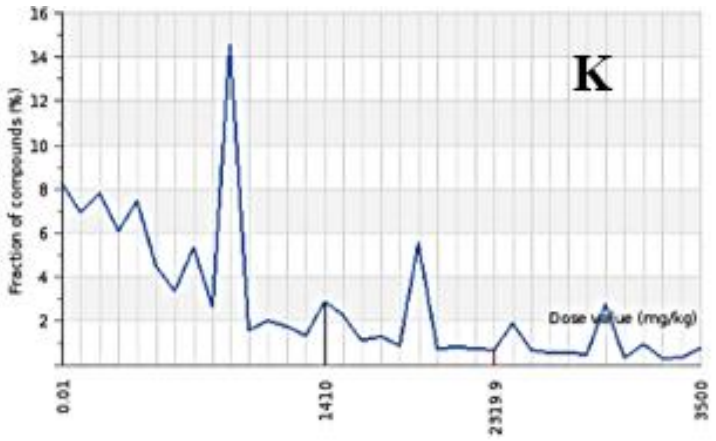


Fig. 1. Graphical representation of predicted dose value distribution for studied compounds (A = Deltamethrin; B = Cypermethrin; C = Cyhalothrin; D = Bifenthrin; E = Resmethrin; F = Fenvalerate; G = Permethrin; H = Allethrin, Cinerin I and Jasmolin I; I = Pyrethrin I; J = Pyrethrin II and K = Cinerin II and Jasmolin II)

The present predictive results indicated that all synthetic pyrethroids are toxic as per LD_{50} values (5.0 mg/Kg) obtained by the webserver (ProTox-II) but only Deltamethrin was obtained fatal as per LD_{50} value. According to several researchers, the LD_{50} value of Deltamethrin ranged from 30 to 140 mg/Kg as per body weight (b.w.) in rats, with a minimum toxic dose of 10 mg/kg bw causing mild salivation while the LD_{50} ranged from 19 to 34 mg/Kg b.w. in mice after gavage Deltamethrin mixed with vegetable oil or polyethylene glycol (Zhang et al., 1991; Poonam et al., 2013; Rehman et al., 2014). The present prediction revealed lower LD_{50} value in relation to earlier study.

In Table 3, the prediction of organ toxicity with special reference to liver toxicity or hepatotoxicity was observed. Among 14 compounds, two compounds such as Fenvalerate and Deltamethrin were showed hepatotoxic active and probability scores were 0.55 and 0.50 while all other 12 compounds such as Pyrethrin I, II, Jasmolin I, II, Cinerin I, II, Allethrin, Resmethrin, Permethrin, Bifenthrin, Cyhalothrin and Cypermethrin were observed non-hepatotoxic or hepatotoxic inactive with probability scores of 0.71, 0.73, 0.75, 0.76, 0.72, 0.74, 0.71, 0.66, 0.79, 0.61, 0.51 and 0.53 respectively.

The immunotoxicity end points of studied 14 compounds only Permethrin was observed immunotoxic active with a probability score of 0.75 while other 13 compounds viz. Pyrethrin I, II, Jasmolin I, II, Cinerin I, II, Allethrin, Resmethrin, Bifenthrin, Fenvalerate, Cyhalothrin, Cypermethrin and Deltamethrin were obtained immunotoxic inactive with probability scores of 0.96, 0.97, 0.97, 0.98, 0.96, 0.97, 0.98, 0.99, 0.90, 0.85, 0.68, 0.93 and 0.64 respectively (Table 3).

In the present predictive results, Deltamethrin and Fenvalerate showed hepatotoxic potential, which is supported by other researchers that these 2 synthetic pyrethroids found hepatotoxic in rat model during experimental study (Waheed and Mohammed, 2012; Rjeibi et al., 2016; Khalatbary et al., 2017; Chrustek et al., 2018). It was found in the present predictive results that Permethrin is immunotoxic, which has evidenced from previous experimental study on male Wistar rats after chronic exposure to Permethrin (Gabbianelli et al., 2009). On the other

hand, Banerjee et al. (1996) studied that pesticides are immunotoxic and impaired immune response.

Table 3. Prediction of organ toxicity and immunotoxicity end points of studied compounds.

Sl. No.	Compounds name	Hep	P	Imm	P
1.	Pyrethrin I	I	0.71	I	0.96
2.	Cinerin I	I	0.72	I	0.96
3.	Jasmolin I	I	0.75	I	0.97
4.	Pyrethrin II	I	0.73	I	0.97
5.	Cinerin II	I	0.74	I	0.97
6.	Jasmolin II	I	0.76	I	0.98
7.	Allethrin	I	0.71	I	0.98
8.	Resmethrin	I	0.66	I	0.99
9.	Permethrin	I	0.79	A	0.75
10.	Bifenthrin	I	0.61	I	0.90
11.	Fenvalerate	A	0.55	I	0.85
12.	Cyhalothrin	I	0.51	I	0.68
13.	Cypermethrin	I	0.53	I	0.93
14.	Deltamethrin	A	0.50	I	0.64

Hep = Hepatotoxicity; Imm = Immunotoxicity; I = Inactive; A = Active and P = Probability

In Table 4, the prediction of genotoxicity with special reference to cytotoxicity, mutagenicity and carcinogenicity were observed. Among 14 compounds all compounds such as Pyrethrin I, II, Jasmolin I, II, Cinerin I, II, Allethrin, Resmethrin, Permethrin, Bifenthrin, Fenvalerate, Cyhalothrin, Cypermethrin and Deltamethrin were observed non-cytotoxic or cytotoxic inactive with probability scores of 0.74, 0.71, 0.71, 0.70, 0.74, 0.71, 0.76, 0.76, 0.77, 0.65, 0.67, 0.70 and 0.67 respectively.

In case of mutagenicity endpoints, 9 compounds such as Pyrethrin I, II Cinerin I, II, Jasmolin I, II, Allethrin, Resmethrin and Permethrin were observed mutagenic active with probability scores of 0.76, 0.71, 0.70, 0.66, 0.63, 0.60, 0.76, 0.60 and 1.00 respectively while 5 compounds viz. Bifenthrin, Fenvalerate, Cyhalothrin, Cypermethrin and Deltamethrin were

obtained mutagenic inactive with probability scores of 0.55, 0.95, 0.83, 1.00 and 0.81 respectively (Table 4).

All the studied 14 compounds were obtained carcinogenic inactive with probability scores 0.58, 0.58, 0.60, 0.64, 0.64, 0.66, 0.58, 0.65, 0.72, 0.78, 0.86, 0.78, 0.93 and 0.82 respectively (Table 4).

Table 4. Prediction of genetic toxicity end points of studied compounds.

Sl. No.	Compounds name	Cytt	P	Mutg	P	Carcig	P
1.	Pyrethrin I	I	0.74	A	0.76	I	0.58
2.	Cinerin I	I	0.74	A	0.70	I	0.58
3.	Jasmolin I	I	0.71	A	0.63	I	0.60
4.	Pyrethrin II	I	0.71	A	0.71	I	0.64
5.	Cinerin II	I	0.71	A	0.66	I	0.64
6.	Jasmolin II	I	0.70	A	0.60	I	0.66
7.	Allethrin	I	0.74	A	0.76	I	0.58
8.	Resmethrin	I	0.76	A	0.60	I	0.65
9.	Permethrin	I	0.76	A	1.00	I	0.72
10.	Bifenthrin	I	0.77	I	0.55	I	0.78
11.	Fenvalerate	I	0.65	I	0.95	I	0.86
12.	Cyhalothrin	I	0.67	I	0.83	I	0.78
13.	Cypermethrin	I	0.70	I	1.00	I	0.93
14.	Deltamethrin	I	0.67	I	0.81	I	0.82

Cytt = Cytotoxicity; Mutg = Mutagenicity; Carcig = Carcinogenicity; I = Inactive; A = Active and P = Probability

In the present prediction, cytotoxicity was not observed but in earlier experimental study revealed that cis-Bifenthrin showed cytotoxicity in two cell lines namely Hela and CHO in cell viability test experimentally (Cui et al., 2009). Earlier experimental results showed that three pyrethroids such as Resmethrin, Permethrin and Fenvalerate were not found mutagenic in *S. typhimurium*in the presence or absence of a rat liver activation system but Allethrin was found mutagenic with TA100, TA104 and TA97 strains and suggested metabolic activation (S9 mix) in relation to its activity, especially with TA100 and TA104 strains. In the present prediction

some contradictory results obtained, Resmethrin and Permethrin along with Pyrethrin I, II, Jasmolin I, II, Cinerin I, II, Allethrin observed mutagenic active while similarity was observed only for Fenvalerate (Herrera and Laborda, 1988). Moreover, according to researcher, few compounds are found mutagenic (Pluijmen et al., 1994). The previous experimental studies were also revealed that synthetic pyrethroids may cause tumour but did not develop carcinogenicity in mice and rat models (Cabral et al., 1990), which has similarities in the present prediction.

For Tox21-nuclear receptor signalling pathways, several parameters such as AhR, AR, AR-LBD, Aro, ER, ER-LBD and PPAR-Gamma were predicted for Pyrethrin I, II Cinerin I, II, Jasmolin I, II, Allethrin, Resmethrin, Permethrin, Bifenthrin, Fenvalerate, Cyhalothrin, Cypermethrin and Deltamethrin (Table 5). All the studied fourteen compounds were obtained Ahr inactive with probability scores 0.99, 0.93, 0.98, 0.92, 0.99, 0.93, 0.99, 0.98, 0.99, 0.97, 0.98, 0.98, 0.99 and 0.99 respectively. For other parameter AR, all the studied compounds were obtained AR inactive with probability scores 0.99, 0.99, 0.98, 0.99, 0.98, 0.98, 0.99, 0.99, 0.99, 0.99, 1.00, 0.99, 1.00 and 1.00 respectively. In case of AR-LBD parameter, all were found AR-LBD inactive and the probability scores 0.99, 0.98, 0.98, 0.97, 0.98, 0.97, 0.99, 0.99, 1.00, 0.99, 1.00, 1.00, 1.00 and 0.98 were obtained. The parameter Aromatase or Aro was found inactive for all the studied compounds and probability scores 0.98, 0.96, 0.98, 0.96, 0.98, 0.97, 0.98, 0.98, 0.99, 0.96, 0.94, 0.99, 0.99 and 0.95 were obtained. In case of the parameters ER and ER-LBD, it was observed inactive for both cases and probability scores 0.92, 0.94, 0.91, 0.94, 0.91, 0.93, 0.92, 0.96, 0.96, 0.92, 0.94, 0.99, 0.98 and 0.98 for ER and 0.99, 0.98, 0.99, 0.97, 0.98, 0.97, 0.99, 0.98, 1.00, 0.95, 0.99, 0.99, 1.00 and 0.99 for ER-LBD were recorded. For the parameter as PPAR-Gamma, it was also found inactive by fourteen compounds and probability scores were recorded 0.97, 0.97, 0.97, 0.97, 0.98, 0.98, 0.97, 0.98, 0.99, 0.93, 0.99, 0.99, 0.99 and 0.94 respectively.

Table 5. Prediction of Tox21-nuclear receptor signalling pathways of studied compounds

Sl. No.	Compounds name	Tox21-Nuclear receptor signalling pathways							
		Ahr	P	AR	P	AR-LBD	P	Aro	P
1.	Pyrethrin I	I	0.99	I	0.99	I	0.99	I	0.98
2.	Cinerin I	I	0.98	I	0.98	I	0.98	I	0.98
3.	Jasmolin I	I	0.99	I	0.98	I	0.98	I	0.98
4.	Pyrethrin II	I	0.93	I	0.99	I	0.98	I	0.96
5.	Cinerin II	I	0.92	I	0.99	I	0.97	I	0.96
6.	Jasmolin II	I	0.93	I	0.98	I	0.97	I	0.97
7.	Allethrin	I	0.99	I	0.99	I	0.99	I	0.98
8.	Resmethrin	I	0.98	I	0.99	I	0.99	I	0.98

9.	Permethrin	I	0.99	I	0.99	I	1.00	I	0.99
10.	Bifenthrin	I	0.97	I	0.99	I	0.99	I	0.96
11.	Fenvalerate	I	0.98	I	1.00	I	1.00	I	0.94
12.	Cyhalothrin	I	0.98	I	0.99	I	1.00	I	0.99
13.	Cypermethrin	I	0.99	I	1.00	I	1.00	I	0.99
14.	Deltamethrin	I	0.99	I	1.00	I	0.98	I	0.95

		ER	**P**	**ER-LBD**	**P**	**PPAR-Gamma**	**P**
1.	Pyrethrin I	I	0.92	I	0.99	I	0.97
2.	Cinerin I	I	0.91	I	0.99	I	0.97
3.	Jasmolin I	I	0.91	I	0.98	I	0.98
4.	Pyrethrin II	I	0.94	I	0.98	I	0.97
5.	Cinerin II	I	0.94	I	0.97	I	0.97
6.	Jasmolin II	I	0.93	I	0.97	I	0.98
7.	Allethrin	I	0.92	I	0.99	I	0.97
8.	Resmethrin	I	0.96	I	0.98	I	0.98
9.	Permethrin	I	0.96	I	1.00	I	0.99
10.	Bifenthrin	I	0.92	I	0.95	I	0.93
11.	Fenvalerate	I	0.94	I	0.99	I	0.99
12.	Cyhalothrin	I	0.99	I	0.99	I	0.99
13.	Cypermethrin	I	0.98	I	1.00	I	0.99
14.	Deltamethrin	I	0.98	I	0.99	I	0.94

AhR = Aryl hydrocarbon Receptor; AR = Androgen receptor; AR-LBD = Androgen Receptor Ligand Binding Domain; Aro = Aromatase; ER = Estrogen Receptor Alpha; ER-LBD = Estrogen Receptor Ligand Binding Domain; PPAR-Gamma = Peroxisome Proliferator Activated Receptor Gamma; I = Inactive; A = Active and P = Probability

The present predictive results indicated inactivity for all the parameters such as AhR, AR, AR-LBD, Aro, ER, ER-LBD and PPAR-Gamma under nuclear receptor (NR) signalling pathways on different compounds. The experimental study was characterized the potential ER, AR, and TR activities of nine pyrethroids and two metabolites by using CV-1 cells and MDA-

kb2 cell line. The results revealed that these compounds exhibited not only weak estrogenic activities, but also antiestrogenic, antiandrogenic, and anti-TH activities via ER, AR, and TR, which support present prediction (Du et al., 2010). According to Kolodkin et al. (2010), NR signalling occurs to maintain development, cellular growth, inflammation and metabolism and ligand distribution appeared dynamic with few NRs found predominantly in the nucleus (pregnane X receptor and peroxisome proliferator-activated receptor gamma), while some are located either in both compartments (vitamin D receptor and mineralocorticoid receptor) or mostly in the cytoplasm (glucocorticoid receptor and androgen receptor). The present results indicated the inactivity of studied compounds may not lead to carcinogenesis, which obtained in Table 4.

In Table 6, Tox21-stress response pathways parameters such as nrf2/ARE, HSE, MMP, p53 and ATAD5 for studied compounds were predicted. Among 14 compounds, 8 compounds such as Pyrethrin I, II, Cinerin I, II, Jasmolin I, II, Allethrin and Resmethrin were obtained nrf2/ARE and HSE active with probability score 1.00, 0.85, 0.79, 0.70, 0.72, 0.64, 1.00 and 0.92 while rest 6 compounds viz. Permethrin, Bifenthrin, Fenvalerate, Cyhalothrin, Cypermethrin and Deltamethrin were found inactive with probability score 0.95, 0.94, 0.93, 0.97, 0.96 and 0.95 and 0.98, 0.94, 0.93, 0.97, 0.98 and 0.95 respectively. For MMP parameter, all 11 compounds such as Pyrethrin I, II, Cinerin I, II, Jasmolin I, II, Allethrin, Resmethrin, Permethrin, Bifenthrin and Cypermethrin were obtained MMP inactive with probability scores 0.86, 0.84, 0.87, 0.81, 0.79, 0.81, 0.86, 0.90, 0.86, 0.52 and 0.59 but 3 compounds viz. Fenvalerate, Cyhalothrin and Deltamethrin were found MMP active with probability score 1.00, 1.00 and 0.99 respectively. For p53 and ATAD5 parameters, all 14 compounds such as Pyrethrin I, II, Cinerin I, II, Jasmolin I, II, Allethrin, Resmethrin, Permethrin, Bifenthrin, Fenvalerate, Cyhalothrin, Cypermethrin and Deltamethrin were obtained inactive with probability scores 0.93, 0.86, 0.94, 0.86, 0.93, 0.85, 0.93, 0.99, 0.99, 0.94, 0.99, 0.99, 0.98 and 0.96 for p53 parameter as well as 0.99, 0.97, 0.99, 0.97, 0.99, 0.91, 0.99, 0.99, 0.99, 0.98, 0.99, 0.99, 1.00 and 0.98 respectively for ATAD5 parameter.

Table 6. Prediction of Tox21-stress response pathways of studied compounds.

Sl. No.	Compounds name	Tox21- Stress response pathways									
		nrf2/ARE	P	HSE	P	MMP	P	p53	P	ATAD5	P
1.	Pyrethrin I	A	1.00	A	1.00	I	0.86	I	0.93	I	0.99
2.	Cinerin I	A	0.79	A	0.79	I	0.84	I	0.94	I	0.99
3.	Jasmolin I	A	0.72	A	0.72	I	0.87	I	0.93	I	0.99
4.	Pyrethrin II	A	0.85	A	0.85	I	0.81	I	0.86	I	0.97
5.	Cinerin II	A	0.70	A	0.70	I	0.79	I	0.86	I	0.97

6.	Jasmolin II	A	0.64	A	0.64	I	0.81	I	0.85	I	0.91
7.	Allethrin	A	1.00	A	1.00	I	0.86	I	0.93	I	0.99
8.	Resmethrin	A	0.92	A	0.92	I	0.90	I	0.99	I	0.99
9.	Permethrin	I	0.95	I	0.98	I	0.86	I	0.99	I	0.99
10.	Bifenthrin	I	0.94	I	0.94	I	0.52	I	0.94	I	0.98
11.	Fenvalerate	I	0.93	I	0.93	A	1.00	I	0.99	I	0.99
12.	Cyhalothrin	I	0.97	I	0.97	A	1.00	I	0.99	I	0.99
13.	Cypermethrin	I	0.96	I	0.98	I	0.59	I	0.98	I	1.00
14.	Deltamethrin	I	0.95	I	0.95	A	0.99	I	0.96	I	0.98

nrf2/ARE = Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element; HSE = Heat shock factor response element; MMP = Mitochondrial Membrane Potential; p53 = Phosphoprotein (tumour supressor); ATAD5 = ATPase family AAA domain-containing protein 5; I = Inactive; A = Active and P = Probability

Several cellular stress in response pathways have been investigated individually through *in vitro* studies, and the major signalling components and molecular mechanisms have been identified by researchers. Adaptive stress response pathways are signal transduction pathways that ultimately resulted in the transcriptional activation of cytoprotective genes (Simmons et al., 2009). The compounds such as Pyrethrin I, II, Cinerin I, II, Jasmolin I, II, Allethrin and Resmethrin were obtained nrf2/ARE and HSE active that caused reactive oxygen species (ROS), ultimately oxidative stress in the cell for former case as antioxidant responsive element (ARE) (Kang et al., 2005; Kensler et al., 2007; Simmons et al., 2009) while in the second case, another stress response pathway i.e. heat shock factor response element (HSE), which caused transcriptional upregulation of a family of genes called as heat shock proteins and occurred protein denaturation because chemical insult (Voellmy, 1994; Boellmann et al., 2004; Voellmy and Boellmann, 2007; Simmons et al., 2009). In the present prediction, 8 compounds were obtained harmful for cellular stress and may alter molecular mechanisms after chronic exposure. Another stress response pathway i.e. mitochondrial membrane potential (MMP), 3 compounds namely Fenvalerate, Cyhalothrin and Deltamethrin were obtained active. It is well-known that mitochondria consist double membrane, which provides the energy to the cell through oxidative phosphorylation and prevent apoptosis (Hill et al., 2018). According to Parikh et al. (1987), yeast mitochondria have adapted a mitochondria-to-nucleus signal transduction pathway termed the retrograde response to induce the transcription of nuclear-encoded mitochondrial genes, and alleviate mitochondrial stress. Moreover, mitochondrial stress by toxins may lead to various diseases (Meyer et al., 2018). In recent research by Richter et al. (2019) emphasized that toxins inhibit the mitochondrial protein synthesis and block with the stress response. Other two parameters such as p53 or Phosphoprotein (tumour suppressor) and ATPase family AAA domain-containing protein 5 (ATAD5) observed inactive for all the

studied compounds. The p53 gene controls the cell cycle arrest, carcinogenesis, DNA damage, apoptosis, etc. and inactivity in the present prediction showed no incidence of carcinogenesis obtained in Table 4. On the other hand, ATAD5 is involved in DNA damage response. This is also involved in a RAD9A-related damage checkpoint, a pathway which is important in determining whether DNA damage is compatible with cell survival or whether it requires cell elimination by apoptosis (Ishii et al., 2005). The inactivity of studied compounds revealed that DNA damage may repair due to no stress response of ATAD5.

4. CONCLUSION

In conclusion, the present predictive results are suitable for academician, researchers, industries, etc. those who are making drugs and environmental chemicals. This web server helps faster screening of large numbers of compounds within short duration as well as without animal testing. This study emphasizes narrow range of compounds, which further easily study in future experimental assay to validate the present prediction.

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