Indian Journal of Animal Sciences **81** (11): 1093–1098, November 2011

Ameliorative efficacy of ashwagandha (*Withania somnifera*) in pesticides intoxicated cockerel

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Received: 28 August 2010; Accepted: 4 June 2011

ABSTRACT

The present study was undertaken to know the ameliorative efficacy of aswagandha in pesticides intoxicated cockerels. Ninety, 8-week old male White Leghorn cockerels were randomly divided into 9 groups of 10 birds each. Group 1 was considered as control and group 2 (endosulfan100 ppm), 3 (chlorpyriphos 250 ppm), 4 (deltamethrin 100 ppm), 5 (fenvalarate 250 ppm), 6 (endosulfan100 ppm + ashwagandha 100 ppm), 7 (chlorpyriphos 250 ppm + ashwagandha 100 ppm), 8 (deltamethrin 100 ppm + ashwagandha 100 ppm), 9 (fenvalarate 250 ppm + ashwagandha 100 ppm) were considered as treated groups. They were fed medicated ration for 24 weeks. Blood samples (4–6 ml) were collected at 12 and 24 week interval from wing vein of cockerels using sterilized disposable syringes in a heparinised test tube and in other test tube without anticoagulant for separating serum for estimation of different biochemical parameters. There was significant increase in total protein, serum bilirubin, serum urea, serum creatinin, serum cholesterol, serum K, AST, ALT and ALP in pesticides intoxicated cockerel at 12 and 24 weeks interval and simultaneously feeding of aswagandha subsides the levels in comparison to control. Similarly significantly decreases the serum Ca, Na, erythrocyte acetylcholine esterase and plasma acetylcholine esterase in pesticides intoxicated cockerel at 12 and 24 weeks interval and simultaneously feeding of aswagandha subsides the levels in comparison to control.

Key words: Aswagandha, Cholpyriphos, Cockerels, Deltamethrin, Endosulfan, Fenvelares, *Withania somnifera*

Endosulfan, an organochlorine insecticide of cyclodiene group, a well known insecticide and, is used against invertebrates. It has a higher solubility in water and hence lower affinity for lipids. As a result of its biomagnifications in food chain is comparatively very less and hence lower chances of cumulative toxicity (WHO 1984). Chronic exposure to endosulfan in rats inhibited 5 HT uptakes. It might also induce monoamine alterations (Lakshmana and Raju 1994). Endosulfan enhances the action of metabolizing enzymes activity (Tyagi *et al.* 1984, Narayan *et al.* 1985), lowers plasma and RBC acetyl cholinesterase activity but elevates brain acetyl cholinesterase activity (Barnard *et al.* 1984). Hepatic microsomal enzymes are involved in the metabolism of xenobiotics (Dixit 2007). Several insecticides either inhibit or potentiate the activity of metabolizing enzymes (Backette and Hayes 1993, Hugqing *et al.* 1996).

Medicinal values of ashwagandha (*Withania somnifera*) have been scientifically proven for various activities including antioxidant, anti-inflammatory (Anbalagan and sadique 1981, 1984), immunomodulatory (Ghosal *et al.* 1989), and antistress (Bhattacharya *et al.* 1997). In view of these facts, the present investigation was carried out to assess the effect of simultaneous administration of pesticides and *Withania somnifera* on biochemical parameters and to know the ameliorative efficacy of ashwagandha in pesticide intoxicated cockerels.

MATERIALS AND METHODS

Animal: White Leghorn cockerals, 8-week-old, procured commercially were used in this study. The animals were kept in uniform management conditions and offered feed and water *ad lib.* till the completion of the study. All the birds were dewormed once before the study, using fenbendazole @ 5 mg/kg body weight.

Chemical: Pure salt of endosulfan, chlorpyrifos, deltametnrin and fenvalerate were procured. Roots of ashwagandha (*Withania somnifera*) were procured from

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Experimental design: Male White Leghorn (90), 8-week-old cockerels were randomly divided into 9 groups of 10 birds each. Group 1 was control, and group 2 (endosulfan100ppm), 3 (chlorpyriphos 250ppm), 4 (deltamethrin 100ppm), 5 (fenvalarate 250ppm), 6 (endosulfan100ppm + ashwagandha 100ppm), 7 (chlorpyriphos 250ppm + ashwagandha 100ppm), 8 (deltamethrin 100ppm + ashwagandha 100ppm), 9 (fenvalarate 250ppm + ashwagandha 100ppm) were considered as treated groups. They were fed medicated ration for 24 weeks. Blood samples (4–6 ml) were collected at 12 and 24 week interval from wing vein of cockerels using sterilized disposable syringes in a heparinised test tube and in other test tube without anticoagulant for separating serum for estimation of total protein (Doumas 1975). Albumin (Reinhold 1953) and serum globulin were estimated by substracting albumin levels from that of total proteins. A:G ratio was enumerated by dividing the serum albumin value with that of serum globulin expressed as g/L. Other parameters estimated were serum glucose (mmol/L) by standard method, total serum bilirubin (mmol/L) (Faulkner and Meites 1982), serum creatinine (µmol/L) (Bowers 1980), serum urea (mmol/L) (Wybenga *et al*. 1971), serum cholesterol (mmol/L) (Allain *et al.* 1974), serum calcium (mmol/L) (Kessier 1964), serum potassium and sodium (mmol/L) (Maruna 1958), acetylcholinesterase (AChE) (U/min/ml) in plasma and erythrocytes (Fishman and Green 1961), aspartate transaminase (AST), alaine transaminase (ALT), alkaline phosphatase (ALP) (U/L) (Bower and Mc Comb 1972). All the values were expressed as mean±SEM. Statistical analysis was done by 1-way and 2-way analysis of variance test. Complete randomized design was used. Inter group comparisons were made by least significant difference. Statistically, significant difference was recorded at 1 and 5% level of significance (Das 2000).

RESULTS AND DISCUSSIONS

There was significant (P <0.01) decline in total serum protein (TSP) content in pesticide alone treated group both at 12 and 24 weeks and pesticide + ashwagandha fed groups revealed a significant (P <0.01) increase in TSP as compared to control and pesticide fed group at 12 at 24 weeks intervals (Tables 1, 2).

A significantly higher A:G ratio in pesticide alone and pesticide + ashwagandha treated cockerels was observed as compared to control at both time intervals. A significant (P <0.01) fall was observed in A:G ratio of pesticide + ashwagandha treated group except for deltamethrin and fenvalerate as compared to pesticide alone group at 12 week interval.

The decrease in the total serum protein and albumin

Table 1 a. Effect of feeding pesticides and ashwagandha at different dietary levels for 12 weeks on biochemical parameters in cockerels (n,10)

Group	Parameters (mean± SE; n=10)								
	Dietary levels (ppm)	Total protien (g/L)	Albumin (g/L)	Globulin (g/L)	A:G	Serum glucose (mmol/L)	Serum bilirubin (mmol/L)	Serum creatinine (mmol/L)	Serum urea (mmol/L)
Control	0	52.975±0.233^{g,A}	27.870±0.077^{c,A}	25.105±0.2466^{e,A}	1.106±0.012^{a,A}	12.930±0.03^{e,A}	1.705±0.004^{b,A}	45.034±0.044^{a,A}	0.634±0.001^{a,A}
Endosulfan	100	47.470±0.264^{d,A}	27.900±0.090^{c,A}	19.270±0.0715^{b,A}	1.438±0.010^{d,B}	11.340±0.065^{d,B}	2.251± 0.012^{f,A}	46.460±0.095^{b,c,A}	0.788±0.0008^{f,A}
Chlopyriphos	250	46.340±0.103^{c,A}	27.860±0.139^{c,A}	18.680±0.163^{a,A}	1.488±0.020^{e,B}	10.950±0.034^{c,B}	2.155±0.010^{e,A}	48.100±0.376^{d,e,A}	0.811±0.0007^{g,A}
Deltamethrin	100	44.600±0.138^{a,A}	26.140±0.047^{a,A}	18.480±0.153^{a,A}	1.411±0.013^{c,d,B}	9.150±0.022^{b,B}	2.470± 0.013^{h,A}	52.287±0.320^{f,A}	0.779±0.001^{e,A}
Fenvelarates	250	45.110±0.050^{b,A}	26.320±0.059^{a,b,A}	18.790±0.058^{a,A}	1.395±0.007^{c,B}	8.970±0.030^{a,B}	2.585±0.021^{i,A}	51.280±0.251^{f,A}	0.774±0.002^{e,A}
Endosulfan + ashwagandha	100 +100	50.200±0.101^{f,A}	28.340±0.182^{d,A}	21.870±0.210^{d,A}	1.292±0.021^{b,B}	11.450±0.061^{d,A}	2.070±0.015^{d,A}	45.826±0.045^{a,b,A}	0.761±0.0002^{d,A}
Chlorpyriphos+ ashwagandha	250 +100	48.450±0.152^{e,A}	28.090±0.048^{d,A}	20.360±0.126^{c,A}	1.374±0.008^{c,B}	11.070±0.026^{c,B}	2.002±0.011^{c,A}	47.269±0.993^{c,d,A}	0.786±0.0004^{f,A}
Deltamethrin+ ashwagandha	100 + 100	46.280±0.059^{c,A}	26.590±0.116^{b,A}	19.690±0.127^{b,A}	1.347±0.014^{c,B}	9.200±0.029^{b,B}	2.262±0.016^{f,A}	48.172±0.144^{d,e,A}	0.721±0.0003^{c,A}
Fenvelarates+ ashwagandha	250 + 100	45.210±0.072^{b,A}	26.430±0.053^{b,A}	18.780±0.117^{a,A}	1.402±0.011^{c,d,B}	9.050±0.022^{a,B}	2.345±0.035^{g,A}	48.422±0.099^{e,A}	0.709±0.001^{b,A}

• Different small alphabets differ significantly (P<0.01) between rows within a column; different capital alphabets differ significantly (P<0.01) between columns within a row.

Table 1 b. Effect of feeding pesticides and ashwagandha at different dietary levels for 12 weeks on biochemical parameters in cockerels (n,10)

Groups	Parameters (mean± SE; n=10)									
	Dietary levels	Serum cholesterol (mmol/L)	Serum calcium (mmol/L)	Serum sodium (mmol/L)	Serum potasium (mmol/L)	Serum erythrocyte AChE(U/mil/ml)	Serum plasmaAChE (U/mil/ml)	AST(U/L)	ALT(U/L)	ALP(U/L)
Control	0	2.325 ±0.052^{a,A}	2.66 ±0.03^{a,b,A}	158.1 ±2.12^{c,d,A}	5.03 ±0.01^{c,A}	2226.740 ±0.068^{f,A}	421.460 ±0.127^{g,A}	155.810 ±0.194^{a,B}	16.870 ±0.032^{a,A}	8.191 ±0.047^{d,B}
Endosulfan	100	2.598 ±0.003^{c,d,A}	2.65 ±0.01^{a,B}	156.16 ±1.99^{b,A}	6.00 ±0.06^{e,A}	2221.500 ±0.341^{c,B}	400.250 ±0.098^{e,A}	165.420 ±0.107^{d,A}	29.525 ±0.069^{i,,B}	10.950 ±0.045^{e,A}
Chlopyriphos	250	2.643 ±0.004^{d,e,f,,A}	2.89 ±0.05^{c,A}	154.14 ±2.06^{a,b,A}	5.4 ±0.06^{d,A}	1523.100 ±0.900^{a,B}	250.580 ±0.157^{a,B}	172.110 ±0.027^{i,A}	31.505 ±0.149^{j,B}	12.263 ±0.036^{g,A}
Deltamethrin	100	2.656 ±0.007^{e,f,A}	2.72 ±0.29^{b,B}	152.30 ±2.0^{a,A}	4.82 ±1.12^{b,A}	2225.200 ±0.200^{e,A}	397.720 ±0.398^{d,B}	169.620 ±0.077^{g,A}	24.650 ±0.145^{h,A}	13.150 ±0.037^{h,A}
Fenvelarates	250	2.664 ±0.009^{f,A}	2.70 ±0.23^{a,b,B}	153.30 ±5.96^{a,b,A}	4.6 ±2.12^{a,A}	2223.300 ±0.213^{d,A}	395.650 ±0.236^{c,A}	170.079 ±0.023^{h,A}	23.025 ±0.191^{f,B}	11.270 ±0.055^{f,A}
Endosulfan + ashwagandha	100 +100	2.578 ±0.001^{c,A}	2.65 ±0.03^{a,B}	156.65 ±1.02^{b,c,d,A}	6.00 ±1.12^{e,A}	2223.500 ±0.341^{d,A}	411.480 ±0.431^{f,A}	162.170 ±0.044^{c,A}	22.375 ±0.130^{e,B}	7.646 ±0.019^{b,A}
Chlorpyriphos+ ashwagandha	250 +100	2.586 ±0.009^{c,B}	2.88 ±0.03^{c,B}	155.21 ±2.12^{a,b,c,A}	5.5 ±1.0^{d,A}	1655.100 ±1.376^{b,B}	311.490 ±0.640^{b,B}	167.990 ±0.043^{f,A}	23.625 ±0.100^{g,B}	7.333 ±0.008^{a,A}
Deltamethrin+ ashwagandha	100 + 100	2.609 ±0.001^{c,d,e,A}	2.71 ±0.08^{a,b,B}	153.33 ±3.33^{a,b,A}	4.92 ±0.02^{b,c,A}	2225.800 ±0.249^{e,f,A}	398.530 ±0.381^{d,B}	166.081 ±0.024^{e,A}	19.315 ±0.100^{d,B}	7.250 ±0.012^{a,A}
Fenvelarates+ ashwagandha	250 + 100	2.614 ±0.001^{c,d,e,B}	2.69 ±1.0^{a,b,B}	154.23 ±3.09^{a,b,A}	4.80 ±1.0^{b,A}	2224.100 ±0.233^{d,e,A}	396.300 ±0.213^{c,A}	165.840 ±0.092^{e,A}	17.760 ±0.151^{c,B}	7.261 ±0.030^{a,A}

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content in the birds exposed to pesticide can be attributed to the hepatic dysfunction and damage to the kidney tubules, which resulted in elimination of more protein from the body and finding was corroborated with findings in calves (Shukla 1991), birds (Singh 1997, Kumar 2000), rats (Ebert *et al.* 1985), and in rat and mice (Gomes 1999, Anand 2000, Rawat 2002); along with hypoglycemia caused by administration of different pesticides in chicks (Mitra 1975, Singh 1997).

The serum glucose levels (mmol/L) did not vary significantly (P<0.01) with the time course. There was a significant (P<0.01) depreciation in serum glucose levels of cockerels of pesticide alone and pesticide + ashwagandha treated groups in comparison to control at the interval of 12 and 24 weeks. A significant reduction in serum glucose levels was observed following prolonged administration of all pesticides used in this study. Hypoglycemia observed in this investigation might have resulted due to depletion of glycogen or disruption of enzymic activity involved in the glycogenolysis and gluconeogenesis as a consequence of hepatotoxic effect of the insecticide. These results are comparable with hypoglycemia caused by administration of different pesticides in chicks (Singh 1997). Serum bilirubin (mmol/L) was significantly (P<0.01) increased in all pesticide treated groups.

However, cockerels fed on ashwagandha + pesticide revealed a significant (P<0.01) reduction in bilirubin levels as compared to pesticide alone group and this was further significantly (P<0.01) decreased with time. The serum urea significantly (P<0.01) increased in all the groups as compared to control. There was significant (P<0.01) decline in urea concentration in pesticide + ashwagandha treated group as compared to pesticide alone group. Serum creatinine level significantly (P<0.01) increases in all pesticide groups as well as pesticide + ashwagandha treated groups. There was no significant change in level of cholesterol in all pesticide treated groups as well as pesticide + ashwagandha treated group at 12 weeks but it varied (P<0.01) at 24 weeks in comparison to control. The values of pesticide + ashwagandha group were significantly (P<0.01) lower than the pesticide alone group at 12 and 24 weeks intervals. A significant increase in level of bilirubin, urea and creatanine and cholesterol in pesticide and pesticide + aswagandha treated group was indication of nephrotoxic effect of pesticides. This finding is corroborated with finding of Pickering (1982, Singh 1997, Krishnappa *et al.* 2000). The serum calcium levels significantly (P<0.01) increased in all pesticide alone and pesticide+ashwagandha medicated groups in comparison to control. Serum potassium level (mmol/L) significantly (P<0.01) increased with endosulfan and chlorpyrifos however, it declined (P<0.01) in deltamethrin and fenvalerate treated groups at 12 and 24 weeks intervals (Table 1b and 2b). There was a significant (P<0.05) decrease in serum sodium level in all pesticide treated as well as pesticide + ashwagandha treated groups as

compared to control at 12 and 24 weeks intervals. The decreased sodium levels in the serum might be due to the excessive loss of sodium through faeces and urine. The absorption of sodium might have been hampered in the birds administered with pesticide. Endosulfan also causes the influx of sodium in the axoplasm, which may also contribute to the decreased levels of sodium in the serum (Gupta 1976). This study was justified from the finding of Singh (1997) and Walden (1982). A significant (P<0.01) suppression of erythrocyte acetylcholinesterase activity in pesticide treated groups (more in chlorpyrifos) and pesticide + ashwagandha treated cockerels in comparison to control groups after 12 weeks trial. This suppression increases (P<0.01) with time course. There was a significant (P<0.01) increase in erythrocyte acetylcholinesterase activity in pesticide + ashwagandha treated group as compared to control and pesticide alone group. Similarly, blood acetylcholinesterase activity was significantly (P<0.01) inhibited in all the pesticide and pesticide + ashwagandha (more in chlorpyrifos) groups at 12 week interval. All these values varied significantly (P<0.01) when compared with period. Inhibition of AChE in case of pesticide toxicity is of diagnostic importance and is used as a biomarker for OP toxicity (Klaassen 1999). In case of endosulfan toxicity, workers have reported a decline in AChE activity in the plasma and RBC but not in the brain (Barnard 1984).

A significant (P<0.01) elevation in the levels of AST activity of all pesticides were observed both at 12 and 24 weeks in comparison to control. Significant (P<0.01) and higher increase was observed in the activity of ALT in chlorpyrifos> endosulfan >deltamethrin> fenvalerate treated cockerels both at 12 and 24 weeks. There was a significant (P<0.01) increase in pesticide + ashwagandha groups as compared to pesticide alone and control group at both 12 and 24 weeks interval. A significant (P<0.01) appreciation in the alkaline phosphatase activity in pesticide treated groups was noted in comparison to other. The pesticide + ashwagandha treated group did not reveal any change in activity of the enzyme throughout the study.

The serum AST and ALT activities increased following pesticide exposure. These enzymes are used as an indicator for damage to vital organs in the body. The increase in the serum activity of these enzymes in this study indicated the damage to the organs like liver, heart or muscle (Cornelius 1989). Our result were in agreement with those of Krtastav *et al.* (1995) and Choudhary and Joshi (2002). Ashwagandha treated cockerels did not cause any alteration in serum proteins. This was indicative of tonic and hepatoprotective action of ashwagandha. It was also evident from positive effect on weight gain of liver in ashwagandha medicated animals (Aphale *et al.* 1998, Rao *et al.* 1999). Schliebs *et al.* (1997), have also reported an enhanced AChE activity and muscarinic cholinergic receptor binding in nervous tissue following ashwagandha medication in animals.

Table 2 a. Effect of feeding pesticides and ashwagandha at different dietary levels for 24 weeks on biochemical parameters in cockerels (n, 10)

Group	Dietary levels (ppm)	Parameters (Mean± SE; n=10)							
		Total Protien (g/L)	Albumin (g/L)	Globulin (g/L)	A:G	Serum glucose (mmol/L)	Serum bilirubin (mmol/L)	Serum Creatinine (mmol/L)	Serum Urea (mmol/L)
Control									
Control	0	$54.500\pm0.102^{h,B}$	$29.230\pm0.053^{e,B}$	$25.260\pm0119^{f,B}$	$1.153\pm0.007^{b,B}$	$14.250\pm0.104^{h,B}$	$1.711\pm0.003^{a,A}$	$47.056\pm0.996^{a,B}$	$0.652\pm0.0005^{a,B}$
Endosulfan	100	$52.855\pm0.123^{f,B}$	$29.101\pm0.050^{d,e,B}$	$23.845\pm0.137^{d,B}$	$1.212\pm0.008^{d,A}$	$11.200\pm0.029^{f,A}$	$4.356\pm0.018^{b,B}$	$58.123\pm0.031^{c,B}$	$0.821\pm0.0004^{b,B}$
Chlopyriphos	250	$52.100\pm0.100^{e,B}$	$29.080\pm0.038^{d,B}$	$23.020\pm0.120^{c,B}$	$1.261\pm0.007^{f,A}$	$10.270\pm0.036^{d,A}$	$4.242\pm0.024^{g,B}$	$59.030\pm0.008^{c,B}$	$0.845\pm0.001^{i,B}$
Deltamethrin	100	$48.00\pm0.059^{a,B}$	$27.090\pm0.031^{a,b,B}$	$20.910\pm0.076^{a,B}$	$1.289\pm0.005^{g,A}$	$8.650\pm0.060^{b,A}$	$3.855\pm0.015^{e,B}$	$68.561\pm0.507^{f,B}$	$0.786\pm0.001^{f,A}$
Fenvelarates	250	$48.635\pm0.139^{b,B}$	$26.940\pm0.037^{a,B}$	$21.695\pm0.143^{b,B}$	$1.236\pm0.008^{e,A}$	$8.310\pm0.023^{a,A}$	$3.775\pm0.013^{d,B}$	$67.117\pm0.033^{e,B}$	$0.779\pm0.0006^{e,A}$
Endosulfan + ashwagandha	100+100	$54.230\pm0.049^{h,B}$	$29.290\pm0.064^{e,B}$	$24.940\pm0.080^{e,B}$	$1.171\pm0.005^{c,A}$	$11.970\pm0.033^{g,B}$	$4.140\pm0.026^{f,B}$	$54.069\pm0.067^{b,B}$	$0.801\pm0.0009^{g,B}$
Chlorpyriphos+ ashwagandha	250+100	$53.070\pm0.078^{f,g,B}$	$28.870\pm0.197^{c,B}$	$24.00\pm0.086^{d,B}$	$1.206\pm0.004^{d,A}$	$10.660\pm0.042^{e,B}$	$3.829\pm0.010^{e,B}$	$54.655\pm0.161^{b,B}$	$0.818\pm0.0015^{h,B}$
Deltamethrin+ ashwagandha	100+100	$49.120\pm0.075^{c,B}$	$27.170\pm0.021^{b,B}$	$21.950\pm0.074^{b,B}$	$1.234\pm0.004^{e,A}$	$9.120\pm0.029^{c,A}$	$3.487\pm0.011^{c,B}$	$66.143\pm0.022^{e,B}$	$0.753\pm0.004^{c,B}$
Fenvelarates+ ashwagandha	250+100	$50.170\pm0.030^{d,B}$	$27.080\pm0.020^{a,b,B}$	$23.090\pm0.037^{h,B}$	$1.167\pm0.002^{h,c,A}$	$8.580\pm0.029^{b,A}$	$3.410\pm0.002^{b,B}$	$64.287\pm0.0157^{d,B}$	$0.760\pm0.0009^{d,B}$

• Different small alphabets differ significantly (P<0.01) between rows within a column; different capital alphabets differ significantly (P<0.01) between column within a row.

Table 2 b. Effect of feeding pesticides and ashwagandha at different dietary levels for 24 weeks on biochemical parameters in cockerels (n=10)

Groups	Dietary levels	Parameters (mean± SE; n=10)								
		Serum cholesterol (mmol/L)	Serum calcium (mmol/L)	Serum sodium (mmol/L)	Serum potasium (mmol/L)	Serum erythrocyte AChE(U/mil/ml)	Serum plasmaAChE (U/mil/ml)	AST(U/L)	ALT(U/L)	ALP(U/L)
Control	0	2.433 ±0.006^{b,B}	2.67 ±0.04^{b,A}	159.03 ±1.12^{a,A}	5.12 ±0.12^{a,B}	2236.670 ±0.185^{f,B}	422.700 ±0.059^{h,B}	145.180 ±0.051^{a,A}	17.110 ±0.027^{a,b,B}	7.929 ±0.021^{d,A}
Endosulfan	100	2.799 ±0.011^{e,B}	2.30 ±0.14^{a,A}	158.59 ±2.4^{a,B}	6.27 ±0.06^{b,B}	2232.900 ±0.233^{e,A}	401.580 ±0.210^{f,B}	173.110 ±1.010^{d,B}	27.700 ±0.138^{g,A}	11.670 ±0.054^{e,B}
Chlopyriphos	250	2.870 ±0.001^{f,B}	2.90 ±0.05^{d,A}	156.49 ±2.40^{a,B}	5.86 ±0.06^{b,B}	1249.900 ±1.622^{a,A}	230.910 ±0.129^{a,A}	178.070 ±0.026^{f,B}	31.275 ±0.069^{h,A}	12.765 ±0.039^{g,B}
Deltamethrin	100	2.864 ±0.002^{f,B}	2.65 ±0.41^{b,A}	159.73 ±3.02^{a,B}	4.8 ±0.12^{a,A}	2230.00 ±0.258^{d,A}	394.930 ±0.486^{c,A}	175.720 ±0.057^{e,B}	25.230 ±0.108^{f,B}	13.510 ±0.043^{h,B}
Fenvelarates	250	2.849 ±0.005^{f,B}	2.63 ±0.39^{b,A}	160.11 ±2.02^{a,B}	4.75 ±1.12^{a,B}	2226.500 ±0.166^{c,B}	396.200 ±0.326^{d,B}	174.790 ±0.075^{d,e,B}	22.970 ±0.091^{e,A}	12.260 ±0.047^{f,B}
Endosulfan + ashwagandha	100 +100	2.688 ±0.044^{d,B}	2.32 ±0.04^{d,A}	159.50 ±2.02^{a,B}	6.22 ±1.13^{b,B}	2233.900 ±0.233^{e,B}	412.210 ±0.126^{g,B}	169.300 ±0.089^{b,B}	18.121 ±0.038^{c,A}	7.781 ±0.079^{c,B}
Chlorpyriphos+ ashwagandha	250 +100	2.611 ±0.001^{c,A}	2.83 ±0.03^{c,A}	157.12 ±3.21^{a,B}	5.92 ±1.11^{b,B}	1447.900 ±0.737^{b,A}	243.620 ±1.039^{b,A}	174.150 ±0.045^{e,B}	21.065 ±0.827^{d,A}	7.564 ±0.004^{b,B}
Deltamethrin+ ashwagandha	100 + 100	2.712 ±0.0003^{d,B}	2.66 ±0.08^{b,A}	159.12 ±3.33^{a,B}	4.92 ±0.04^{a,A}	2232.800 ±0.5538^{e,B}	397.420 ±0.331^{e,A}	172.070 ±0.021^{c,B}	18.240 ±0.112^{c,A}	7.317 ±0.002^{a,B}
Fenvelarates+ ashwagandha	250 + 100	2.609 ±0.006^{c,A}	2.65 ±1.12^{b,A}	160.00 ±3.02^{a,B}	4.82 ±0.03^{a,A}	2228.00 ±0.149^{c,B}	396.800 ±0.249^{d,e,B}	171.280 ±0.234^{c,B}	17.680 ±0.132^{b,c,A}	7.279 ±0.018^{a,B}

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