



Toxicological studies on *Helicoverpa armigera* in pigeonpea growing in Vidarbha region of Maharashtra, India

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Abstract: Insecticide resistance level in pigeonpea pod borer, *Helicoverpa armigera* (Hubner) to technical grade insecticides collected from major pigeonpea growing districts of Vidarbha viz., Akola, Amravati, Buldhana, Yavatmal and Washim was worked out. LDP indicated LD₅₀ of Cypermethrin in the range of 1.402 to 9.209 ppm with maximum in Yavatmal (9.209 ppm); LD₉₀ within range of 6.021 to 18.427 ppm. LD₅₀ of Quinalphos in the range of 1.303 to 4.789 ppm with maximum in Yavatmal (4.789 ppm); LD₉₀ within range of 3.150 to 14.194 ppm. LD₅₀ of Methomyl in the range of 1.297 to 3.792 ppm with maximum in Yavatmal (3.792 ppm); LD₉₀ within range of 4.993 to 16.737 ppm. LD₅₀ of Indoxacarb in the range of 0.521 to 2.709 ppm with maximum in Yavatmal (2.709 ppm); LD₉₀ within range of 2.819 to 20.947 ppm. LD₅₀ of Spinosad in the range of 0.713 to 2.408 ppm with maximum in Buldhana (2.408 ppm); LD₉₀ within range of 6.413 to 18.349 ppm. The resistance level is visibly high in cypermethrin, moderate to indoxacarb, quinalphos, spinosad and low to methomyl; Yavatmal and Washim strains expressed higher resistance level to cypermethrin, quinalphos and methomyl, whereas Yavatmal and Buldhana strains expressed higher resistance level to indoxacarb and spinosad. The investigation will help to track resistance level in *Helicoverpa armigera* to different groups of insecticides.

Keyword: Cypermethrin, Indoxacarb, Methomyl, Quinalphos, Spinosad

INTRODUCTION

Helicoverpa armigera (Hubner) (Lepidoptera: Noctuidae) is the most dreaded species commonly known as cotton bollworm, pigeonpea pod borer, American bollworm. It is a polyphagous pest of worldwide occurrence inflicting annual crop damage worth US \$1 billion in India (Upendhar *et al.*, 2011). In India this insect has been recorded on more than 200 hosts (Pawar, 1998). About 250 species of insects belonging to 8 orders and 61 families have been found to attack on pigeonpea, among these gram pod borer, *H. armigera* (Hubner), tur plume moth, *Exelastis atomosa* (Walsh) and tur pod fly, *Melanogamyza obtusa* (Mall) are important pod feeders of pigeonpea (Durairaj, 1999) causing considerable losses in grain yield ranging from 3-100 per cent (Sharma *et al.*, 2001). The losses due to *H. armigera* alone contribute upto 50 per cent (Thakre, 2001).

The management of *Helicoverpa* has become increasingly difficult due to indiscriminate and extensive use of chemical insecticides which led to development of resistance to insecticides (Armes *et al.*, 1996; Kranthi *et al.*, 1997; Ramasubramaniam and Regupathy 2004). Insecticide resistance in *H. armigera* in India was first recorded in 1987 when widespread field control failure were reported by farmers growing cotton and pulse crop in Andhra Pradesh (McCaffery *et al.*, 1989). This

trend still exists though, not documented in the literature reviewed. Resistance monitoring is an indispensable prerequisite in designing any IPM programme. Thus, an assessment of resistance profile and mechanism of resistance would be key for the management of *H. armigera* and to decide the management strategy for management of insecticide resistant strains which could help in high production and productivity in pigeonpea. The present investigation was carried out to evaluate the level of resistance to commonly used insecticides in *H. armigera* collected from different locations of Vidarbha that will help to track the level of resistance to different groups of insecticides.

MATERIALS AND METHODS

Test insect: *H. armigera* (Hub.) larvae were collected from different fields of pigeonpea during 2013-14 from five major pigeonpea growing districts of Vidarbha (Amravati, Yavatmal, Buldhana, Akola and Washim). Field collected populations were reared on semi synthetic diet using standard rearing technique (Armes *et al.*, 1992) in laboratory. Eggs collected from mating chamber were allowed to hatch and third instar larvae of F₁ generation were used to evaluate the level of resistance of *H. armigera* to commonly used insecticides.

Method of preparation of artificial semi-synthetic diet (Armes *et al.*, 1992): All ingredients were kept

together, one fraction was prepared by boiling agar upto boiling point in half quantity of water, then yeast was added and the mixture was again boiled. The second fraction containing other ingredients except sorbic acid, vitamin supplement and streptomycin were weighed as per recipe and were homogeneously mixed in mixer. These two fractions were thoroughly mixed together after adding remaining ingredients. The semi-liquid diet was poured in petri-plates. The diet was allowed to cool and then pour into multicellular tray for easy feeding. For the preservation of diets for few days, they were stored in refrigerator. The recipe of ingredients of different diets is given in Table 2.

Insecticide: Five technical grade insecticides from different groups were used, cypermethrin 10 EC (synthetic pyrethroid), quinalphos 25 EC (organophosphate), methomyl 40 SP (carbamate), indoxacarb 15.8 EC (oxidiazin) and spinosad 45 SC (microcyclic lactone).

Bioassay: The bioassays were conducted by topical application using Hamilton micro applicator to evaluate the toxicity of test insecticides (Kranthi *et al.*, 2002).

Topical application method: Graded stock solutions through serial dilution technique of the test insecticide were prepared from the technical grade products by dissolving the required quantities after accurate weighing in acetone. One microlitre of the test insecticide solution was applied on the dorsum of thoracic segment by micro applicator. Three replications were maintained for each concentration with 30 larvae for each concentration and minimum 5-7 insecticide concentration. Larvae were held individually in 12-well tissue culture plates containing artificial diet, at 25 ± 2 °C for 6 d when mortality was recorded. All rearing and bioassay operations were carried out at 25 ± 2 °C under a photoperiod of 12:12 (L:D) hours (Kranthi *et al.*, 2002).

Maintenance of susceptible strain: *H. armigera* were collected from pigeonpea field (unsprayed) from dif-

ferent locations and maintained in laboratory for seven generation without exposure to insecticides.

RESULTS AND DISCUSSION

Log dose probit assays were carried to determine the median lethal concentration and level of resistance of different insecticides in *H. armigera* (Hubner) strains collected from the different locations of Vidarbha, M.S., India, during 2013-14.

Toxicity and level of resistance of insecticides to *H. armigera* (Hubner)

Cypermethrin: Cypermethrin indicated significant levels of resistance in field collected populations from Yavatmal and Washim. LD₅₀ values of Cypermethrin ranged from 1.402 to 9.209 ppm (Table 2). High level of resistance was observed in Yavatmal population. The LD₅₀ of Yavatmal strain was 9.209 ppm, while its LD₉₀ was 18.427 ppm. It was followed by Washim with LD₅₀ value 8.176, Amaravati with LD₅₀ value 7.551, Akola with LD₅₀ value 7.354 and Buldhana with LD₅₀ value 6.803 ppm, respectively. The laboratory strain was found most susceptible amongst the strain tested. LD₅₀ for laboratory strain was 1.402 ppm whereas, LD₉₀ was 6.021 ppm. The fiducial limits at 95 per cent of LD₅₀ were between 0.941 and 2.890 ppm. The DrPDKV strain was followed by laboratory strain having 5.841 ppm LD₅₀ value and LD₉₀ was 10.887 ppm.

Yavatmal strain showed resistance ratio of 6.56 and 1.57 as compare to laboratory and DrPDKV strain, followed by Washim with 5.83 and 1.40, Amaravati with 5.38 and 1.29, Akola with 5.24 and 1.25, Buldhana with 4.85 and 1.16, respectively. The order of toxicity of Cypermethrin to *Helicoverpa armigera* (Hubner) was Yavatmal > Washim > Amaravati > Akola > Buldhana > DrPDKV.

Quinalphos: Quinalphos indicated significant levels of resistance in field collected populations from Yavatmal and Amaravati. LD₅₀ values of Quinalphos ranged from 1.303 to 4.789 ppm (Table 2). High level of resistance was observed in Yavatmal population. The LD₅₀ of Yavatmal strain was 4.789 ppm, while its LD₉₀ was 14.194 ppm. It was followed by Washim with LD₅₀ value 4.250, Amaravati with LD₅₀ value 4.089, Akola with LD₅₀ value 3.968 and Buldhana with LD₅₀ value 3.466 ppm, respectively. The laboratory strain was found most susceptible amongst the strain tested. LD₅₀ for laboratory strain was 1.303 ppm whereas, LD₉₀ was 3.150 ppm. The fiducial limits at 95 per cent of LD₅₀ were between 0.591 and 1.759 ppm. The DrPDKV strain was followed by laboratory strain having LD₅₀ value 3.466 ppm.

Yavatmal strain showed resistance ratio of 3.67 and 1.53 as compare to laboratory and DrPDKV strain, followed by Washim with 3.26 and 1.36, Amaravati with 3.13 and 1.31, Akola with 3.04 and 1.27, Buldhana with 2.66 and 1.11, respectively. The order of

Table 1. Ingredients for the preparation of semisynthetic diet used for rearing of *H. armigera* larvae.

S. N.	Ingredients	Quantity
Part – A		
1	Chickpea flour	160 g
2	Wheat germ	60 g
3	Ascorbic acid	5.3 g
4	Methyl-4-hydroxybenzoate	3.3 g
5	Sorbic acid	1.7 g
6	Aureomycin	2.5 g
7	Formaldehyde (10 % v/v)	13.5 ml
8	Distilled water	550 ml
Part – B		
1	Yeast (Dried)	53 g
2	Agar-agar	16 g
3	Distilled water	550 ml

Table 2. Toxicity and level of resistance of different insecticides to *H. armigera* (Hub) (150 larvae/concentration).

Insecticides	Strain	LD ₅₀ (95% FL)	LD ₉₀ (95% FL)	Slope (±SE)	Chi Square	RR over	
						FS	LS
Cypermethrin	Akola	7.354 (6.444 - 8.450)	12.765 (10.3 -24.123)	5.351 ± 1.42	0.022	1.25	5.24
	Amaravati	7.551 (6.637 - 8.76)	13.014 (10.45- 25.33)	5.420 ± 1.46	0.108	1.29	5.38
	Buldhana	6.803 (5.816 - 7.70)	12.171 (9.885 - 22.45)	5.073 ± 1.35	0.184	1.16	4.85
	Washim	8.176 (7.03 - 9.886)	13.127 (10.52 - 33.16)	6.233 ± 2.04	0.149	1.40	5.83
	Yavatmal	9.209 (7.786 -16.75)	18.427 (12.4- 192.52)	4.254 ± 1.56	0.108	1.57	6.56
	DrPDKV (Field)	5.841 (4.337 - 6.82)	10.887 (9.12 - 22.45)	4.623 ± 1.36	0.230		
Quinalphos	Lab Sus-ceptible	1.402 (0.941 - 2.89)	6.021 (3.138 - 7.954)	4.05 ± 1.85	0.526		
	Akola	3.968 (3.10 - 5.025)	10.521 (7.308 - 28.94)	3.026 ± 0.77	0.320	1.27	3.04
	Amaravati	4.089 (3.27 - 5.354)	12.209 (7.99 - 40.00)	2.697 ± 0.68	0.456	1.31	3.13
	Buldhana	3.466 (2.66 - 4.118)	7.308 (5.795 - 12.44)	3.955 ± 0.91	0.747	1.11	2.66
	Washim	4.250 (3.25 - 5.817)	12.799 (8.114 - 56.37)	2.676 ± 0.75	0.646	1.36	3.26
	Yavatmal	4.789 (3.56 - 7.371)	14.194 (8.53 -121.57)	2.716 ± 0.88	0.043	1.53	3.67
Methomyl	DrPDKV (Field)	3.121 (2.337 - 4.82)	6.887 (5.12 - 22.453)	4.023 ± 1.06	0.267		
	Lab Sus-ceptible	1.303 (0.591 - 1.76)	3.150 (2.545 - 4.352)	3.34 ± 0.85	0.840		
	Akola	3.099 (2.03 - 4.379)	12.058 (7.126 - 67.54)	2.172 ± 0.62	0.514	1.45	2.38
	Amaravati	3.640 (2.446 - 5.51)	13.698 (7.736 -125.3)	2.226 ± 0.70	0.567	1.61	2.66
	Buldhana	2.738 (2.03 - 3.556)	10.151 (6.56 - 29.46)	2.252 ± 0.51	1.589	1.28	2.11
	Washim	3.452 (2.383-5.329)	15.809 (8.367-143.15)	1.939 ± 0.56	0.890	1.70	2.80
Indoxacarb	Yavatmal	3.792 (2.496 - 6.44)	16.737 (8.53 -298.99)	1.987 ± 0.65	0.437	1.77	2.92
	DrPDKV (Field)	2.138 (1.73 - 3.156)	8.151 (6.56 - 19.46)	2.282 ± 0.91	1.589		
	Lab Sus-ceptible	1.297 (0.691 - 1.76)	4.993 (3.617 - 9.922)	2.188 ± 0.50	2.886		
	Akola	1.574 (0.974 -2.263)	9.150 (5.17 -36.887)	1.676 ± 0.39	0.909	1.47	3.02
	Amaravati	1.802 (1.026 - 2.77)	11.285 (5.839 - 73.62)	1.608 ± 0.42	0.435	1.68	3.45
	Buldhana	2.311 (1.31 - 3.547)	10.852 (5.91 - 81.94)	1.907 ± 0.56	0.710	2.15	4.43
Spinosad	Washim	2.148 (1.29 - 3.288)	11.449 (6.096 - 72.53)	1.763 ± 0.47	0.784	2.0	4.12
	Yavatmal	2.709 (1.548 -5.538)	20.947 (8.36 -799.64)	1.442 ± 0.45	0.504	2.52	5.19
	DrPDKV (Field)	1.074 (0.574 -1.963)	7.150 (4.17 -26.887)	1.963 ± 0.39	1.209		
	Lab Sus-ceptible	0.521 (0.38 - 0.948)	2.819 (2.02 - 5.06)	2.07 ± 0.40	1.049		
	Akola	1.694 (0.85 - 2.722)	16.299 (7.48 -154.41)	1.303 ± 0.34	2.040	1.41	2.37
	Amaravati	2.025 (1.12 - 3.135)	14.909 (7.34 -121.43)	1.478 ± 0.39	2.227	1.68	2.84
	Buldhana	2.408 (1.20 - 3.711)	13.523 (6.83 -233.89)	1.709 ± 0.56	0.507	2.01	3.37
	Washim	1.607 (0.94 - 2.414)	13.634 (6.90 -72.681)	1.380 ± 0.32	2.967	1.33	2.25
	Yavatmal	2.334 (1.001- 3.416)	10.340 (5.75- 189.48)	1.982 ± 0.70	1.084	1.94	3.27
	DrPDKV (Field)	1.207 (0.689 - 2.31)	18.349 (9.84 -183.41)	1.113 ± 0.38	1.349		
	Lab Sus-ceptible	0.713 (0.36 - 1.266)	6.413 (3.79 - 19.38)	1.429 ± 0.31	6.677		

toxicity of Quinalphos to *Helicoverpa armigera* (Hubner) was Yavatmal > Washim > Amravati > Akola > Buldhana > DrPDKV.

Methomyl: Methomyl indicated significant levels of resistance in field collected populations from Yavatmal and Amaravati. LD₅₀ values of Methomyl ranged from 1.297 to 3.792 ppm (Table 2). High level of resistance was observed in Yavatmal population. The LD₅₀ of Yavatmal strain was 3.792 ppm, while its LD₉₀ was 16.737 ppm. It was followed by Washim with LD₅₀ value 3.640, Amaravati with LD₅₀ value 3.452, Akola with LD₅₀ value 3.099 and Buldhana with LD₅₀ value 2.738 ppm, respectively. The laboratory strain was found most susceptible amongst the strain tested. LD₅₀ for laboratory strain was 1.297 ppm whereas, LD₉₀ was 4.993 ppm. The fiducial limits at 95 per cent of LD₅₀ were between 0.691 and 1.762 ppm. The LD₅₀ of DrPDKV strain was followed by laboratory strain having values 2.138 ppm.

Yavatmal strain showed resistance ratio of 2.92 and 1.77 as compared to laboratory and DrPDKV strain, followed by Washim with 2.80 and 1.70, Amravati with 2.66 and 1.61, Akola with 2.38 and 1.45, Buldhana with 2.11 and 1.28, respectively. The order of

toxicity of Methomyl to *Helicoverpa armigera* (Hubner) was Yavatmal > Amravati > Washim > Akola > Buldhana > DrPDKV.

Indoxacarb: Indoxacarb indicated significant levels of resistance in field collected populations from Yavatmal and Buldhana. LD₅₀ values of Indoxacarb ranged from 0.521 to 2.709 ppm (Table 2). High level of resistance was observed in Yavatmal population. The LD₅₀ of Yavatmal strain was 2.709 ppm, while its LD₉₀ was 20.947 ppm. It was followed by Buldhana with LD₅₀ value 2.311, Washim with LD₅₀ value 2.148 and Amaravati with LD₅₀ value 1.802, Akola with LD₅₀ value 1.574 ppm, respectively. The laboratory strain was found most susceptible amongst the strain tested. LD₅₀ for laboratory strain was 0.521 ppm whereas, LD₉₀ was 2.819 ppm. The fiducial limits at 95 per cent of LD₅₀ were between 0.382 and 0.948 ppm. The DrPDKV strain was followed by laboratory strain having 1.074 ppm LD₅₀ value and LD₉₀ was 16.299 ppm.

Yavatmal strain showed resistance ratio of 5.19 and 2.52 as compared to laboratory and DrPDKV strain, followed by Buldhana with 4.43 and 2.15, Washim with 4.12 and 2.0, Amaravati with 3.45 and 1.68, Akola with 3.02 and 1.47, respectively. The order of toxic-

ty of Indoxacarb to *Helicoverpa armigera* (Hubner) was Yavatmal > Buldhana > Washim > Amravati > Akola > DrPDKV.

Spinosad: Spinosad indicated significant levels of resistance in field collected populations from Buldhana and Yavatmal. LD₅₀ values of Spinosad ranged from 0.713 to 2.408 ppm (Table 2). High level of resistance was observed in Buldhana population. The LD₅₀ of Buldhana strain was 2.408 ppm, while its LD₉₀ was 13.523 ppm. It was followed by Yavatmal with LD₅₀ value 2.334, Amravati with LD₅₀ value 2.025, Akola with LD₅₀ value 1.694 and Washim with LD₅₀ value 1.607 ppm, respectively. The laboratory strain was found most susceptible amongst the strains tested. LD₅₀ for laboratory strain was 0.713 ppm whereas, LD₉₀ was 6.413 ppm. The fiducial limits at 95 per cent of LD₅₀ were between 0.365 and 1.266 ppm. The LD₅₀ of DrPDKV strain was followed by laboratory strain having values 1.207 ppm.

Buldhana strain showed resistance ratio of 3.37 and 2.01 as compared to laboratory and DrPDKV strain, followed by Yavatmal with 3.27 and 1.94, Amravati with 2.84 and 1.68, Akola with 2.37 and 1.41, Washim with 2.25 and 1.33, respectively. The order of toxicity of Spinosad to *Helicoverpa armigera* (Hubner) was Buldhana > Yavatmal > Amravati > Akola > Washim > DrPDKV.

LDP during 2013-14 indicated LD₅₀ value of Cypermethrin in the range of 1.402 to 9.209 ppm with maximum in Yavatmal (9.209 ppm) and LD₉₀ value within range of 6.021 to 18.427 ppm. LD₅₀ value of Quinalphos in the range of 1.303 to 4.789 ppm with maximum in Yavatmal (4.789 ppm) and LD₉₀ value within range of 3.150 to 14.194 ppm. LD₅₀ value of Methomyl in the range of 1.297 to 3.792 ppm with maximum in Yavatmal (3.792 ppm) and LD₉₀ value within range of 4.993 to 16.737 ppm. LD₅₀ value of Indoxacarb in the range of 0.521 to 2.709 ppm with maximum in Yavatmal (2.709 ppm) and LD₉₀ value within range of 2.819 to 20.947 ppm. LD₅₀ value of Spinosad in the range of 0.713 to 2.408 ppm with maximum in Buldhana (2.408 ppm) and LD₉₀ value within range of 6.413 to 18.349 ppm.

The pesticides continue to play a pivotal role in pest control because of which the situation is accentuated by rapid rise in resistance toward insecticides. Several new insecticide molecules are being introduced in the market for controlling pests. In the present investigation, efforts were made to monitor levels of insecticide resistance in *H. armigera* (Hubner) collected from various locations of Vidarbha, M.S., India.

These findings are in the same line as the results reported by previous workers. Rao *et al.* (2005) carried out Insecticide Resistance studies on *Helicoverpa armigera* (Hubner) against cypermethrin 0.1 µg/µl, fenvalrate 0.2 µg/µl, endosulfan 10 µg/µl, quinalphos 0.75 µg/µl, and methomyl 0.1 µg/µl. Synthetic pyrethroids showed

high resistance, low to moderate against methomyl and quinalphos showed moderate to above moderate level. The *Helicoverpa armigera* has developed 946 folds resistance against cypermethrin whereas, only 13 folds resistance was observed against quinalphos. Sampath kumar *et al.* (2007, 2008) carried out toxicity of indoxacarb and spinosad against spotted boll worm, *Earias vittella* revealed an LD₅₀ value of 0.09270 µg/larva and 0.00188 µg/larva respectively. Ghodaki (2009) reported the resistance development due to constant selection pressure with indoxacarb in *Helicoverpa armigera* was 1238.86 fold as compared to the laboratory susceptible strain. Rao and Grace (2008) carried out the experiment on *H. armigera*. The LC₅₀ (µg/larva) values of indoxacarb, spinosad, methomyl, quinalphos and cypermethrin were 0.22, 0.11, 0.45, 0.53 and 8.70 respectively. Of all the insecticides tested spinosad was the most toxic and cypermethrin was the least toxic.

Nimbalkar *et al.* (2009) studied the Resistance Management in *Helicoverpa armigera*, recorded a maximum LD₅₀ value of 0.070 µg/larva for quinalphos in October with a minimum of 0.025 mg/larva in August in Jalna district. Resistance to quinalphos was greatest in Jalna (2.50-7.00 fold) followed by Aurangabad (3.0-5.0 fold) and Parbhani (3.7-4.9 fold). The LD₅₀ value for cypermethrin was highest, during October (1.459 mg/larvae in Aurangabad) and lowest during August in Parbhani (0.157 µg/larva).

Upendhar *et al.* (2011) conducted bioassay on *H. armigera* larvae of the Mahaboobnagar district and recorded a LD₅₀ of 29.125 µg/larva and 59.609 µg/larva at LD₉₀ for cypermethrin. The LD₅₀ and LD₉₀ values of cypermethrin for Raichur population were 32.481 and 38.172 µg/larva, respectively. Toxicity of cypermethrin to Nagpur population showed that the LD₅₀ and LD₉₀ values were 20.069 and 54.708 µg/larva, respectively. The *H. armigera* larvae of the Mahaboobnagar district recorded a LD₅₀ of 3.651 µg/larva and 10.287 µg/larva at LD₉₀ for methomyl. The LD₅₀ and LD₉₀ values of methomyl for Raichur population was 3.630 and 10.417 µg/larva, respectively, while toxicity of methomyl to Nagpur population showed that the LD₅₀ and LD₉₀ values were 2.652 and 7.214 µg/larva. Sen *et al.*, (2012) reported in *Helicoverpa assulta*, after 13 generations of selection with indoxacarb in the laboratory, the resistance ratio of the third instar larvae increased by 4.19-fold.

Conclusion

The present investigation on the status of insecticide resistance in *Helicoverpa armigera* to commonly used insecticides of different groups viz., Synthetic pyrethroid, organophosphorous, carbamate, oxydiazine and macrocyclic lactones and with different mode of action in major pigeonpea growing districts of Vidarbha, Maharashtra, indicated that the resistance level is visi-

ble as high in cypermethrin, moderate to indoxacarb, quinalphos, spinosad and low to methomyl. In the present investigation, Yavatmal and Washim strains showed higher resistance level to cypermethrin, quinalphos and methomyl; Yavatmal and Buldhana strains showed higher resistance level to indoxacarb and spinosad. On the basis of present findings, it could be concluded that *H. armigera* population of Yavatmal was found most resistant whereas, Dr.Panjabrao Deshmukh Krishi Vidyapeeth population was found susceptible to technical grade insecticides from all the populations collected during 2013-14. Evaluation of insecticide resistance in *H. armigera* revealed highest resistance in technical grade of cypermethrin followed by indoxacarb, quinalphos, spinosad and lowest in methomyl.

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