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Monitoring of resistance to conventional insecticides in field populations of *Aphis gossypii* glover from major cotton growing areas of Vidarbha

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Abstract
The insecticide resistance was assayed for field collected *Aphis gossypii* populations from Yavatmal, Amravati, Akola, Wardha and Buldhana districts of Vidarbha (MS) by leaf dip method. The results indicated that the three insecticides tested viz., Acetamiprid 20 SP, Methyl demeton 25 EC and Dimethoate 30 EC were less toxic to Yavatmal and Amravati populations of *Aphis gossypii* recording higher LC_{50} and LC_{90} values. The relative toxicity studies amongst insecticides reveled that Dimethoate 30 EC was highly toxic to aphid population from five districts with RT values of 1.00 followed by Methyl demeton (0.49 to 0.53) and Acetamiprid (0.31 to 0.39) indicating that the resistance to these insecticides not varied much amongst the five geographical regions. The studies on relative degree of resistance revealed that the *Aphis gossypii* populations from Yavatmal district registered highest degree of resistance with resistance ratios (RR) ranging 1.05 to 1.48 fold against Acetamiprid 1.26 to 1.53 against Dimethoate and 1.24 to 1.47 against Methyl demeton as compared with aphid populations from Buldhana, Wardha, Akola and Amravati districts. The aphid populations from Buldhana district seems to be more susceptible against all the three insecticides tested as compared to aphids populations from other four districts.

Keywords: Resistance, insecticides, aphid

Introduction
Aphis gossypii Glover (Aphidae: Homoptera) commonly known as cotton aphid is cosmopolitan in distribution. It is a polyphagous pest of many plant species including cotton, cucurbits, citrus, vegetables and ornamental plants (Blackman and Eastop, 1985) [2]. Colonies of aphids are often seen clustered on the plants sucking the sap from the undersurface of leaves and tender shoots. This leads to deformation and ultimately wilting and drying of the affected plants. High aphid population may cause stunted and retarded growth and development of seedlings. Late season population can cause stickiness and development of black sooty mould associated with honey dew dropped on cotton fibre (Isely, 1946) [9]. The aphids *Aphis gossypii* Glover infect cotton crop at seedling stage causing significant reduction in crop yields. Early infection of *Aphis gossypii* on cotton causes a delay in vegetative development and seed cotton loss upto 23 per cent is reported (Deguine *et al*. 1994) [4]. Satpute *et al*. (1990) [11] also reported 22.85 per cent losses in cotton due to *A. gossypii* in Vidarbha region of Maharashtra state. Aphids not only reduce the cotton yield but also increase the cost of production (Hardee and O'Brien, 1990) [7]. In view of large infestation due to this pest complex and the huge losses caused by them, this must be kept below ETL. Currently, aerial application of conventional aphicides like dimethoate, methyl demeton and newly introduced acetamiprid and imidacloprid (17.8SL) from the neonicotinoid group were found unable to provide satisfactory control of aphids on cotton. Thus, considering the increasing infestation of aphids accompanied with failure of conventional aphidicides in major cotton growing districts of Vidarbha viz., Akola, Amravati, Buldana, Wardha and Yavatmal with an assumption that the cotton aphids in these districts might have developed resistance, the present investigations were undertaken.

Material and Methods

Collection of field populations of cotton aphids

The field population of cotton aphids *A. gossypii Glover* were collected from the five major cotton growing districts of Vidarbha viz., Akola, Amravati, Buldana, Wardha and Yavatmal. The aphid populations were collected directly from farmers fields who experienced control failures with standard aphicides (recommended insecticides). The heavily infested leaves and shoots of cotton plants along with aphid colonies were picked and carried to laboratory and used on same day for bioassay.

Multiplication of susceptible population of cotton aphids

The susceptible strain of cotton aphid *A. gossypii* (Glover) was established from a single parthenogenetic female on potted cotton seedlings. These potted seedlings were kept free from any insecticidal drift. The sixth generation onward progenies from this caged aphid colonies were used for bioassay studies.

Bioassay techniques used

The bioassay technique with leaf dip assay on agar beds as prescribed by Insecticide Resistance Action Committee (IRAC) was followed in the present investigation. Various steps followed in this bioassay techniques have been described under (IRAC, 2010) [8].

A minimum of 5 to 11 concnetrations of Acetamiprid 20 SP, Methyl demeton 25 EC and Dimethoate 30 EC were prepared in distilled water and poured in small plastic jars. Fresh cotton leaves were cut into round discs and dipped in test liquid. The leaf discs were removed from the test solution after 30 seconds and placed on paper towel for surface drying under mild fan. The surface dried leaf discs were placed upside down on agar bed (25 mm) in the petriplates (30 mm high). The bioassays were conducted using three replicate batches of aphids (i.e. 60 insects) per insecticide concentration for field as well as cage (laboratory) populations of *A. gossypii*. After the aphids were released on treated leaf discs in agar beds, the petriplates were kept upright without lids at ambient laboratory conditions. The mortality of aphids was recorded at 24 and 48 hr after treatments using hand lens. The aphids which are unable to right themselves within 10 seconds once turned on their back were considered as dead. In the event of doubt, the suspected individuals were gently touched using fine camel hair brush and after noting the response the mortality was recorded accordingly. The mortality data in the range of 20-80% from each treatment were considered and subjected to probit analysis (Finney, 1971)

Determination of toxicity of selected insecticides

Based on LC_{50} and LC_{90} values the toxicity of the selected insecticides to *A. gossypii* of each of the five districts were determined. The LC_{50} and LC_{90} values of each of the test insecticides to *A. gossypii* of each of the five districts were considered for computation of relative toxicity values (RT). The relative toxicity was computed relative to the insecticide with lowest LC_{50} value recorded for *A. gossypii* population from respective district.

Determination of degree of resistance developed by cotton aphids to test insecticides

The LC_{50} and LC_{90} values of each test insecticide against aphid population of Akola, Amravati, Buldana, Wardha and Yavatmal districts were taken into consideration for assessing the degree of resistance among the five test populations to each of the test insecticides was calculated using formula given by FAO (1979) [5]

$$\text{Resistance factor (RF)} = \frac{LC_{50} \text{ of resistant population}}{LC_{50} \text{ of susceptible population}}$$

The actual degree of resistance developed by the five aphid populations was calculated by comparing the LC_{90} values of field populations with the corresponding LC_{90} values of laboratory grown population of *A. gossypii*.

Result and Discussion

The LC_{50} and LC_{90} (Table 1) values of Acetamiprid 20 SP against *Aphis gossypii* of five districts in Vidarbha (MS) are 418.40 and 4955.54 (Yavatmal), 389.50 and 6389.10 (Amravati), 358.90 and 3345.43 (Akola), 339.31 and 3662.34 (Wardha) and 293.37 and 4728.89 (Buldhana), respectively indicated that the aphid populations from the five districts differ in their susceptibility to Acetamiprid. Chalam *et al*. (2003) [3] reported LC_{50} values of Acetamiprid as 0.0002 per cent against *A. gossypii* populations of Guntur district (AP-India). The LC_{50} values recorded by Yavatmal populations was highest while that Amravati, Akola and Wardha were moderate and it was lowest for Buldhana population. The LC_{90} value was, however, highest for Amravati population and it was lowest for Akola population indicating the heterogeneity in susceptibility of aphids from different locations consequent upon differential selection pressure due to variability in insecticide consumption in these major cotton growing districts.

The LC_{50} and LC_{90} (Table 1) values of Dimethoate 30 EC against *A. gossypii* of five districts in Vidarbha (MS) are 161.73 and 2044.99 (Yavatmal), 136.66 and 1568.37 (Amravati), 120.95 and 3079.99 (Akola), 105.23 and 1334.46 (Wardha) and 93.33 and 1626.70 (Buldhana), respectively clearly exhibiting variation in susceptibility of the aphid populations from the five districts to Dimethoate. Ahmad and Arif (2008) [1] also reported variation in LC_{50} of Dimethoate in the range of 11.4 to 464 ppm in *A. gossypii* collected from Multan state of Pakistan during 1996 to 2004. However, earlier reports from Kerns and Gaylor (1992) [10] who reported variation in LC_{50}'s of Dimethoate in the range of 5410 to 13275 ppm in aphids from Alabama and Texas Province. The LC_{50} values recorded by Yavatmal and Amravati populations are higher as compared to Wardha and Buldhana districts, while the LC_{90} value recorded by Akola population was highest, singling at higher insecticide consumption in Yavatmal, Amravati and Akola districts.

The LC_{50} and LC_{90} (Table 1) values of Methyl demeton 25 EC against *A. gossypii* of five districts in Vidarbha (MS) are 327.24 and 2939.01 (Yavatmal), 262.31 and 9789.17 (Amravati), 222.16 and 6888.15 (Akola), 183.62 and 2378.77 (Wardha) and 177.24 and 2000.30 (Buldhana), respectively clearly exhibiting indicating the variability in the susceptibility of five aphid populations to Methyl demeton 25 EC. Kerns and Gaylor (1992) [10] also reported variability in the LC_{50} values of *Aphis gossypii* populations collected from Alabama and Texas Province of U.S. to Methyl demeton 25 EC in the range between 667.4 to 3480 ppm. The LC_{50} values recorded by Yavatmal populations is highest, moderate in case of Amravati and Akola, while comparatively lower in case of Wardha and Buldhana populations. The LC_{90} values were, however, highest in case of Amravati population followed by Akola populations.

The mean toxicity of the three test insecticides to the aphid populations of five districts viz., Yavatmal, Amravati, Akola, Wardha and Buldhana revealed the order of toxicity as Dimethoate > Methyl demeton > Acetamiprid both at LC_{50} and LC_{90}. It seems that Dimethoate is the more toxic followed by Methyl demeton and Acetamiprid is less toxic to *A. gossypii* in Vidarbha (MS).

The highest LC 50 against acetamaprid 20 SP and Methyl dematon 25 EC was observed in Yavatmal (418.4 and 237.24) whereas, lowest LC 50 observed in Buldana (293.37 and 177.24). similarly, the highest LC 50 against dimethoate 30 EC was observed in Yavatmal (161.73) whereas, lowest LC 50 observed in Wardha (105.23). It indiacates that the aphid populations of Yavatmal district on the basis of LC50 developed 1.43, 1.73 and 1.85 fold relative resistance to Acetamiprid, Dimethoate and Methyl dematon respectively (Table 2,3,4).

The highest LC 90 against acetamaprid 20 SP and Methyl dematon 25 EC was observed in Yavatmal (418.4 and 237.24) whereas, lowest LC 50 observed in Buldana (293.37 and 177.24). similarly, the highest LC 50 against dimethoate 30 EC was observed in Yavatmal (161.73) whereas, lowest LC 50 observed in Wardha (105.23). It indiacates that the aphid populations of Yavatmal district on the basis of LC50 developed 1.43, 1.73 and 1.85 fold relative resistance to Acetamiprid, Dimethoate and Methyl dematon respectively. Similarly, on the basis of LC90 Amaravati developed 1.91 and 4.89 fold resistance against Acetamiprid 20 SP and methyl dematon 25 EC. Whereas, Akola developed 1.96 fold resistance against Dimethoate 30 EC (Table 2,3,4).

A comparison of the LC90 values of test insecticides for field collected and cage grown aphid population (Table 5) for actual resistance revealed that the aphid populations from Yavatmal, Amravati, Akola, Wardha and Buldhana have developed 21.96, 28.37, 14.82, 16.23 and 20.96 fold relative resistance to Acetamiprid, 7.89,6.05, 11.88, 5.15, 6.27 fold resistance to Dimethoate and 3.20, 7.41, 7.51, 2.59 and 2.18 fold relative resistance to methyl dematon over cage grown (susceptible) aphids.

Table 1: Toxicity of insecticides (LC_{50}) to *Aphis gossypii* Glover from five districts of Vidarbha (MS)

Insecticides	Yavatmal		Amravati		Akola		Wardha		Buldana	
	LC50 (ppm)	LC90 (ppm)	LC50 (ppm)	LC90 (ppm)	LC50 (ppm)	LC90 (ppm)	LC50 (ppm)	LC90 (ppm)	LC50 (ppm)	LC90 (ppm)
Acetamiprid 20 SP	418.4	4955.54	389.5	6389.1	358.9	3345.43	339.31	3662.34	293.37	4728.89
Dimethoate 30 EC	161.73	2044.99	136.66	1568.37	120.95	3079.99	105.23	1334.46	93.33	1626.7
Methyl demeton 25 EC	327.24	2939.01	262.31	9789.17	222.16	6888.15	183.62	2378.77	177.24	2000.3

Table 2: Relative degree of resistance among aphid populations of five districts to Acetamaprid 20 SP

District	LC50 (ppm)	RF at LC 50 in comparision with aphid population of				LC 90 (ppm)	RF at LC 90 in comparision with aphid population of				RF in comparision with aphid population	
		Buldana	Wardha	Akola	Amravati		Buldana	Wardha	Akola	Amravati	cage grown	Recommanded dose
Yavatmal	418.4	1.43	1.23	1.17	1.07	4955.54	1.05	1.35	1.48	1.29	21.96	33.03
Amravati	389.5	1.33	1.15	1.09	--	6389.1	1.35	1.74	1.91	--	28.31	42.59
Akola	358.9	1.22	1.06	--	--	3345.43	1.41	1.09	--	--	14.82	22.3
Wardha	339.31	1.16	--	--	--	3662.34	1.29	--	--	--	16.23	24.41
Buldana	293.37	--	--	--	--	4728.89	--	--	--	--	20.96	31.52

Table 3: Relative degree of resistance among aphid populations of five districts to Dimethoate 30 EC

District	LC50 (ppm)	RF at LC 50 in comparision with aphid population of				LC 90 (ppm)	RF at LC 90 in comparision with aphid population of				RF in comparision with aphid population	
		Buldana	Wardha	Akola	Amravati		Buldana	Wardha	Akola	Amravati	cage grown	Recommanded dose
Yavatmal	161.73	1.73	1.54	1.34	1.18	2044.99	1.26	1.53	1.51	1.3	7.89	2.04
Amravati	136.66	1.46	1.3	1.13	-	1568.37	1.04	1.18	1.96	-	6.05	1.57
Akola	120.95	1.3	1.15	-	-	3079.99	1.89	2.31	-	-	11.88	3.08
Wardha	105.23	1.13	-	-	-	1334.46	1.22	-	-	-	5.15	1.33
Buldana	193.33	-	-	-	-	1626.7	-	-	-	-	6.27	1.63

Table 4: Relative degree of resistance among aphid populations of five districts to Methyl dematon 25 EC

District	LC50 (ppm)	RF at LC 50 in comparision with aphid population of				LC 90 (ppm)	RF at LC 90 in comparision with aphid population of				RF in comparision with aphid population	
		Buldana	Wardha	Akola	Amravati		Buldana	Wardha	Akola	Amravati	cage grown	Recommanded dose
Yavatmal	327.24	1.85	1.78	1.47	1.25	2939.96	1.47	1.24	2.34	3.33	3.20	3.67
Amravati	262.31	1.48	1.43	1.18	--	9789.17	4.89	4.12	1.42	--	7.41	12.24
Akola	222.16	1.25	1.21	--	--	6888.15	3.44	2.90	--	--	7.51	8.61
Wardha	183.62	1.04	--	--	--	2378.77	1.19	--	--	--	2.59	2.97
Buldana	177.24	--	--	--	--	2000.30	--	--	--	--	2.18	2.50

Table 5: Toxicity of insecticides to cage grown population of Aphids *Aphis gossypii* Glover

S. No	Insecticides	LC50	Fiducial limit	LC90	Fiducial limit	Slope	X2 (Heterogenety)
1	Acetamiprid 20 SP	20.94	14.58-30.07	225.66	90.43-563.08	1.24	1.63
2	Dimethoate 30 EC	23.49	16.04-34.39	259.34	96.43-698.96	1.22	0.43
3	Methyl demeton 25 EC	16.10	8.65-29.58	916.78	192.83-4358.57	0.72	1.91

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