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# RELATIVE SUSCEPTIBILITY OF LIFE STAGES OF COTTON WHITEFLY *BEMISIA TABACI* (GENN.) TO PYRIPROXYFEN

Kelageri S S, Mahapatro G K[1]*, Subramanian S, Chitra Srivastava and Rajna S

Division of Entomology, ICAR-Indian Agricultural Research Institute (IARI), New Delhi 110012, India
[1]IARI Regional Station, Pune 411067, Maharashtra, India
*Email: gagan_gk@rediffmail.com (corresponding author)

**ABSTRACT**

**The relative susceptibility of lifestages of seven *Bemisia tabaci* (Genn.) populations from major cotton growing regions in India to pyriproxyfen has been studied. The results revealed that adults were unaffected at significantly high concentration ie., 200 mg/ l (field recommended dose, <10% mortality), hence adult bioassay was not done. Indore and Amravati populations were the most and least susceptible ones for both egg and nymphal stages; and all the populations were found susceptible to pyriproxyfen with RR ratio of <5, except for Amravati one, revealing low level of resistance with RR ratio (5.04- egg; 5.09- nymph) computed deploying the $LC_{90}$ and $LC_{50}$ values.**

**Key words:** *Bemisia tabaci*, pyriproxyfen, bioassay, $LC_{90,}$ $LC_{50}$, adults, egg, nymph, juvenile hormone analogue, relative resistance ratio

The cotton whitefly *Bemisia tabaci* (Gennadius) is a pest of global significance affecting wide range of crops including field, vegetable, fruit and ornamental crops (Kanakala and Ghanim 2019; Horowitz et al., 2020). Indirectly *B. tabaci* affects crops through vectoring more than 114 virus species (Simon, 2003). Use of insecticides is the major control measure against *B. tabaci,* although the rapid development of insecticide resistance by *B. tabaci* has resulted in frequent pest outbreaks. It has evolved resistance to most of the commonly used insecticides (Basit 2019, Horowitz et al., 2020; Mota-Sanchez and Wise, 2019). Involvement of biorational insecticides in the spray schedule reduces selection pressure and encourages natural enemies. Juvenile hormone analogues (JHAs) are the synthetic analogues of juvenile hormone, and these are considered to be effective and environment friendly (Mohandass et al., 2006). Pyriproxyfen is a pyridine based juvenile hormone analogue ie., 4-phenoxyphenyl (RS)-2-(2-pyridyloxy) propyl ether, it targets JH binding site receptors in insects by mimicking the action of juvenile hormone and thus keeping it in the immature stage (Sullivan and Goh, 2008). Pyriproxyfen has been proven as an effective molecule for managing *B. tabaci* with strong ovicidal action, inhibiting adult emergence and translaminar activity against eggs; and the egg hatchability of the treated female gets suppressed (Ishaaya and Horowitz, 1992). In this study the relative susceptibility of seven *B. tabaci* populations from cotton growing regions of India to pyriproxyfen has been evaluated.

## MATERIALS AND METHODS

*Bemisia tabaci* populations were collected from seven cotton growing locations and reared on cotton (*Gossypium hirsutum* L.) in the Insect Proof Climate Control Chamber, Division of Entomology, IARI (27±2$^0$C, 60-70% RH, and photoperiod 14:10- L:D). The whiteflies collected from *Leucaena leucocephala* from Pusa campus, reared under laboratory condition, served as the susceptible check. The details of populations are- Amravati (21.02°N 77.48°E), Guntur (16.22°N,80.30°E), Hisar (29.09°N, 75.87°E), Indore (22.8°N,75.73°E), Ludhiana (30.54°N, 75.48°E), New Delhi (28.64°N,77.17°E), Sriganganagar (29.54°N,73.54°E), and susceptible (laboratory-28.38°N 77.09°E) one. Commercial formulation of pyriproxyfen 10 EC (Lano®, Sumitomo Chemical India) procured from the market was diluted with deionized water to make 1% stock solution for use in bioassay. Seven concentrations with three replications were set and studies carried out following the modified Insecticide Resistance Action Committee (IRAC) protocols (https://irac-online.org/). All the important lifestages viz., egg, nymph (N2) and adults were used; lethal effects in egg and nymphal stage (2$^{nd}$ instar) were tested following the IRAC method No. 016 (formally method No 12c) (https://irac-online.org/), whereas adults were tested with leaf dip bioassay- modified IRAC method by Naveen et al. (2017). The estimates of lethal concentrations and 95% confidence intervals were determined by log–dose probit analysis using

PoloPlus 2.0 (LeOra Software, Petaluma, CA). Relative resistance ratios were computed to classify these- 5.0 < RR ≤ 10.0, as low level of resistance; (10.0 < RR ≤ 40.0) as moderate level; and RR ≥40.0 as a high level of resistance (Liu et al., 2010). Correlation coefficient was worked out to understand the relationship between RR and slope of the equation.

## RESULTS AND DISCUSSION

The susceptibility studies were carried out keeping the laboratory susceptible population as the baseline. Pyriproxyfen did not cause significant mortality in adults even at very high concentration i.e. < 10 % mortality at 200 mg/ l (maximum field recommended dose) with the laboratory susceptible populations; hence, adult bioassay was not undertaken for the field populations. The $LC_5$ against egg and nymph given in Table 1 reveal that control mortalities in all the bioassays were <7%, and the $LC_{50}$ and $LC_{90}$ values for the susceptible population was 0.018 and 0.083 mg/ l. The RR ratio for egg was maximum for the populations from Amravati and Sriganganagar. There was no significant difference between the $LC_{50}$ and $LC_{90}$ value of laboratory susceptible population and field populations except for Sriganganagar and Hisar ones considering the overlap of 95% fiducial limits. The results suggest a high amount of homogeneity in the response of populations to pyriproxyfen at egg stage. In nymphal bioassay the control mortalities were <9%, and $LC_{50}$ and $LC_{90}$ values for the susceptible population were 0.054 and 0.176 mg/ l. The RR ratio for egg stage was maximum for Amravati population followed by Ludhiana one. There was a significant difference between the $LC_{50}$ value of laboratory susceptible and field populations except for Indore one; similarly there was significant difference between the $LC_{90}$ value of laboratory susceptible and field populations except for Indore, New Delhi and Guntur populations; and the overlap of 95% fiducial limits suggest the existence of heterogeneity in the response of these to pyriproxyfen at nymphal stage. Correlation coefficients indicate non-significant values between slope and RR values for both egg (n=8, r=–0.265, p=0.526) and nymph (n=8, r=0.502, p=205) stages at p=0.05, indicating the heterogeneous response across individuals of the samples. According to the resistance level classification given by Liu et al. (2010), the populations studies were found to be susceptible to pyriproxyfen at both egg and nymphal stages except for Amravati population at egg stage.

Pyriproxyfen showed limited efficacy against adults of *B. tabaci*, but maintaining the insect in its immature stage, with suppression of embryogenesis and adult formation (Ishaaya and Horowitz 1992). Pyriproxyfen is known to cause the external deformities in emerged adults such as twisted wings and legs in case of *Plodia interpunctella* (Ghasemi et al., 2010), apart from external deformities pyriproxyfen reduces the size of ovaries due to the reduction in the synthesis and supply of lipid and protein (Ghasemi et al., 2010). There are many reports of pyriproxyfen resistance in *B. tabaci*- Egg bioassays by Devine et al. (1999) reported very high level of resistance (6500-fold) among Israeli populations; low to moderate level of resistance (11-fold) in Chinese populations (Luo et al., 2010); moderate resistance from Alhassa Oasis (Saudi Arabia) (Hajjar et al., 2019); and high resistance (89.71-folds) from cotton field populations of Arizona (Ma et al., 2010) and Australian (96.9 fold) (Hopkinson et al., 2019). Similarly with nymphal bio assay studies, Basit et al. (2013) reported low level of resistance from Pakistan; moderate level of resistance (30.08-fold) from West Bengal, India was observed (Roy et al., 2019); and very high level of resistance (1100-fold) in Israeli populations (Devine et al., 1999). Pyriproxyfen resistance in *B. tabaci* might be a case of metabolic resistance involving cytochrome P450 monooxygenases (P450s) and glutathione S-transferases (GSTs) (Ma et al., 2010; Ghanim et al., 2007; Nauen et al., 2015).

The present results are in contrast with the resistance development data available from other parts of the world, as it has been observed that the Indian populations are highly susceptible to the pyriproxyfen at both egg and nymphal stages. This might be due to the less selection pressure exerted as pyriproxyfen is not among the mainstream insecticides. Another factor is the dominance of B biotype of *B. tabaci* in the Indian subcontinent, as confirmed by the earlier studies (Ellango et al., 2015; Mandali et al., 2016). It was observed that cases of strong resistance to pyriproxyfen have been associated with the Q rather than the B biotype (Dennehy et al., 2005; Horowitz and Ishaaya, 2014). This fact derives support from the data that in Israel considerable reduction in pyriproxyfen resistance was observed since 2009. Since then studies had shown a significant shift in the biotype ratios i.e. the B biotype has become predominate over the Q (Crowder et al., 2011; Horowitz and Ishaaya, 2014). Recent studies involving the *B. tabaci* populations from major cotton growing regions of India revealed the $LC_{50}$ values of 52 to 956 and 26 to 194 mg/ l for imidacloprid and thiamethoxam; while pyrethroids viz., cypermethrin

Table 1. Log dose probit mortality data for pyriproxyfen against egg stage of different *Bemisia tabaci* populations

| S. No. | Population | df | Slope± SE | χ2 | $LC_{50}$ value mg/ l (CI 95%) | Fiducial limit for $LC_{50}$ (mg/ l) | RR for $LC_{50}$ | $LC_{90}$ value mg/ l (CI 95%) | Fiducial limit for $LC_{90}$ (mg/ l) | RR for $LC_{90}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| Lab Susceptible | | 3 | 1.929± 0.104 | 2.764 | 0.018 | 0.010 to 0.031 (a) | 1.00 | 0.083 | 0.042 to 1.538(a) | 1.00 |
| 1 | Amravati | 3 | 2.171± 0.095 | 1.261 | 0.064 | 0.026 to 0.115(a) | 3.56 | 0.418 | 0.188 to 16.826(a) | 5.04 |
| 2 | Guntur | 3 | 1.552± 0.089 | 3.150 | 0.038 | 0.031 to 0.045(a) | 2.11 | 0.253 | 0.178 to 0.426(a) | 3.05 |
| 3 | Hisar | 3 | 1.850± 0.095 | 1.146 | 0.052 | 0.043 to 0.063(b) | 2.89 | 0.258 | 0.181 to 0.458(a) | 3.11 |
| 4 | Indore | 4 | 2.566± 0.085 | 2.175 | 0.020 | 0.010 to 0.040 (a) | 1.11 | 0.135 | 0.057 to 6.133(a) | 1.63 |
| 5 | Ludhiana | 3 | 1.623± 0.082 | 1.781 | 0.049 | 0.027 to 0.083(a) | 2.72 | 0.303 | 0.145 to 3.328(a) | 3.65 |
| 6 | New Delhi | 3 | 1.556± 0.079 | 3.134 | 0.030 | 0.023 to 0.039(a) | 1.67 | 0.201 | 0.123 to 0.484(a) | 2.42 |
| 7 | Sriganganagar | 3 | 1.555± 0.087 | 2.112 | 0.056 | 0.035 to 0.089(b) | 3.11 | 0.371 | 0.179 to 3.038(a) | 4.47 |

Log dose probit mortality data for pyriproxyfen against nymphal stage (2nd instar) of different *Bemisia tabaci* populations

| S. No. | Population | df | Slope± SE | χ2 | $LC_{50}$ value mg/ l (CI 95%) | Fiducial limit for $LC_{50}$ (mg/ l) | RR for $LC_{50}$ | $LC_{90}$ value mg/ l (CI 95%) | Fiducial limit for $LC_{90}$ (mg/ l) | RR for $LC_{90}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| Lab Susceptible | | 3 | 1.565± 0.269 | 2.824 | 0.054 | 0.041 to 0.073(a) | 1.00 | 0.176 | 0.105 to 0.316(a) | 1.00 |
| 1 | Amravati | 3 | 2.543± 0.416 | 0.874 | 0.275 | 0.193 to 0.396(c) | 5.09 | 0.878 | 0.540 to 4.244(b) | 4.99 |
| 2 | Guntur | 4 | 3.562± 0.541 | 2.863 | 0.155 | 0.136 to 0.176(b) | 2.87 | 0.355 | 0.285 to 0.517(a) | 2.02 |
| 3 | Hisar | 4 | 3.603± 0.589 | 0.985 | 0.173 | 0.151 to 0.195(b) | 3.20 | 0.393 | 0.318 to 0.575(b) | 2.23 |
| 4 | Indore | 3 | 2.362± 0.397 | 0.485 | 0.079 | 0.065 to 0.095(a) | 1.46 | 0.275 | 0.206 to 1.024(a) | 1.56 |
| 5 | Ludhiana | 4 | 3.236± 0.569 | 2.142 | 0.191 | 0.169 to 0.216(c) | 3.54 | 0.424 | 0.340 to 0.634(b) | 2.41 |
| 6 | New Delhi | 3 | 2.949± 0.488 | 0.062 | 0.137 | 0.117 to 0.159(b) | 2.54 | 0.373 | 0.285 to 0.614(a) | 2.12 |
| 7 | Sriganganagar | 3 | 3.880± 0.599 | 1.150 | 0.187 | 0.166 to 0.210(c) | 3.46 | 0.400 | 0.327 to 0.568(b) | 2.27 |

Chi-square values non-significant- p=0.05 (table value 3df=7.81, 4df=9.49); Letters in parentheses indicate significant difference in lethal concentrations and deltamethrin showed $LC_{50}$ values of 10 to 1362 and 10 to 760 mg/ l respectively. Similarly OP insecticides triazophos, monocrotophos and chlopyriphos showed $LC_{50}$ values ranging from 53 to 1429, 88 to 3934, 12 to 220 mg/ l, respectively (Naveen et al., 2017). Novel insecticides fipronil and flonicamid showed $LC_{50}$ values of 6.56 to 20.80 and 23.35 to 749.91 mg/ l (Romila et al., 2019) and for cyantraniliprole it was 1.80 to 4.57 mg/ l (Rajna et al., 2021). Comparison of these $LC_{50}$ values with those of pyriproxyfen from the present study viz., 0.018 to 0.064 mg/ l for egg and 0.054 to 0.275 mg/l for nymph reveal the supremacy of the pyriproxyfen, suggesting that it can be used as a stage specific insecticide in IRM programmes.

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### AUTHOR CONTRIBUTION STATEMENT

CS, MGK, and SS conceived and designed research, KSS and RS conducted experiments and analyzed data, KSS wrote manuscript and MGK corrected manuscript.

### CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

### REFERENCES

Basit M, Saeed S, Saleem M A, Denholm I, Shah M. 2013. Detection of resistance, cross-resistance, and stability of resistance to new chemistry insecticides in *Bemisia tabaci* (Homoptera: Aleyrodidae). Journal of Economic Entomology 106(3): 1414-22.

Basit M. 2019 Status of insecticide resistance in *Bemisia tabaci*: resistance, cross resistance, stability of resistance, genetics and fitness costs. Phytoparasitica 47: 207-225.

Crowder D W, Horowitz A R, Breslauer H, Rippa M, Kontsedalov S, Ghanim M, Carriere Y. 2011. Niche partitioning and stochastic processes shape community structure following whitefly invasions. Basic and Applied Ecology 12: 685-694.

Dennehy T J, Degain B A, Harpold V S, Brown J K, Morin S, Fabrick J A, Byrne F J, Nichols R L. 2005. New challenges to management of whitefly resistance to insecticides in Arizona. University of Arizona, Tucson. http://hdl.handle.net/10150/215014

Devine G J, Ishaaya I, Horowitz A R, Denholm I. 1999. The response of pyriproxyfen-resistant and susceptible *Bemisia tabaci* Genn (Homoptera: Aleyrodidae) to pyriproxyfen and fenoxycarb alone and in combination with piperonyl butoxide. Pesticide Science 55: 405-411.

Ellango R, Singh S T, Rana V S, Gayatri Priya N, Raina H, Chaubey R, Naveen N C, Mahmood R, Ramamurthy V V, Asokan R. Rajagopal R. 2015. Distribution of *Bemisia tabaci* genetic groups in India. Environmental Entomology 44(4): 258-1264.

Ghanim M, Kontsedalov S, Czosnek H. 2007. Tissue-specific gene silencing by RNA interference in the whitefly *Bemisia tabaci* (Gennadius). Insect Biochemistry and Molecular Biology 37(7): 732-738.

Ghasemi A, Sendi J J, Ghadamyari M. 2010. Physiological and biochemical effect of pyriproxyfen on Indian meal moth *Plodia interpunctella* (Hubner) (Lepidoptera: Pyralidae). Journal of Plant Protection Research 50(4): 416-422.

Hajjar J M, Almarzouk I, Alhudaib K. 2020. Biotype and status of insecticide resistance of whitefly *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) in Alhassa Oasis, Eastern Province of Saudi Arabia. Entomological Research 50(2): 74-81.

Hopkinson J, Pumpa S, Brunschot S V, Fang C, Frese M, Tay W T, Walsh T. 2019. Insecticide resistance status of *Bemisia tabaci* MEAM1 (Hemiptera: Aleyrodidae) in Australian cotton production valleys. Austral Entomology 59(1): 201-214.

Horowitz A R, Ishaaya I. 2014. Dynamics of biotypes B and Q of the whitefy *Bemisia tabaci* and its impact on insecticide resistance. Pest Management Science 70:1568-1572.

Horowitz R A, Ghanim M, Roditakis E, Nauen R, Ishaaya I. 2020. Insecticide resistance and its management in *Bemisia tabaci* species. Journal of Pest Science 93: 893-910.

Ishaaya I, Horowitz A R. 1992. Novel phenoxy juvenile hormone analog (pyriproxyfen) suppresses embryogenesis and adult emergence of sweet potato whitefly (Homoptera: Aleyrodidae). Journal of Economic Entomology 85(6): 2113-2117.

Kanakala S, Ghanim M. 2019. Global genetic diversity and geographical distribution of *Bemisia tabaci* and its bacterial endosymbionts. Plos One 14(3): 1-21 e0213946.

Liu FY, Li H, Qiu J, Zhang Y, Huang L, Li H, Wang G, Shen J. 2010. Monitoring of resistance to several insecticides in brown planthopper (*Nilaparvata lugens*) in Huizhou. Chinese Bulletin of Entomology 47: 991-993.

Luo C, Jones C M, Devine G, Zhang F, Denholm I, Gorman K. 2010. Insecticide resistance in *Bemisia tabaci* biotype Q (Hemiptera: Aleyrodidae) from China. Crop Protection 29: 429-434.

Ma W, Li X, Dennehy T J, Lei C, Wang M, Degain B A, Nichols R L. 2010. Pyriproxyfen resistance of *Bemisia tabaci* (Homoptera: Aleyrodidae) Biotype B: Metabolic Mechanism. Journal of Economic Entomology 103(1): 158-65.

Mandali R, Lakshmi K V, Sujatha M. 2016. Survey and biotype identification of whitefly, *Bemisia tabaci* transmitting tomato leaf curl virus in Andhra Pradesh, India. Journal of Agricultural Technology 12(7.1): 1687-1694.

Mohandass S M, Arthur F H, Zhu K Y, Throne J E. 2006. Hydroprene; Mode of action, current status in stored pest management, insect resistance, and future prospects. Crop Protection 25: 902-909.

Mota-Sanchez D, Wise J C. 2019. The Arthropod Pesticide Resistance Database. Michigan State University. http://www.pesticideresistance

Nauen R, Wolfel K, Lueke B, Myridakis A, Tsakireli D, Roditakis E, Tsagkarakou A, Stephanou E, Vontas J. 2015. Development of a lateral flow test to detect metabolic resistance in *Bemisia tabaci* mediated by CYP6CM1, a cytochrome P450 with broad spectrum catalytic efficiency. Pesticide Biochemistry and Physiology 121: 3-11.

Naveen N C, Chaubey R, Kumar D, Rebijith K B, Rajagopal R, Subrahmanyam B, Subramanian S. 2017. Insecticide resistance status in the whitefly, *Bemisia tabaci* genetic groups Asia-I, Asia-II-1 and Asia-II-7 on the Indian subcontinent. Scientific Reports 7: 40634.

Rajna S, Mahapatro G K, Subramanian S, Subhash C, Sudhakar K. 2021. Susceptibility of imidacloprid resistant whitefly *Bemisia tabaci* (Gennadius) to cyantraniliprole. Indian Journal of Entomology. e20407. DOI: 10.5958/IJE.2021.21

Romila A, Chitra S, Subramanian S, Telem R S, Rajna S. 2019. Relative toxicity of fipronil and flonicamid against *Bemisia tabaci* (Gennadius) on cotton. Indian Journal of Entomology 81 (4): 733-739.

Roy D, Bhattacharjee T, Biswas A, Ghosh A, Sarkar S, Mondal D, Sarkar P K. 2019. Resistance monitoring for conventional and new chemistry insecticides on *Bemisia tabaci* genetic group Asia-I in major vegetable crops from India. Phytoparasitica 47(1): 55-66.

Simon B, Cenis J L, Demichelis S, Rapisarda C, Caciagli P, Bosco D. 2003. Survey of *Bemisia tabaci* (Hemiptera: Aleyrodidae) biotypes in Italy with the description of a new biotype (T) from *Euphorbia characias*. Bulletin of Entomological Research 93: 259-264.

Sullivan J J, Goh K S. 2008. Environmental fate and properties of pyriproxyfen. Journal of Pesticide Science 33(44): 339-350.

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