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Monitoring of resistance in *Spodoptera litura* (Fabricius) (Noctuidae: Lepidoptera) of Kurnool district of Andhra Pradesh to diamide group of insecticides

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Abstract
Resistance in Kurnool population of *S. litura* was monitored during 2016-17 and 2017-18 by leaf dip assay conducted at RARS, Lam. The insecticides used in the bioassay are diamide group of insecticides *viz.*, chlorantraniliprole, flubendiamide, Avermectin insecticide: emamectin benzoate, Carbamate insecticide: thiodicarb, IGR: Novaluron, Organophosphorous group: chlorpyriphos and a pyrethroid: cypermethrin. The LC_{50} values recorded were 0.072, 0.023, 0.103, 0.120, 0.113, 1.486 and 2.570 ppm during 2016-17 and 0.093, 0.031, 0.113, 0.132, 0.108, 1.580 and 3.047 respectively during 2017-18. The Resistant Ratios (RR) ranged from 1.63 to 2.11 for chlorantraniliprole, 1.53 to 2.06 for flubendiamide, 1.51 to 1.66 for emamectin benzoate, 3.07 to 3.38 for thiodicarb, 3.4 to 3.27 for novaluron, 25.62 to 27.24 for chlorpyriphos and 38.35 to 45.4 for cypermethrin.

Keywords: *Spodoptera litura*, kurnool population, diamides, resistance, resistance ratio

Introduction
The tobacco caterpillar, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) which was earlier known to be a sporadic pest has become a destructive polyphagous pest in the recent past. It feeds on wide variety of crops including tomato, rice, corn, eggplant, potato, sweet and hot peppers, tobacco (Zhou *et al*., 2010) [37], cotton, okra, cabbage, cauliflower, radish, peanut and other legumes (Atwal and Dhaliwal, 2009, Navasero and Navasero 2011) [6, 21]. The *S. litura* accounts for up to 17.71 and 70.00 per cent yield losses in groundnut (Anon., 1983) and blackgram (Krishnaiah *et al*., 1983) [14] in Karnataka and Andhra Pradesh, respectively. *S. litura* was one of the first pests of agricultural importance in India to develop resistance to insecticides. By 1965 resistance to Benzene Hexachloride (BHC) was reported in field population from Rajasthan (Srivastava and Joshi, 1965) [29] and by the early 1970s to endosulfan and carbaryl in Haryana (Verma *et al*., 1971) [33] and West Bengal (Mukherjee and Srivastava 1970). In the early 1980s, populations in the state of Andhra Pradesh were found to be resistant to lindane, endosulfan, carbaryl and malathion (Ramakrishnana *et al*., 1984; Mehrotra, 1991 [23, 17] expressed that *S. litura* developed multiple resistance to insecticides because of high level of resistance to synthetic pyrethroids.

Diamides are a new group of insecticides that have been classified as ryanodine recptor modulators (MOA group 28) (IRAC 2014). Insect ryanodine receptors (RyR) are calcium channels located in the sarcoplasmic reticulam. Diamides cause prolonged channel opening and uncoordinated muscle contraction in intoxicated pest insects subsequently leading to death (Teixeira and Andaloro 2013) [31]. At present, diamide insecticides are being used progressively on different crops and cropping systems of Andhra Pradesh. However, after several years of field applications, cases of resistance development to diamides have been reported for some lepidopteran species from several countries. High levels of diamide resistance are present in the tomato leafminer, *Tuta absoluta* (Roditakis *et al*., 2015) [24] Resistance to chlorantraniliprole has been reported in the rice stem borer *Chilo suppressalis*, the cutworm *Spodoptera litura*, and the beet armyworm *Spodoptera exigua* (Su *et al*. 2012; Che *et al*. 2013; Gao *et al*. 2013) [30, 10]. Further, resistance has been reported for *C. suppressalis* to flubendiamide (Wu *et al*. 2014) [35]. Selection pressure for resistance development can only increase in the absence of viable and sustainable integrated pest management strategies. Therefore, generating baseline data on diamide insecticides would be helpful in effective implementation of resistance monitoring and management tactics in frequently treated pests (Teixeira and Andaloro 2013) [31] at present, diamide insecticides are being used progressively on different crops and cropping systems of Andhra Pradesh. However, after several years of field applications, cases of resistance development to diamides have been reported for some lepidopteran species from several countries. High levels of diamide resistance are present in the tomato leafminer, *Tuta absoluta* (Roditakis *et al*., 2015) [24] Resistance to chlorantraniliprole has been reported in the rice stem borer *Chilo suppressalis*, the cutworm *Spodoptera litura*, and the beet armyworm *Spodoptera exigua* (Su *et al*. 2012; Che *et al.* 2013; Gao *et al*. 2013) [30, 10]. Further, resistance has been reported for *C. suppressalis* to flubendiamide (Wu *et al*. 2014) [35]. Selection pressure for resistance development can only increase in the absence of viable and sustainable integrated pest management strategies. Therefore, generating baseline data on diamide insecticides would be helpful in effective implementation of resistance monitoring and management tactics in frequently treated pests (Teixeira and Andaloro 2013) [31].

Material and Methods

S. litura (Fab.) egg masses were collected from the groundnut fields of Kurnool district of Andhra Pradesh and kept in wide mouthed plastic containers without lid and mouth tied with gauss cloth and secured with a rubber band. The freshly hatched larvae were fed on castor leaves. The larvae were reared in a hygienic conditions in BOD under laboratory conditions (25 ± 2 ºC and 65 ± 5% RH). The larvae were fed with fresh castor leaves continuously and the plastic containers were cleaned regularly to avoid NPV and other contaminations. When larvae pupates, pupae were transferred to another container and were provided with cotton balls dipped in honey on which the adults feed after emerging from pupa. The adults were transferred to a different container a, the males and females were sexed in 1:2 ratio for mating. After mating the adults lays eggs in masses. The egg masses were transferred to different containers and provided with fresh castor leaves. The third instar larvae of F1 were used for bioassay.

Rearing of susceptible/ lab strain of *S. litura* in the lab

Egg masses of *S. litura* were collected from the fields and they are cultured in the laboratory upto 14 generations. The third instar larvae of F_{14} is used for bioassay

Test insecticides

Commercial formulations of the insecticides *viz*., chlorpyriphos 50EC, thiodicarb 75 WP, cypermethrin 25EC, emamectin benzoate 5SG, novaluron 10 EC, flubendiamide 39.35 SC and chlorantraniliprole 18.75 SC were used as toxicants and different serial dilutions were prepared to test the status of insecticide resistance in Kurnool populations of *S. litura* in Andhra Pradesh

Leaf dip method bioassay

The castor leaves were cut into discs of 6 cm and dipped in test insecticides for 30 seconds and shade dried. The discs were placed in a petri plates. The test insects were preconditioned for about 5-6 h without food under laboratory conditions. After starvation, ten healthy and active third instar caterpillars of F1 generation were released for feeding onto the castor leaves. The experiment was replicated thrice.

Mortality data

Mortality of *S.litura* larvae was recorded at 24, 48, 72, 96, 120 and 144 hours after treatment (HAT). The per cent mortality was calculated and subjected to probit analysis (Finneys method, 1971). LC_{50}, LC_{90} at 144 HAT heterogeneity (χ^2), intercept (a), slope of the regression line (b), regression equation and fudicial limits were calculated using SPSS software for probit analysis.

Results and Discussion

The LC_{50} values of the lab strain were 0.015, 0.033, 0.039, 0.044, 0.058, 0.067 and 0.068 ppm for flubendiamaide, novaluron, thiodicarb, chlorantraniliprole, chlorpyriphos, cypermethrin and emamection benzoate respectively

Diamides: The LC_{50} and LC_{90} values for chlorantraniliprole were 0.072 and 1380 ppm during 2016-17 and 0.093 and 489.2 ppm during 2017-18 compared to the lab strain indicating no or negligible resistance development. And for flubendiamide, LC_{50} and LC_{90} values were 0.023 and 181 ppm during 2016-17 and 0.031 and 157.1 ppm during 2017-18. The Resistance Ratios for *Spodoptera litura* of Kurnool population for chlorantraniliprole and flubendiamide were less than two (Table 1 and 2) during both years of study.

The results are in concordance with Ahmad and Gull (2017) observed very low resistance (3.1 to 7.9) levels in due to chlorantraniliprole during 2009-10 and (13 to 17 folds) during 2012-2013, very low (3.3-6.7 folds) for flubendiamide from 2009 to 2012, the Gujranwala population of 2013 exhibited a moderate level of flubendiamide resistance (24 folds). Lai *et al*., 2011 [16] reported 17.1 folds resistance in DG10 srain, eight populations showed low resistance levels (RF=5-10) and only WH09 strain was susceptible to chlorantraniliprole against *S. exigua* of China. Low levels of resistance to chlorantraniliprole was reported in *Plutela xylostella* (Wang *et al*., (2010) [34] up to 5 folds, 5.8 folds in *Cnaphalocrosis medinalis* (Zeng *et al.*, 2011) [36] and 39 folds resistance in *Choristoneura rosaceana* in WEB population (Lahm *et al.*, (2007) [15]. High resistance levels for Italian strain to the extent of 1,742 folds but for Greece strain were 11 fold, suggesting that resistance was low for this strain, but increasing over time (Roditakis and Vasakis, 2015) [24]. Muthusamy *et al*., 2014 [20] observed that ten generations after selection with chlorantraniliprole, *S. litura* has developed 80 fold resistance to chlorantraniliprole compared to lab selected strain. It is evident from the present study and experiences from China and Italy on different insect pests like *Spodoptera exigua* and *Tuta absoluta*, repeated use of same insecticide on successive generations on the same pest results in development of insecticide resistance. To avoid resistance to diamides in *Spodoptera litura*, group 28 insecticides should be rotated with insecticides possessing different modes of action as the Kurnool population is still susceptible to both the diamides

Emamectin benzoate: The LC_{50} and LC_{90} values were 0.103 and 1195 ppm and 0.113 and 811 ppm during 2016-17 and 2017-18 respectively. Resistance monitoring studies revealed nil or negligible resistance in Kurnool populations of *S. litura* to emamectin benzoate (RF=1.5 and 1.6 during 2016-17 and 2017-18) and susceptible to emamectin benzoate. The present results are in accordance with Ahmad *et al*., 2015 [2] who studied the development of resistance in 5 five populations of *S. litura* from Pakistan and found that from 1997 to 2003 the pest did not develop any resistance but during 2005-2010, a low level of resistance was reported in six populations

(RF<10). Ahmad *et al*., 2008 [26] out of 19 populations, five populations showed moderate level of resistance with RR (resistance ratio) from 15- to 21-fold compared with Lab-PK, whereas 11 populations were tolerant to emamectin with RR of 2–10-fold only. Three revealed high level of resistance (32.7-77.0-fold) and by Ahmad and Gull (2017) [1] 2.8-7.9 folds.

Thiodicarb

The LC_{50} and LC_{90} values were 0.120 and 61.9 ppm during first year of study and 0.132 and 1025 ppm during second year. The pest has developed a resistance of 3.07-3.38 folds for thiodicarb. The results documented in this study are in accordance with Ahmad *et al*., (2007) [3] assessed 18 field populations of *S. litura* from Pakistan and found that seven populations exhibited a very low resistance, five populations a low resistance, two populations a moderate resistance and three populations a high resistance to thiodicarb. In a similar kind of study with field populations of *Helicoverpa armigera*, Hussain *et al* (2014) reported low resistance ratios (5.60-11.50-fold). On contrary, Saleem *et al*. (2008) [26] reported the increase in levels of resistance to thiodicarb from 20 to 102 folds in *S. litura* population of Okara, Pakistan. Tong *et al.*, (2013) [32] reported high levels of resistance *ie*., 38.6 to 425 times to thiodicarb against *S. litura* of China as the insecticide was used very frequently in the region (8 times a month), Shad *et al*. (2012) [27] also reported high levels of resistance to thiodicarb against this pest in Pakistan (78-396 folds) The low level of resistance observed to thiodicarb can be attributed rotation of molecule with other new chemistries like novaluran, lufenuron, chlorantraniliprole and flubendiamide consequence of vigorous IRM/IPM dissemination activities of the ANGR Agricultural University and Ag. Department of the Govt. of Andhra Pradesh. Therefore, thiodicarb can be judiciously used with other molecules against *Spodoptera litura* on different crops in the near future too.

Novaluron

The LC_{50} and LC_{90} values were 0.113 and 42.7 ppm during 2016-17 and 0.108 and 325.7 ppm during 2017-18. *S. litura* has developed 3.4 and 3.27 folds resistance compared to lab strain. Present results were in accordance with Hussain *et al.* (2014) has recorded a low RR of 5.98-11.83-fold for the methoxyfenozide, an insect growth regulator against *H. armigera*. Very low (RF <5) resistance levels obtained in the present study against *Spodoptera litura* in Andhra Pradesh and other reports on low resistance levels in *Helicoverpa armigera* with metoxyfenozide is a testimony to the fact Insect Growth Regulators are best fit IRM/IPM tools since they manage polyphagous insect pests like *Spodoptera* and *Helicoverpa* by dual action of ingestion and contact.

Chlorpyriphos

During 2016-17, The LC_{50} and LC_{90} for chlorpyriphos were 1.486 and 5983 ppm and 1.580 and 715 ppm Tobacco caterpillar of Kurnool has developed low resistance to a fold of 25.6 and 27.2 compared to lab strain Aruna Sri (2010) [5] documented very low resistance in *S. litura* on cotton upto 6.33 to Guntur population and 5.46 for Prakasam strain upto 5.72 similarly Sreelakshmi *et al.*, (2017) [28] observed 2.62 to 6.14 folds to chlorpyriphos in Kerala population. However, Kranthi *et al*., 2002 [13] reported RFs of 129 and 127 in Warangal and Mahaboobnagar populations of *Spodoptera litura*. They opined that high resistance levels indicated in their study may be due to heavy use of chlorpyriphos on groundnut during that period in central part of India. Gandhi *et al*., 2016 [9] monitored resistance in *S. litura* to conventional insecticides from seven regions of India and observed that the *S. litura* population has noticed resistance to a folds of 2.5 (Indore) to 88.4 (Adilabad). This pest has developed varying degrees of resistance to chlorpyriphos across the country depending on the quantum of usage chlorpyriphos in the respective geographical regions. Hence, the judicious use of the chemical is suggested against *Spodoptera litura* as there is every possibility of control failures due to resistance development in the pest. Chemical can be considered in the management schedule, if other IRM tools are not available, if needed or judiciously recommended only once in a season for time being on populations where in low level of resistance was encountered.

Cypermethrin

The LC_{50} and LC_{90} values were 2.570 and 6679 ppm and 3.047 and 10590 ppm during both the years respectively. During 2016-17, Kurnool population has recorded low level of resistance upto 38 and high level of 45.4 folds during 2017-18. Armes *et al*., 1997 [4] studied the resistance monitoring in 32 strains of the tobacco caterpillar, *Spodoptera litura*, collected from 8 locations in Andhra Pradesh from 1991 to 1996 and reported resistance levels ranging from 0.2 to 197 folds. Likewise, Kranthi *et al*., 2002 [13], documented high resistance levels of 148 and 133 during 1997 and 1998 in *Spodoptera litura* collected from cotton grown in Mahaboobnagar dist. and attributed to the heavy use of cypermethrin on groundnut in the region during that period. Prasad *et al*, 2008 [22] reported Resistance Ratios as high as 557 for cypermethrin in Guntur population of *Spodoptera litura* collected from cotton during *Kharif* 2005. 163-287 folds resistance reported by Huang *et al*., (2006) from China, Saleem *et al*., (2008) [26] noted 5 to 83 folds resisatnce Chandrayudu *et al*., 2015 documented RRs as low as 25.8 for cypermethrin but ArunaSri (2010) [5] reported upto 16.21 folds resistance in Guntur population of Andhra Pradesh. As well aa Karuppaiah *et al*., (2017) [12] reported very low of 2.2 folds. From Thailand, 4.73 to 6.78 folds by Ruttanaphan *et al*., (2018) [25]. In conclusion, *S. litura* of Kurnool district of Andhra Pradesh is still susceptible to novel chemicals like chlorantraniiprole, flubendiamide and emamectinbenzoate, developed very low resistance to novaluron and thiodicarb and moderate resistance to chlorpyriphos and high resistance to cypermethrin. Hence, the novel chemicals should be rotated with each other having different modes of action as an important IRM tool, judicious use of cypermetrin and restricted use of cypermethrin.

Table 1: Toxicity of chemicals to third instar larvae of *S.litura* of Kurnool district during 2016-17

Chemical	LC 50 in ppm/larvae (95% FL)	Sus. Strain LC 50 in ppm/larvae (95% FL)	LC_{90} ppm / larva (95% FL)	Heterogeneity (χ^2)	Slope ± S.E (b)	Regression equation Y = a+bx	RF
Chlorantraniliprole 18.5 SC	0.072 (0.012-0.26)	0.044 (0.02-0.252)	1380 (191.7-45402)	0.097	0.341±0.080	Y=4.241+0.341x	1.63
Flubendiamide 39.35 SC	0.023 (0.003-0.083)	0.015 (0.001-0.080)	181 (33.4-2928)	0.157	0.541±0.087	Y=6.238+0.541x	1.53
Emamectin benzoate 5 SC	0.103 (0.021-0.347)	0.068 (0.010-0.231)	1195 (169-32918)	0.280	0.311±0.083	Y=3.761+0.311x	1.51
Thiodicarb 75 WP	0.120 (0.031-0.317)	0.039 (0.007-0.144)	61.9 (18-410)	0.678	0.435±0.095	Y=4.603+0.435x	3.07
Novaluron 10 EC	0.113 (0.031-0.28)	0.033 (0.002-0.151)	42.7 (14.39-215.3)	0.370	0.471±0.095	Y=4.941+0.471x	3.4
Chlorpyriphos 50 EC	1.486 (0.502-4.3)	0.058 (0.008-0.244)	5983 (837 -148939)	0.228	0.061±0.083	Y=0.740+0.061x	25.62
Cypermethrin 25EC	2.570 (0.94-6.82)	0.067 (0.005-0.245)	6679 (1263-80833)	0.510	0.154±0.083	Y=1.856+0.154	38.35

Table 2: Toxicity of chemicals to third instar larvae of *S.litura* of Kurnool district during 2017-18

Chemical	LC 50 in ppm/larvae (95% FL)	Sus. Strain LC 50 in ppm/larvae (95% FL)	LC_{90} ppm / larva (95% FL)	Heterogeneity (χ^2)	Slope ± S.E (b)	Regression equation Y = a+bx	RF
Chlorantraniliprole 18.5SC	0.093 (0.012-0.340)	0.044 (0.02-0.252)	489.2 (83.9-12625)	0.204	0.355±0.204	Y=3.93+0.355x	2.11
Flubendiamide 39.35 SC	0.031 (0.006-0.105)	0.015 (0.001-0.080)	157.1 (31.7-2054)	0.090	0.521±0.087	Y=5.997+0.521x	2.06
Emamectin benzoate 5 SC	0.113 (0.025-0.357)	0.068 (0.010-0.231)	811 (130-16740)	0.488	0.315±0.083	Y=3.782+0.488x	1.66
Thiodicarb 75 WP	0.132 (0.030-0.417)	0.039 (0.007-0.144)	1025 (159-22559)	0.263	0.290±0.083	Y=3.499+0.290x	3.38
Novaluron 10 EC	0.108 (0.028-0.312)	0.033 (0.002-0.151)	325.7 (67-3928)	0.159	0.356±0.085	Y=4.188+0.356x	3.27
Chlorpyriphos 50 EC	1.580 (0.630-3.65)	0.058 (0.008-0.244)	715 (183.8-6039)	0.339	0.096±0.0.094	Y=1.023+0.096x	27.24
Cypermethrin 25EC	3.047 (0.97-8.55)	0.067 (0.005-0.245)	10590 (1714-204576)	0.806	0.175±0.091	Y=1.923+0.175x	45.4

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