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Monitoring insecticide resistance to leaffolder, *Cnaphalocrocis medinalis* (Guenee) (Lepidoptera: Pyralidae) in rice growing regions of Tamil Nadu

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Abstract
Leaffolder, *Cnaphalocrocis medinalis* (Guenee) is a prominent and important insect pests of rice and more insecticides are being used for the management in the farmers' holdings. In the present study, level of insecticide resistance in rice leaffolder was assessed by collecting leaffolder from different rice growing tracts of Tamil Nadu and using the data on discriminating dose (DD) fixed on susceptible population. The results revealed that the per cent resistance varied from 53.34 to 80.00, 51.8 to 63.34, 10.66 to 26.67 and 43.34 to 61.34 respectively for insecticides chlorpyriphos, cartap hydrochloride, chlorantraniliprole and profenophos. The order of frequency of resistance to different insecticides were chlorpyriphos > cartap hydrochloride > profenophos > chlorantraniliprole. Irrespective of the insecticides tested, high resistance was noticed in Bhavanisagar population. The order of resistance levels to chlorpyriphos, cartap hydrochloride, chlorantraniliprole and profenophos in the four major rice growing locations in Tamil Nadu were Bhavanisagar > Aduthurai > Coimbatore >Trichy.

Keywords: *Cnaphalocrocis medinalis*, insecticide, resistance, seedling dip, bioassay, Rice

Introduction
Out of the eight species of leaffolder attacking rice, the most wide spread and important one is *Cnaphalocrocis medinalis* (Guenee) [Pyralidae: Lepidoptera] which attack rice in different crop growth stage [6]. *C. medinalis* considered earlier as a pest of minor importance have increased in abundance in late 1980's and have become a major pest in many parts of the world [3]. *C. medinalis* has been reported to attain the major pest status in some important rice growing areas of India [7]. Second instar leaffolder larva glues the growing rice leaves longitudinally and feeds the green foliage voraciously which results in papery dry leaves. Feeding often results in stunting, curling or yellowing of plant green foliage. Loss caused by the insect pest to rice crop is in surmountable [3]. In certain extend, *C. medinalis* causes 63 to 80 per cent yield losses in rice [17].

The need for higher levels of production for feeding huge population has caused expanded planting areas and more crops per year to be harvested. In any cropping system, agrochemicals become the prime control method because they ease in adoption, cost effectiveness, relative availability than other tactics. At all times, chemical spray was considered as a major way to control the pest. The heavy use of pesticides and high fertilizer rates seem to favour leaffolder out breaks. Frequent and serious out breaks have reported in many Asian countries, including China, India, Indonesia, Philippines, Malaysia, Korea, Japan, Sri Lanka and Vietnam [13]. The control of rice insect pests has been completely relied on extensive use of chemical insecticides, which leads to development of resistance to major group of insecticides. It already has been documented in Japan [11] China [23] and India [5].

Resistance of agricultural pests to agrochemicals is now recognized as far as one of the toughest challenges to sustaining worldwide production of food crops. The increasing insecticide resistance is threatening the impressive gains in control of insect pests problems. One of the most important problems in confronting the pest resistance is the ignorance in the early detection and measurement of resistance to insecticide in an insect species. Evidence of resistance is observed through reduced insect mortalities following the use of insecticidal formulations and application techniques which previously provided effective control. Monitoring of resistance helps to evaluate the impact of resistance management strategies besides help in avoiding ineffective molecule and assists in making a proper recommendation

of alternative molecules. Nowadays a number of insects are developing resistant towards the different group of insecticides. However, there is almost report of insecticides resistance in major pests like *Helicoverpa armigera*, *Plutella xylostella*, *Nilaparvata lugens* and *Chillo spp. Spodoptera litura* to different insecticides by many researchers [2, 8, 9, 14, 16]. So this issue now became a global level threat to chemical control of insects. Keeping the current scenario in our mind we have decided to find out the level of insecticide resistance in case of *C. medinalis* to different insecticides like chlorpyriphos, cartap hydrochloride, profenophos and chlorantraniliprole in few rice growing tracts of Tamil Nadu.

Materials and methods

Assessing the level of resistance

The tentative discriminating doses (DD) for the susceptible population based on baseline toxicity data arrived were used for detecting the level of insecticide resistance of rice leaf folder collected from different rice growing tracts of Tamil Nadu. The tentative discriminating doses (DD) by seedling dip method to third instar larvae were 46, 40, 4 and 36 ppm for chlorpyriphos, cartap hydrochloride, chlorantraniliprole and profenophos respectively [18].

Insects

Field populations of rice leaffolder were collected from major rice growing regions of Tamil Nadu to assess the susceptibility to different insecticides. The test insects were collected from Aduthurai, Trichy, Bhavanisagar and Coimbatore during 2016. The collected larvae were cultured to the immediate generation at the Entomology glass house, Paddy Breeding Station, TNAU, Coimbatore on susceptible rice variety Taichung Native-1 (TN-1) plants.

Insecticides used

Commercial formulations of insecticides used in the bioassay studies were: [Profenophos 50 EC; Organophosphate, Syngenta Co Ltd.], [Chlorpyriphos 20 EC; Organophosphate, Dow Agro Sciences], [Cartap hydrochloride 50 SP; Nereis toxin analogues, Takeda chemical Industry] and [Chlorantraniliprole 18.5 SC; Diamide, Syngenta Co Ltd.].

Bioassay method

Bioassays experiments were performed in the laboratory with the four populations collected from Tamil Nadu. Third instar larvae of F_1 generation were exposed to different insecticides *viz*., chlorpyriphos, cartap hydrochloride, chlorantraniliprole and profenophos by using seedling dipping method developed by Zheng *et al.* [24]. Serial dilutions were done for the test compounds using distilled water to arrive concentration for treatments. Distilled water alone was used as control treatment. Four weeks old rice seedling were immersed in insecticidal suspension for 30 seconds by turning the pot upside down and dipping the leaves and stem into the solution. The seedlings were then allowed to dry until no residual water observed. Filter papers were placed into the base of a small petri dish (7 cm dia.), and then the filter papers were hydrated by adding 1 ml of water. The seedlings were cut and 5 cm sections of the leaves were layered onto the filter paper in petri-dish. Approximately 35 leaf cut sections were used in each petri-dish and ten third-instar larvae were transferred onto each petri-dish with a small paint brush. The Petri dishes were stored at 25°C room temperature and 60% relative humidity. This experiment was done by using all insecticides along with water as control with four repeated replications.

Statistical Analysis

Larval mortalities were assessed after 24 and 48 hours. Larvae were considered as dead if there was no coordinated movements.The mortality values were converted to percentages and adjusted for control mortalities using Abbott's formula [1]. The data was subjected to probit analysis [12] after converting the observed mortality into corrected mortality by using Abbott's formula. The standard error was calculations were done by using the formula by Regupathy and Dhamu [20].

The resistance percentage (RP) was worked out by the formula

$$\text{Per cent resistance (RP)} = 1 - \frac{\text{No. of dead insects}}{\text{No. of insects tested}} \times 100$$

The standard error was calculated as given below,

$$\sqrt{\frac{p\,(100-p)}{n-1}}$$

Where,
p = per cent insect surviving in discriminative dose
n = total no. of insects tested

Results and discussion

Comparative levels of resistance among the insecticides

The results revealed that rice leaffolder populations developed resistance to all four insecticides tested. The resistance percentage was determined for four field collected populations *viz*., Aduthurai, Trichy, Bhavanisagar and Coimbatore (Table 1). Among the field collected rice leaffolder populations, Bhavanisagar population was found to be the most resistant and recorded 80.00, 63.33, 26.67and 61.34 per cent resistance to chlorpyriphos, cartap hydrochloride, chlorantraniliprole and profenophos respectively followed by Aduthurai population which recorded 62.77, 55.00, 21.34 and 50.00 per cent, respectively. The Coimbatore leaffolder population recorded with 53.34, 51.80, 18.00 and 46.72 percent resistance. Resistance level was low in Trichy population irrespective of the insecticides tested and was 51.33, 48.66, 10.66 and 43.34 per cent, respectively. Irrespective of location, the order of resistance level to the four selected insecticides is as follows.
Chlorpyriphos > Cartap hydrochloride > Profenophos > Chlorantraniliprole

Assessing the level of resistance at various rice growing regions

Monitoring insecticide resistance is the corner stone of resistance management strategies. The development of resistance is complex and is influenced by a variety of interacting factors, including the extent of selection pressure, the mode of inheritance and migratory behaviour of adults [15, 21]. In the present study, the level of insecticide resistance was estimated using discriminating dose (DD) technique by adopting seedling dip bioassay method because the contact and ingestion toxicity by the insecticide to the larva more closely resembled the field conditions [10].

Chlorpyriphos

The LC_{95} value obtained for the highly susceptible leaffolder population [18] was used to derive the per cent survival for the

field collected populations. The LC_{95} of chlorpyriphos, 46 ppm was used to test the resistance percentage for different field collected populations. The resistance percentage to chlorpyriphos varies slightly among the four field populations. Among the field collected populations, the population from Bhavanisagar was found to be the most resistant to chlorpyriphos (80.00%). The level of resistance was low in Trichy population (51.33%) compared to the Aduthurai population (62.67%) and Coimbatore population (53.34%) (Table 1) (Fig.1). Anandan and Regupathy [4] also revealed the extent of resistance to chlorpyriphos in Coimbatore population of *C. medinalis* and
M. patnalis varied from 6.0 to 16.0 per cent and 6.67 to 16.67 per cent, respectively. The resistance level in *C. medinalis* to chlorpyriphos in various places in their study was in the following order, Aduthurai > Bhavanisagar = Madurai > Ambasamudram > Thirur.

Cartap hydrochloride

Among the populations tested, the leaffolder from Bhavanisagar fields indicated high resistance percentage (63.34%). The population from Aduthurai (55.00%), Coimbatore (51.80%) and Trichy (48.66%) were comparatively less susceptible (Table 1) (Fig. 1). Cartap hydrochloride showed higher degree of resistance to *C. medinalis* population. This may be due to the indiscriminate use of cartap hydrochloride in the particular rice ecosystem for control of leaffolder or other insect pests. There were reports suggesting development of resistance by way of increased selection pressure. Even the rabi season results indicated elevated resistance percentage results in 4.25 fold resistance confirming increased usage of cartap hydrochloride in kharif season [19].
The LD_{50} and LD_{90} values of cartap hydrochloride to *C. medinalis* were 0.0814 and 0.3703 µg larvae^{-1}, respectively. The chi-square value and the slope of the log dose probit line was 1.94724. The resistance percentage at the field recommended concentration (0.01%) indicated 3.70 fold resistance. However, at LD_{50} level, comparison of the toxicity of cartap hydrochloride with the conventional insecticides, to which the pest has developed resistance, revealed that cartap hydrochloride was 2.76, 2.41 and 1.58 times toxic than endosulfan, carbofuran and cypermethrin, respectively. While at LD_{90} level it was 1.30, 1.28, and 1.13 times toxic than carbofuran, cypermethrin, and endosulfan, respectively in *kharif* season. Whereas in *rabi* season the third instar larvae of *C. medinalis* recorded LD_{50} and LD_{90} values of 0.0700 and 0.4249 µg larvae^{-1}at 24 HAT [22].

Chlorantraniliprole

Among the field populations tested for resistance, Bhavanisagar population was highly resistant to all the chemicals tested compared to other locations. Among different locations, for the newer compound chlorantraniliprole more resistance percentage was registered to Bhavanisagar population (26.67%) followed by Aduthurai (21.34%), Coimbatore (18.0%) and Trichy (10.66%). Among all insecticides tested chlorantraniliprole was found effective in controlling leaf folder pest. It is showing low level of insecticides resistance percentage as compare to others insecticides due to having unique mode of action. There is also report in China from three locations of five populations were assayed for susceptibility towards chlorantraniliprole and results of the LC_{50} values showed a low level 5.8-fold of sensitive variation (LC_{50} values ranges from 0.204 to 1.186 mg^{-1}) by using seedling dipping method and which exhibited greater efficacy against *C. medinalis* [24].

Table 1: Insecticide resistance in field populations of *Cnaphalocrocis medinalis*

Locations	Chlorpyriphos	Cartap hydrochloride	Chlorantraniliprole	Profenophos
	Resistance Percentage ± SE n=100	Resistance Percentage ± SE n=100	Resistance Percentage ± SE n=100	Resistance Percentage ± SE n=100
Aduthurai	62.67 ± 8.19 (52.337)	55.60 ± 7.64 (47.869)	21.34 ± 6.96 (27.508)	50.00 ± 3.46 (45.000)
Trichy	51.33 ± 4.66 (45.764)	48.66 ± 5.92 (44.236)	10.66 ± 5.78 (19.062)	43.34 ± 8.81 (41.168)
Bhavanisagar	80.00 ± 6.35 (63.434)	63.34 ± 3.33 (52.733)	26.67 ± 6.67 (31.090)	61.34 ± 1.34 (51.550)
Coimbatore	53.34 ± 6.67 (46.911)	51.80 ± 7.38 (46.031)	18.00± 2.00 (25.104)	46.72 ± 6.66 (43.088)

Figures in the parentheses are arcsin transformed values.
RP - Resistance Percentage; SE – Standard error

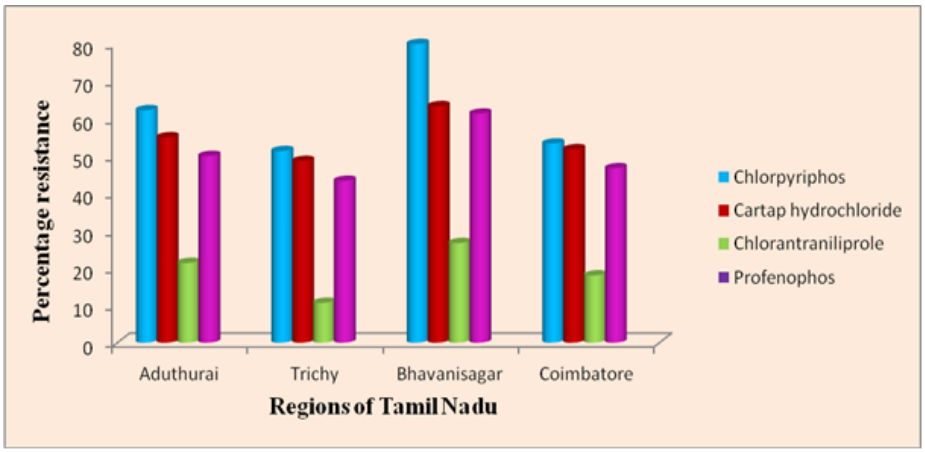

Fig 1: Insecticide resistance in different regions of Tamil Nadu

Profenophos

The LC_{95} value of profenophos, 36 ppm [18] was used to test the resistance percentage for the field collected populations. The resistance percentage to profenophos varies slightly among the four field populations. Among the field collected populations, the population from Bhavanisagar was found to be the most resistant to profenophos (61.34%). The level of resistance was low in Trichy population (43.34%) compared to the Aduthurai population (50.00%) and then Coimbatore population (46.72%).

The rising risk of insecticide resistance of *C. medinalis* should not be over- looked, hence insecticide resistance management strategy should be initiated as early as possible. Establishment of baseline toxicity data is fundamental for any insecticide resistant management strategies. Results in this study have provided resistance percentage of *C. medinalis* to several insecticides in different locations within Tamil Nadu.

Conclusion

Thus, the present study revealed that among all the insecticides tested against *C. medinalis*, chlorantraniliprole was found effective in controlling the pest. The newer compound like chlorantraniliprole was showing less level of resistance percentage among the different locations of Tamil Nadu. So it is advisable that chemical like chlorantraniliprole can be used in management of leaf folder pest and also chemicals like chlorpyriphos, cartap hydrochloride and profenophos should be avoided for rational management of target pest due its high level of resistance percentage. So there is need of continuous resistance monitoring at regular interval is highly recommended to detect possible changes in pest sensitivity before selecting chemicals to tackle the serious pest problem.

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