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DETECTABLE SYSTEM OF RESISTANCE OF VERTIMEC (ABAMECTIN) WITHIN THE TWO-SPOTTED SPIDER MITE, *TETRANYCHUS URTICAE* KOCH

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Abstract - Augmented resistance of pesticides remains a principal issue for unsatisfactory impacts on the environment. So, there is a need to distinguish it faster by innovated devices and then find effective tools to reduce it as soon as possible, especially for mites such as *Tetranychus urticae*. Detectable tools, which are powered with solar energy, were used to discover Vertimec resistance depending on the assurance of the changes of electromagnetic field forces in comparison with sensitive mites. Acquired results with certain differences of electromagnetic forces were affirmed by evaluation of resistance proportions (RRs) which recorded 8.52- and 4.53-folds for resistant strains of Vertimec (Abamectin) on cotton and soybean, respectively, upon Vertimec LC50s. Furthermore, they were 5.97- and 4.29- folds for field Vertimec strains on cotton and soybean, respectively. However, there were no significant differences between resistan and field strains; they appeared in comparing the mentioned strains with susceptible mites. It may be well concluded that pesticides' resistance is now available to be detected by utilizing a simple and quick framework in the field.

***Keywords* -** Detector, Electromagnetic, *Tetranychus urticae*, Resistance and Vertimec.

I. INTRODUCTION

Resistance of pesticides is an essential subject particularly when it is related to the two spotted spider mite, *Tetranychus urticae*. It is vital to take the most important components of climate changes as top priority when resistance is the issue which agriculturists should give a hand to. In Egypt, with elevated ratios of CO_2 and UV pro rata with pesticides' resistance specifically of spider mites, it is difficult to control them with pesticides (Nahar *et* al. 2005) due to their short life cycle, reproduction high capacity, and ability to create protection from miticides (Georghiou 1990).

Even with the broad utilization of chemical pesticides, pests existed a noteworthy explanation behind crop production calamity worldwide. Common used pesticides caused numerous ecological, agricultural, medical, and financial problems. Imperatively, development of resistance occurred as a result of the overwhelming utilization of synthetic insecticides and the selection pressure on insects and mites.

Particular metabolic pathways utilized by insects to change over hindrances into less poisonous structures or their expulsion from the framework are featured. Utilizing the proteomics approach, responsible proteins affected by pesticides in insects and then their alterations by pesticides' resistance could be distinguished (Dawkar et al., 2013).

The available tool to assay pesticides' resistance is depending on Immunochromatographic dip-stick-format kits. They were created to distinguish resistance to carbamates and organophosphates and others. Strips were upon polyclonal antisera versus resistance of pesticides which are related to esterase isozymes isolated from insects. They were simply used by farmers anywhere (Kranthi, 2005), but the problem which is related to cost and accuracy still exists.

Using electromagnetic fields as traps or even to attract or eject pests, is an ordinary known process. It was started by Gerharz (1991) who situated the electromagnetic field nearby or in a pest control. A gadget was chosen from the gathering comprising insect traps, insect teasing stations containing a pest poison, and pointer stations. For instance, a wide assortment of regular pest traps for slithering insects could be retrofitted with an electromagnetic field generator. Devices could have the generator to a focal area of a trap with an electromagnetic field generator, contained or not, a chemical or biological attractant.

So, there is a need to get a priceless device that would be able to get fast and accurate results to help farmers to discover the problem in the beginning before its development. In the same time, there will be a new use of electromagnetic fields to detect pesticides' resistance in order to protect the environment of more quantities of acaricides.

Mites with certain changes in their bodies provided electric changes and then with the present magnetism in the instrument, electromagnetics field are available to react effectively with exposed mites. Nevertheless, resulted electromagnetic fields' forces and their Axis Graph Magnetic Meter were assessed by the innovated instrument in order to detect pesticides' resistance easily and vastly as presented in this paper.

II. MATERIALS AND METHODS

- **Innovated Instrument Components:**

The prototype of the instrument is shown in Image (1) with all of its components: A solar panel with its attachments and a DC-Motor. Electromagnetic field resulted from two magnets and with the passage of electric current, the required field was gained. Whenever the current is gone through mites, the electrophysiological differences appeared in changes of voltages at LCD monitor. Microcontroller system (PIC16F627A) was used to detect the resistance through electrochemical changes in the resistant strain in comparison with the other sensitive one. The sensor, UGN3503U was used to determine the magnetic field strength and varying voltage provided at output proportions which are picked up to the fielstrength. Also, Electromagnetic interference (EMI) detector was attached to the present circuit to provide and confirm accurate readings of the final electromagnetic field strength. Comparison appeared in the magnetic field force and the current conductance with attention to any differences in food resource, weather conditions, etc.

- **Maintenance of *Tetranychus urticae* strains:**

1. Susceptible strain

Colonies of the spider mite, *T.urticae* were raised under lab conditions (25 ± 2^0C, 60±5%RH and 12 hours light/12 hours dark) at Plant Protection Research Institute for several years without exposure to any contaminations or pesticides.

Image (1) Instrument Prototype Parts: 1-Solar Panel 2-Container of battery, microcontroller system (PIC16F627A), UGN3503U, EMI and others 3-Power 4- Blue Light Emitting Diodes (LEDs) 5- Red Light Emitting Diodes (LEDs)	

2. Resistant strain

Original colony of the spider mite, *T.urticae* was set up from mites gathered from castor oil plants without exposing to pesticides. It was raised under laboratory conditions (25 ± 2^0C, 60±5%RH and 12 hours light/12 hours dark) to assess the action of Vertimec (Abamectin 18 g L^{-1} EC) against *T.urticae* grown-up females. The leaf-dip technique presented by Dittrich (1962) was utilized. All treatments were done under laboratory conditions and each was replicated thrice . Likewise, control discs were dipped in water only. Mortality percentages were determined and corrected by using Abbott's formula (1925). Pooled data were subjected to probit analysis (POLO PC) (LeOra software, 1994). The original strain females were selected for Vertimec for 20 generations according to Yang et al. (2002) with some modifications. 1000 adult females of this colony started this selection. Every two generations, LC50s and LC90s were evaluated. New LC50 was applied as subsequent selection pressure. The next selection transferred to untreated leaves. LC50 estimations of the selected strain were compared to those of the susceptible strain. LC50 of Field colony was got after exposure to the recommended concentrations of Vertimec under certain values of UV and CO2 which interacted with sprayed pesticide on infested cotton and soybean with *T.urticae*. Then, the resistance ratio (RR) was computed. Field colony was got after exposure to the recommended concentrations of Vertimec under certain values of UV and CO2 which interacted with sprayed pesticide on infested cotton and soybean with *T.urticae*.

- **Determination of electromagnetic fields:**

Samples of *T.urticae*, about 100 adult females, were placed inside the instrument. Resistan and field strains were compared with the control which was reared on cotton and soybean leaves. Differences of electromagnetic fields' forces and Axis Graphs appeared and were recorded by the microcontroller system.

- **Data Analysis**

SPSS (V.16) was used to show differences among resistant, field and susceptible strains under electromagnetic' forces instrument. Jonckheere-Terpstra Test, Friedman Test, Kendall's W and others were used to test significance between resistance and susceptible cases at probability with 5% and 1%.

III. RESULTS AND DISCUSSION

Data revealed that strength of electromagnetic fields was changed particularly with significant differences between resistant and susceptible mites as appeared in Figure (1). Direct determination recorded high strength of electromagnetic fields in case of resistant mites to Vertimec on cotton followed by resistant strain on soybean with 45.37 and 28.14 G, respectively.0020 Therefore, there were specific differences in Axis Graph Magnetic Meter resulted of electromagnetic fields through both resistant and susceptible of adult females of *T.urticae* on certain crops as appeared in Figure (2).

Jonckheere-Terpstra Test showed that Std. Deviation of J-T Statistic=.957* among grouping variables of mites. Therefore, both Chi-Square and Median were =3.000a and 28.140 ^{a}. While, Kruskal-Wallis Test showed that Chi-Square=2.000 ^{b} at 5%. Proximity Matrix proved that a dissimilarity matrix with 51.587 as euclidean distance among variables while a similarity matrix was determined by the correlation between vectors of values was -.997.

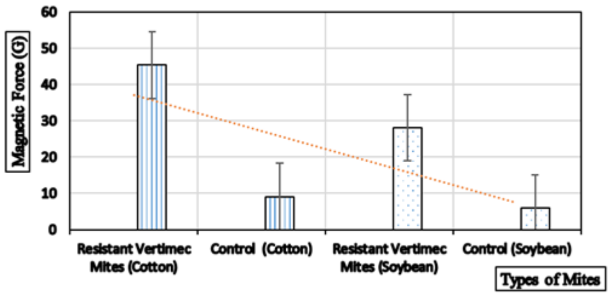

Fig .1. Force of resulted electromagnetic fields through both resistant and susceptible of adult females of *T.urticae* on certain crops

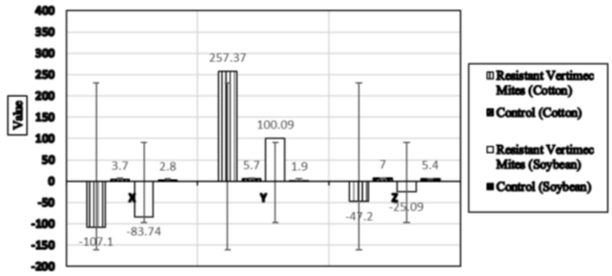

Fig .2. Axis Graph Magnetic Meter resulted of electromagnetic fields through both resistant and susceptible of adult females of *T.urticae* on certain crops

Subsequently, toxicity of Vertimec was compared both of field and resistant strains with the susceptible strain of *T.urticae* on cotton. Whereas, LC_{50} values were 1542.91, 1915.47and 258.45μLL^{-1}, respectively, which showed that LC50 of cotton field strain exposed to moderately levels of CO_2 and UV recorded so close value to that of 20th generation of the laboratory resistant strain. The same situation was in the case of $LC_{90's}$ which recorded 14207.39, 16328.37and 1420.74μLL^{-1}, respectively. Relative to the laboratory strain (S), the resistant ratios (RR) to Vertimec for *T.urticae* laboratory resistant strain and field strain, shown at Table (1). RR's at Vertimec $LC_{50's}$ were 5.97- folds and 8.52- folds, respectively. The same trend occurred in case of assessment of Vertimec resistance in *T.urticae* strain on soybean.

Data showed that LC50 values were 4013.25, 4220.17 and 198.82 μLL^{-1}, respectively, which showed that LC50 of cotton field strain exposed to moderately levels of CO_2 and UV recorded a so close value to that of 20th generation of the laboratory resistant strain. The same situation was in the case of $LC_{90's}$ which recorded 17210.74, 19102.35 and 780.34 μLL^{-1}, respectively. Relative to the laboratory strain (S), the resistant ratios (RR) to Vertimec for *T.urticae* laboratory resistant strain and field strain are shown at Table (1).

RR's at Vertimec $LC_{50's}$ were 5.97- folds and 8.52-folds, respectively. The same trend occurred in case of assessment of Vertimec resistance in *T.urticae* strain on soybean. Data showed that LC_{50} values were 4013.25, 4220.17 and 198.82 μLL^{-1}, respectively, which showed that LC_{50} of cotton field strain exposed to moderately levels of CO2 and UV recorded a so close value to that of 20th generation of the laboratory resistant strain. The same situation was in the case of $LC_{90's}$ which recorded 17210.74, 19102.35 and 780.34 μLL^{-1}, respectively. Relative to the laboratory strain (S), the resistant ratios (RR) to Vertimec for *T.urticae* laboratory resistant strain and field strain are shown at Table (2). RR's at Vertimec $LC_{50's}$ were 4.29- folds and 4.53- folds, respectively. According to Hayashi scale (1983), RRs of both strains resistant to Vertimec LC_{50}, infested cotton and soybean, could be ranked as low resistance. Friedman Test proved the highest significant effect of certain crops on LC50s values at 1% (Chi-Square =12.684**). Kendall's Coefficient of Concordance (Kendall's W^a) = .705** with highly significant Chi-Square =12.684 at 1%.

Table 1. Assessment of Vertimec toxicity against certain strains of adult females of *T.urticae*

T.urticae Strains	Crops	Vertimec Lethal Concentrations				LC_{90}/LC_{50} Ratio
		LC_{50}		LC_{90}		
		Main μLL^{-1}	*RR_{50}	Main μLL^{-1}	*RR_{90}	
Field	Cotton	1542.91^a	5.97	14207.39^a	9.99	9.21
Resistant		1915.47^a	8.52	16328.37^a	11.49	8.52
Susceptible		258.45^b		1420.74^b		5.50
Field	Soybean	4013.25^a	4.29	17210.74^a	22.10	4.29
Resistant		4220.17^a	4.53	19102.35^a	24.48	4.53
Susceptible		198.82^b		780.34^b		3.92

*Resistance ratio (RR) =LC50 or LC90 of Resistant Strain/ LC50 or LC90 of Susceptible Strain.

Pesticides resistance in *Tetranychus urticae* is a portent which caused by many reasons. One of their causes is the introduction of exceeding levels of UV and CO_2, which could assume a critical part to get a resistant field strain. Vertimec, an articulated miticide, LC50 of the research center safe strain which is kept up under selection pressure till F40 and the resistant field strain in comparison with susceptible strain were 2099.38, 200.01 and 50.822 μLL^{-1}, respectively. Additionally, the raised esterases and mixed function oxidases (MFO) in both the laboratory and the field resistant strains had basically characterized the impact of extensive radiation of UV on the surpassed resistance levels recorded for the two strains (Abd El-Wahab and Taha, 2014).

Consequently, expanded CO_2 and global warming can be relied upon to positively influence the concoction barrier flagging framework in plants. Those factors will render them more vulnerable pest assault. The expanded number of produced generations every year and incessant populace flare-ups of potential pests require ceaseless uses of high measure of pesticides. It will boost exposed mites and insects to create pesticides' resistance vastly (Petzoldt and Seaman, 2007). Further, prolongation of insects' lifespan is prolonged under high CO_2 and temperature. Besides, such climate variations will stabilize such insecticide resistant in assortments of pests in their populations. Therefore, all mentioned will make more prominent harm plants even under broad pesticides measures. Also, a few classes of pesticides have been appeared to be less powerful in controlling pests at higher temperatures (Musser and Shelton, 2005).

Vertimec (Abamectin) resistance in *T. urticae* was also reported by several authors (Beers et al., 1998). Stumpf and Nauen (2002) found that MFO (cytochrome P450-dependent monooxygenase) activity was higher in many strains of *T.urticae* than the susceptible strain. Consequently, Abamectin resistance was strongly synergized by PBO (piperonyl butoxide) and DEM (diethyl maleate), suggesting that MFO and GST (glutathione S-transferases) might be involved in abamectin resistance. Astonishingly, levels of cytochrome P450 monooxygenases, specifically, CYP6CM1 in mix with a simple to utilize counter acting agent identification framework permit a quick, dependable, and extremely touchy detection of pesticides' resistance to insects, and certainly to *Bemisia tabaci*, to Neonicotinoid and Pymetrozine (Nauen et al., 2013). Nevertheless, Li et al. (2016) demonstrated that RRs were ranged between 6.51 and 6.03 for *Myzus persicae* (Sulzer) infested tobacco, to certain pesticides in China. Results demonstrated that resistance ratios were RR = 6.51 and 6.03which meant populaces have created minor imperviousness to Imidacloprid. One populace (NC) has achieved a high resistance level to Cyhalothrin (RR = 41.28), five populaces indicated medium level ($10.36 \leq RR \leq 20.45$), and the other six stayed powerless ($0.39 \leq RR \leq 3.53$). As respects Carbosulfan, three populaces have created medium resistance, four populaces indicated just minor resistance, and the other five ($0.81 \leq RR \leq 3.97$) were as yet vulnerable. Populace SZ built up a medium level (RR = 14.83) to Phoxim, the other 11 were vulnerable ($0.29 \leq RR \leq 2.41$). To examine the potential resistance system, restraint impacts of synergists and detoxifying compound exercises were identified. The outcomes showed that the MFO was the most vital detoxifying catalyst presenting Imidacloprid resistance, and CarE was most imperative to Cyhalothrin, Carbosulfan and Phoxim. Our examination gave an exhaustive overview of insecticide resistance of *M. persicae* in Chongqing, and proposed that distinctive districts should take comparing administration to postpone the insecticide resistance advancement and draw out the convenience of insecticides.

Furthermore, magnetic fields were tested on mites, *Tetranychus urticae* and *Polyphagotarsonemuslatus latus* which infested tomato leaves and some leaves were passed through 500 Gauss magnetic field and others were sprayed with magnetic water (Al-Ani, 2010). Results showed the significant decrease of mites' individuals and raised numbers of mites' eggs. That was explained upon hyperactivation of some enzymes in exposed mites to lay down more eggs. Even though, magnetic water showed its effect on the preparation of spray liquids against on the viability of plant protection agents as shown by Wachowiak and Kierzek (2002). They reported raised effectiveness in the control of *Phytophthora sp*. infested potato, after the use of these fungicides diluted in magnetic water. Likewise, magnetic water incremented the efficacy of acaricides against *Tetranychus urticae*. They were Talstar, Omite, Magus and Omite by the use of one and three magnetizers, one magnetizer and two semi-rings (Górski et al., 2009).

In addition, development of exposure methods to certain magnetic field is developed at this paper. Electromagnetic field as shown could play an important role to detect resistance of pesticides in mites through an electromagnetic interference (EMI) detector by translating electrophysiological changes that occurred mainly at glutamate-gated chloride channels (GluCls) and γ-aminobutyric acid-gated rdl and glutamate-gated GluClα chloride channels (GABACl) and resulted to Vertimec' resistance in mites (Riga et al.,2017). Even though and according to other studies which suggested detoxification enzymes with no effective target site were able to cause abamectin resistance in field *T. urticae* populations (Çağatay et al.,2018).

Near future, all recorded readings would be collected and stored at the cloud to be available whenever needed through Internet of Things system (IOT). Besides, data base was connected and joined with resistance ratios (RRs) which were gained from both fields and laboratory' data. Such system will be easier to be used by farmers wherever they are being. But utilizing the electromagnetic fields depending on physiological differences in resistant mites is appeared to be newly emergence trial through this paper. Besides, the solution of metabolic resistance in mites is already done by exposure to specific colors of light emitting diodes (LEDs). Each color could be linked to reduce metabolic pesticides resistance in specific pest on certain plant (Abd El-Wahab,2015; Abd El-Wahab and Abouhatab,2014; Abd El-Wahab and Bursic,2014; Abd El-Wahab et al.2014). Hence, LEDs with different colors are able to be connected with the full innovated system to do the two required steps: firstly, to detect the pesticides' resistance and secondly to stop or reduce it effectively.

IV. CONCLUSION

To conclude, as the pesticides' resistance is a big problem that leads to disasters affecting the environment, there is a new innovated instrument to detect and then reduce it. The solution is depending on differences in electromagnetic forces in resistant strains of *T.urticae* in comparison with susceptible strain. Then, resistance ratios have confirmed the presence of formed resistance. As a consequence, such device is capable to detect resistance to pesticides and farmers can count on it efficiently.

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