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Original Article

Evaluation of acaricide resistance on two spotted spider mite (tetranychus urticae, coch) in the central rift valley of Ethiopia

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ABSTARCT

Spider mite has been one of the major constraints of tomato production in the central rift valley of Ethiopia. The main obstacle in spider mite control in the area is associated with failure of control of tomato spider mite using the available pesticides from time to time. The objective of this study was to evaluate the level of resistance of two spotted spider mite for different acaricides in the central rift valley of Ethiopia. To determine the level of resistance, spider mite populations were collected from four major locations of tomato production areas in central rift valley of Ethiopia such as “Zewai”, “Meki”, “Adama”, and “upper Awash”, leaf dipping assay was performed for different concentrations of dicofol, profenos, endosulfan, abamectin, lambda-cyhalothrin, amitraz. Probit analysis was used to determine the $LC_{50.}$ RF was calculated to asses the development of resistance in the test populations using the “Hawassa” population for they have not been exposed for pesticedes as a control. The RF ranged from 1.79 to 3.11for dicofol, 0.92 to 2.26 for profenose, 1.37 to 1.67 for abamectine, 1.34 to 1.71 for endosulfan, 0.88 to 2.01 for lambda-cyhalothrin 1.99 to 4.47 for amitraz (p<5). The highest RF was observed in “Upper Awash” population of *T. urticea*. In the *T. urticae* population of Upper Awash showed resistance to dicofol, profenos, lambda-cyhalothrins and amitraz whereas in Adama and Meki, there is resistance to dicofol, profenos and amitraz. On the other hand, *T. urticae* population from Zewai is susceptible to all acaricides except amitraz. All the studied populations were susceptible to abamectin and endosulfan. In general, the result suggests that the emergence of resistance in the *T. urticae* populations for most of the acaricides in the rift valley of Ethiopia and should be reduced through finding appropriate resistance management mechanism.

Keywords: Acaricide resistance, LC_{50}’, RF, T. urticae, Central rift valley of Ethiopia

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INTRODUCTION

Resistance to insecticides is the development of an ability in a strain of insects to tolerate doses of toxicants which would prove lethal to the majority of individuals in the normal population of the same species (Anonymous, 1957). T. urticae individuals can develop resistance to insecticides and acaricides (Hoyt *et al.,* 1985; Keena and Granett, 1987; Sawicki and Denholm, 1987; Schoknecht and Otto, 1992; Herron and Rophail, 1998; Gorman *et al.,* 2001). The first failure in the chemical control of spider mite occurred in the early fifties when resistance against organophosphates such as ethyl parathion was detected in green house in the United States and Europe (Helle, 1962). After that, T. urticae resistance to different insecticides and acaricides has been widely reported all over the world (Tian *et al.,* 1992; Campos *et al.,* 1996; Sawicki and Denholm, 1987; Nauen *et al.,* 2001). It has been also reported the outbreak of two spotted spider mite in Ethiopia in the past decade around the central rift valley of Ethiopia in cotton and tomato farms. Following to the wide distribution of acaricide resistance in two spotted spider mite worldwide, attempts have been made on the promotion of integrated pesticide resistance management program that would otherwise lead to environmental degradation and low productivity in many ways. But there is no information on the level of acaricide resistance for two spotted spider mite in Ethiopia to support the resistance management program. Therefore, this study presents the level of resistance of two spotted spider mite populations to the commonly used acaricides in the major tomato producing areas of the central rift valleys of Ethiopia, a case of, two-spotted spider mite populations of "Upper awash", "Adama", "Meki" and "Zewai".

METHODOLOGY

This study was conducted in the sample population of five different populations of T. urticae Koch that were collected from tomato plants in the central rift valley of Ethiopia such as "Adama", "Meki, Zewai", "Upper Awash" and "Hawassa" in the year 2011. A susceptible strain was obtained from Hawassa area where there is no history of acaricide application in the area. Six acaricides; dicofol, profenose, abamectin, endosulfan, lambda-cyhalothrins and amitraz were used at a rate of 25%, 12.5%, 6.25%, 3.125%, 0.78% and 0.39 % of the active ingredients from the recommended dose. Micro pipet 5-1000 µl capacity was used to measure parts per million of the six acaricides, the actual recommended dose and the concentration of acaricide tested are depicted in table 2.1. In bioassays, a leaf-dipping method was used to assess the resistance levels (Tian *et al.,* 1992). Leaf discs of 20 mm in diameter were excised from bean plant (Phaseolus vulgaries) leaves and immersed for five seconds in each suspension of an acaricide solution. The immersed leaves were allowed to dry for 20 minutes and placed upside down on a petridish size of 90 mm in diameter. Ten adult spider mites from the test stock population were transferred using soft "horse hairs" to avoid mechanical damage on the spider mite and covered using a perforated transparent foil.

The experiment was conducted in CRD design. A total of 645 tests (6 Acaricides X 3 replication X 5 Spider mite populations X 7 concentration) and Water (5X3) were used as a control the treatments on the study. Mortality was assessed after 24 hrs of the treatment. Mites that could walk at least one body length after a gentle probe with a fine brush were scored alive. Bioassay data were corrected; the corrected mortality data were analyzed to obtain LC_{50} values and their 95% confidence Limits (CL) and Resistance factors (RF).

Parts per million of acaricides was calculated as;

Total solution per liter = recommended amount of H_2O and rate of the acaricide

Here; Recommended rate= a.i of the acaricide + amount of inert Material

There fore; a.i of the acaricide (in µl) / Total solution (in Lt) = Parts per Million

Statistical analysis

Percent mortality was calculated using;

$$\text{Percent mortality} = \frac{\text{Mortality in the treatment}}{\text{No of mites in the treatment}} * 100\%$$

The data were corrected using Abbott's formula which states (Abbott, 1925):

$$\text{Corrected Mortality} = \frac{\%\ \text{Responded in the treatment} - \%\ \text{responded in control}}{100 - \%\ \text{responded in the control}} * 100$$

All the data from each concentration mortality experiment were subjected to probit analysis. LC_{50} with their 95 % confidence level (CL) and slops ± S.E. of regression were estimated (Finney, 1964) using computer software SPSS (Statistical Package for Social Studies).

Table 1: Parts per million of acaricide concentration used in the bioassay and the recommended dose (µl of the concentration per Lt of solution)

Common name	Trade name	RD a.i	25%	12.5%	6.25%	3.12%	1.56%	0.78%	0.39%
Profenose	Selecron 720	719	179.75	89.88	44.94	22.47	11.23	5.62	2.80
Abamectin 18 g/Lt	Abalon	5.40	1.35	0.68	0.34	0.17	0.08	0.04	0.02
Amitraz	Byby 20 EC	199.8	49.95	24.98	12.49	6.24	3.12	1.56	0.78
Dicofol	Mitigan 18.5 EC	457.0	114.2	57.12	28.56	14.28	7.14	3.57	1.78
Edosulfan	Thionex 35 EC	349.65	87.41	43.71	21.85	10.93	5.46	2.73	1.36
Lambda-cyhalothrin	Karate 17.5 EC	175	43.75	21.86	10.94	5.47	2.73	1.37	0.68

Note: RD: recommended dose, a.i: active ingredient

RESULTS AND DISCUSSION

Resistance of T. urticea *populations to Dicofol*

The percent mortality in the control using water was less than 10% in all mortality tests.

The LC_{50} for **dicofol** were 2.68, 4.8, 6.39, 7.24 and 8.34 for the populations of Hawassa, Zewai, Adama, Meki and Upper Awash respectively; whereas the resistance factors were 1.79, 2.38, 2.70 and 3.11 times higher for the Zewai, Adama, Meki and Upper Awash populations respectively relative to Hawassa population (Table 2). The LC_{50} of Upper Awash population was significantly higher than the populations from Hawassa and Zewai (P-value < 0.05). Similarly, the resistance factor for Upper Awash population tended to be higher than the rest of the populations; indicating that the spider mite population that was found in Upper Awash area was more resistant to dicofol than the populations from the Zewai and Hawassa areas. On the other hand, the population from Hawassa scored a significantly lower LC_{50} compared to the other populations except Zewai (P-value < 0.05) and also showed a lower resistance factor; indicating the lower resistance of the Hawassa population to dicofol. The LC_{50} for Adama and Meki populations were also significantly higher than the control, Hawassa population (P-value < 0.05).

Table 2: Probit analysis for Dicofol resistance, in the five population of *T. urticae* (n*=210)

Population	Slope (SE)	LC_{50} (95%CL) **	RF***
Upper awash	1.78(0.214)	8.34 (6.26 - 10.90)^{a}	3.11
Meki	1.50(0.195)	7.24 (5.13 - 9.79)^{a,b}	2.70
Adama	1.61(0.208)	6.39 (4.55 - 8.55)^{a,b}	2.38
Zewai	2.22(0.293)	4.8 (3.64 - 6.11)^{b,c}	1.79
Hawassa	1.47(0.234)	2.68 (1.50 - 3.91)^{c}	-

*n= sample size for each population of Spider mite

**Same letter signifies no significant difference between the two populations at p < 0.05

***RF (resistance factor) = LC50 of the test population / LC_{50} of Hawassa population

Resistance of T. urticea *populations to Profenose*

Concerning the LC_{50} for profenose, the populations of Hawassa scored a value of 9.32; whereas Zewai, Meki, Upper Awash and Adama populations scored LC_{50} values of 8.55, 19.39, 19.55 and 21.07 with resistance factor of 0.92, 2.08, 2.09 and 2.26, respectively (Table 3). The lethal concentration fifty of Hawassa and Zewai populations were significantly lower than the other populations (p-value<0.05). Likewise the resistance factor of Zewai populations were less than half of the other populations; which indicate the lower resistance of Zewai population to profenose and the presence of resistance to profenose in the populations from Upper Awash, Meki and Adama relative to Hawasa population.

Table 3: Probit analysis for Profenose resistance, in the five population of *T. urticae* (n*=210)

Population	Slope (SE)	LC_{50} (95%CL) **	RF***
Adama	1.11(0.16)	21.07 (14.12 - 31.21)[a]	2.26
Upper awash	1.39(0.179)	19.55 (14.05 - 26.96)[a]	2.09
Meki	1.43(0.18)	19.39 (14.05 - 26.57)[a]	2.08
Zewai	1.24(0.183)	8.55 (5.32 - 12.30)[b]	0.92
Hawassa	1.56(0.206)	9.32 (6.53 - 12.58)[b]	-

*n= sample size for each population of Spider mite
**Same letter signifies no significant difference between the two populations at $p < 0.05$
***RF (resistance factor) = LC_{50} of the test population / LC_{50} of Hawassa population

Resistance of* T. urticea *populations to Abamectin and Endosulfan

In the bioassay; for **abamectin** the LC_{50} values ranged from 0.05 for Hawassa population to 0.08 for Upper Awash with resistance factor ranging from 1.37 for Adama to 1.67 for Upper Awash ; whereas for **endosulfan** the LC_{50} values ranged from 4.85 for Hawassa population to 8.28 for Upper Awash with resistance factor ranging from 1.34 for Zewai to 1.71 for Upper Awash. In general, there was no evidence for the presence of differences in resistance, based on LC_{50}, towards abamectin and endosulfan among the tested populations (p-value<0.05 and n=210 for each population). However, logistic curve for both chemicals showed tendency for lower resistance of the Hawassa population and higher resistance of the Upper Awash population; the concentration against mortality curves of abamectin and endosulfan for the Upper Awash population were consistently lower than the curves for the Hawassa population (Figure 1 and Figure 2).

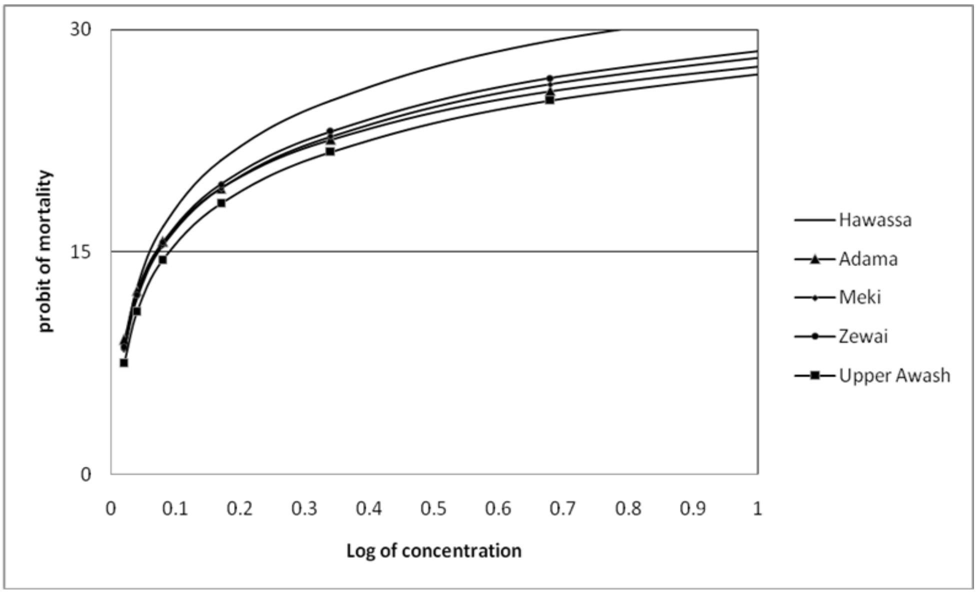

Figure 1: Logistic curve of Abamectin indicating the lethal concentration fifty of the tested T. urticae population.

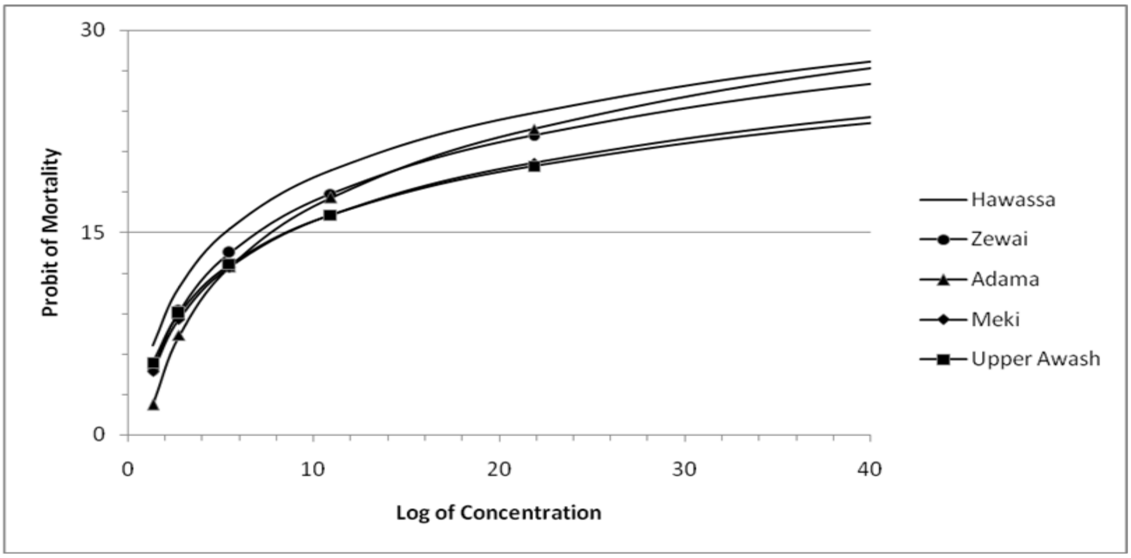

Figure 2: Logistic curve of endosulfan indicating the lethal concentration fifty of the tested T. urticae population

Resistance of* T. urticea *populations to Lambda-cyhalothrins

The LC_{50} values of lambda-cyhalothrins for the populations of Hawassa, Zewai, Meki, Adama and Upper Awash were 2.42, 2.12, 3.87, 4.02 and 4.85, respectively; while the resistance factors were 0.88 for Zewai, 1.60 for Meki, 1.66 for Adama and 2.01 for Upper Awash populations (Table 4). For the Upper Awash population, the resistance factor was the highest; similarly, the LC_{50} value was significantly higher than the values for Zewai and Hawassa populations (p-value<0.05). In contrast, the population from Zewai has the lowest resistance factor and also a significantly lower LC_{50} value compared to the populations of Upper Awash, Adama and Meki (p-value<0.05). The result showed that the resistance to lambda-cyhalothrin was two times higher for the Upper Awash population compared to Zewai populations.

Table 4: Probit analysis for Lambda-cyhalothrins resistance, in the five population of *T. urticae* (n*=210)

Population	Slop ± SE	LC_{50} (95%CL) **	RF***
Upper awash	2.27 (0.25)	4.85 (3.84- 6.11)[a]	2.01
Adama	1.36 (0.18)	4.02 (2.84- 5.57)[a,b]	1.66
Meki	2.44 (0.28)	3.87 (3.09 - 4.83)[a,b]	1.60
Zewai	1.76 (0.23)	2.12 (1.52 - 2.80)[c]	0.88
Hawassa	1.35 (0.19)	2.42 (1.61 - 3.69)[b,c]	-

*n= sample size for each population of Spider mite
**Same letter signifies no significant difference between the two populations at p< 0.05
***RF (resistance factor) = LC_{50} of the test population / LC_{50} of Hawassa population

Resistance of* T. urticea *populations to Amitraz

Concerning **amitraz**, the LC_{50} value recorded for Hawassa population was 1.39; whereas Zewai, Adama, Meki, and Upper Awash populations scored LC_{50} values of 2.78, 3.33, 4.56 and 6.22 with resistance factor of 1.99, 2.39, 3.28 and 4.47, respectively (Table 5). The lethal concentration (LC_{50}) of all the tested populations were significantly higher than Hawassa population; whereas Upper Awash population had a significantly higher LC_{50} than both Hawassa and Zewai populations (p-value<0.05). The study indicates the development of resistance to amitraz in the test populations of, Upper Awash, Meki, Adama and Zewai (Table 6,7).

Table 5: Probit analysis for Amitraz resistance, in the five population of *T. urticae* (n*=210)

Population	Slope (SE)	LC_{50} (95%CL) **	RF***
Upper Awash	1.17 (0.17)	6.22 (4.25 - 9.10)[a]	4.47
Meki	1.20 (0.17)	4.56 (3.06 - 6.54)[a,b]	3.28
Adama	1.66 (0.21)	3.33 (2.42 - 4.41)[a,b]	2.39
Zewai	1.66 (0.21)	2.78 (1.99 - 3.70)[b]	1.99
Hawassa	1.96 (0.29)	1.39 (0.94 - 1.85)[c]	-

*n= sample size for each population of Spider mite
**Same letter signifies no significant difference between the two populations at $p< 0.05$
***RF (resistance factor) = LC_{50} of the test population / LC_{50} of Hawassa population

Table 6: Probit analysis for Endosulfan resistance, in the five population of *T. urticae* (n*=210)

Population	Slope (SE)	LC_{50} (95%CL) **	RF***
Upper awash	1.16(0.17)	8.28 (5.55 - 12.00)[a]	1.71
Meki	1.24(0.17)	8.09 (5.56 - 11.48)[a]	1.67
Adama	2.15(0.25)	7.06 (5.52 - 8.95)[a]	1.46
Zewai	1.58(0.20)	6.50 (4.72 - 8.69)[a]	1.34
Hawassa	1.76(0.22)	4.85 (3.55 - 6.38)[a]	-

*n= sample size for each population of Spider mite
**Same letter signifies no significant difference between the two populations at $p< 0.05$
***RF (resistance factor) = LC_{50} of the test population / LC_{50} of Hawassa population

Table 7: Probit analysis for Lambda-cyhalothrins resistance, in the five population of *T. urticae* (n*=210)

Population	Slop $\pm$ SE	LC_{50} (95%CL) **	RF***
Upper awash	2.27 (0.25)	4.85 (3.84- 6.11)[a]	2.01
Adama	1.36 (0.18)	4.02 (2.84- 5.57)[a,b]	1.66
Meki	2.44 (0.28)	3.87 (3.09 - 4.83)[a,b]	1.60
Zewai	1.76 (0.23)	2.12 (1.52 - 2.80)[c]	0.88
Hawassa	1.35 (0.19)	2.42 (1.61 - 3.69)[b,c]	-

*n= sample size for each population of Spider mite
**Same letter signifies no significant difference between the two populations at $p< 0.05$
***RF (resistance factor) = LC_{50} of the test population / LC_{50} of Hawassa population

Generally, *T. urticae* resistance to different insecticides and acaricides has been widely reported all over the world (Tian *et al.,* 1992; Campos *et al.,* 1996; Sawicki and Denholm, 1987; Nauen *et al.,* 2001). Different studies revealed the development of resistance against dicofol, bromopropylate, bifenthrin (Ay, 2005), propargite, amitraz, abamectin (Ay and Gurkan, 2005), cyhexatin (Tian *et al.,* 1992), dimethoate (Sawicki and Denholm, 1987), chlorpyrifos (Demircan and Yılmaz, 2005) and bifenthrin (Ay and Gurkan, 2005) in T. *urticae* populations from different areas. Similarly, in this study, *T. urticae* populations collected from the four study sites in the central rift valley of Ethiopia showed resistance to the commonly used acaricides in these areas such as dicofol, profenose, lambda-cyalothrin and Amitraz. *T. urticae* population from Upper Awash showed a higher resistance factor to most of the acaricides in comparison with the other populations; the resistance factor for the Upper Awash population was the highest for dicofol, abamectin, endosulfan, lambda-cyhalothrins and amitraz with the second highest for profenos next to Adama population. In contrast, the *T. urticae* populations of Hawassa and Zewai areas showed relatively lower resistance factor for all of the acaricides tested. The LC_{50} of Zewai population was comparable with the LC_{50} of the control, Hawassa population, for all acaricides except amitraz; indicating the development of resistance to amitraz in the *T. urticae* from Zewai area. Further more, *T. urticae* populations from Meki and Adama were observed to have resistance to dicofol, profenos, and amitraz; the LC_{50} of Adama and Meki populations for these acaricides was significantly higher than the control population. In general, the resistance to acaricides was the highest for the *T. urticae* population from Upper Awash area followed by the populations from Adama and Meki areas.

Spider mite (*Tetranychus* spp.) is becoming important in irrigated tomato production in the central rift valley of Ethiopia in the past decade and the main control option available is pesticide use (Abate and Ayalew, 2009). This continuous use of the same insecticides over an extended period of time may result in the development of resistance in some pests. Resistance against agricultural chemicals is directly related with the frequency of pesticide use since frequent application increases the selection pressure of the resistant genotypes (Hoyt *et al.,* 1985; Campos *et al.,* 1996; Herron and Rophail, 1998). The increasing trend in pesticide application could be a possible cause as well as consequence of susceptibility loss in this study.

Maintaining unsprayed wild host reservoirs help to maintain dilution of resistant genotypes in the population (Campos *et al.,* 1996). Comins, (1977) also suggested that migration of susceptible individuals above a critical rate will greatly retard the development of resistance. In this study, the intensive farming system in Upper Awash agro-industry with the use of chemicals on large farm land did not favor the maintenance and migration of susceptible individuals to the farm. Fallowing will also enhance susceptibility of insects through cross breeding (Ramasubramanian, 2004). However, the use of irrigation enabled farmers in the study areas to produce tomato through out the year, which resulted in application of chemicals continuously that increased the selection pressure from pesticides and could contribute for the development of resistance (Regupathy *et al.,* 2004).

In addition, since an individual pest is less likely to be resistant for two or more groups of pesticides, rotation of chemical application is recommended to decrease the rate of resistance development (Beers *et al.,* 1998). Moderation of chemical use together with use of treatment threshold is also important considerations to prevent emergence of pest resistance to pesticides. However, in the central rift valley of Ethiopia, cultivation of vegetables for commercial purpose has increased during the past few years; the condition is expected to increase farmers' accesses to and dependency on the nearly available pesticides unless possible intervention measures are taken.

CONCLUSION

From the study, it was found out that there is resistance to acaricide in some of the populations of *T. urticae* in the central rift valley. In the *T. urticae* population of Upper Awash showed resistance to dicofol, profenos, lambda-cyhalothrins and amitraz whereas in Adama and Meki, there is resistance to dicofol, profenos and amitraz. On the other hand, *T. urticae* population from Zewai is susceptible to all acaricides except amitraz. All the studied populations were susceptible to abamectin and endosulfan. The level of resistance detected in this study is considerable in view of the short history spider mite problem in these areas. It is therefore recommended to carried to detect the extent of accaricide resistance and management practices of the farmers used and discriminating concentrations need to be determined for quick and reliable monitoring of resistance in the future. Moreover further studies on the methods of control for T. urticea control focusing on modification of the habitat and enhance natural control agents, and threshold levels for application of insecticides should be done to enhance resistance management program in the area.

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