



Attraction of *Tetranychus urticae* to garlic treated with chlorfenapyr to control dry bulb mite, *Aceria tulipae*

إنجذاب الـ *Tetranychus urticae* للثوم المعامل بالكورفينابير لمكافحة
أكاروس جفاف رؤوس الثوم *Aceria tulipae*

BY

Rania A. Abd El-Wahab

Nahla H. Harraz

Sheren A Shoman

Plant Protection Research Institute, Agricultural Research
Center, EGYPT

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ABSTRACT:

Chlorfenapyr is a potent pesticide that showed its effect against several mites that infested many crops. There are two recommended compounds under commercial names Selpa and Brometa with ٣٦% a.i, to control the eriophyid garlic mite, *Aceria tulipae* at ٥٥ ml/100 L water. Initial kill values of Selpa (Chlorfenapyr1) were 64.42 and 66.55% in 2021 and 2022, resp., and for Brometa (Chlorfenapyr2) 64.00 and 62.48% for the same arrangement. Both compounds were compared with Quick (Abamectin1.3%+Bifenthrin8.8%), which its initial kill percentages recorded 61.95 and 65.34% for 2021 and 2022, resp. In the same trend, the latent effect of both chlorfenapyr compounds recorded higher percentages in comparison with Quick with 91.92 and 91.95 % for Selpa (Chlorfenapyr1), 91.1 and 90.96 % for Brometa (Chlorfenapyr2), and 88.90 and 88.88% for Quick in 2021 and 2022, resp., versus *A. tulipae*. Further, the general reduction showed an increase than Quick with 3.5 and 4.2 % for Selpa (Chlorfenapyr1) and with 2.7 and 3.2% for Brometa in 2021 and 2022, resp., against *A. tulipae*. Inconsistently, the peculiar phenomenon caused by chlorfenapyr in the two seasons of the study was the increase of *Tetranychus urticae* infestation in garlic treated with Chlorfenapyr compared even by Quick (Abamectin1.3%+Bifenthrin8.8%), or control also. The general infeststion increase percentages of Selpa (Chlorfenapyr1) and Brometa (Chlorfenapyr2) were 33.71 and 31.51% in 2021, resp. Likewise, the general increase percentages of chlorfenapyr1 and chlorfenapyr2 were 31.81 and 30.31 % in 2022, resp. Results were explained by the difference in decreased activity of antioxidant enzymes levels between *Aceria tulipae* and *Tetranychus urticae*.

المستخلص:

الكلورفينابير مبيد فعال للآفات أظهر تأثيره ضد العديد من الأكاروسات التي تصيب العديد من المحاصيل. هناك نوعان من المركبات الموصى بها تحت الإسمين التجاريين Selpa و Brometa بنسبة ٣٦٪ من العنصر الفعال للسيطرة على أكاروس الثوم الإيروفيد ، *Aceria tulipae* عند ٥٥ مل / ١٠٠ لتر ماء . كانت قيم القتل الأولية لـ Selpa (Chlorfenapyr1) 64.42 و ٦٦,٥٥٪ في ٢٠٢١ و ٢٠٢٢ ، على التوالي و 64.00 Brometa (Chlorfenapyr2) و ٦٢,٤٨٪ لنفس الترتيب، حيث سجلت نسب القتل الأولية Quick (Abamectin1.3 + Bifenthrin8.8٪) تمت مقارنة كلا المركبين مع مبيد ٦١,٩٥ و ٦٥,٣٤٪ لعامي ٢٠٢١ و ٢٠٢٢ ، على التوالي .في نفس الاتجاه ، سجل التأثير الكامن لكلا مركبي chlorfenapyr نسباً أعلى بالمقارنة مع Quick مع ٩١,٩٢ و ٩١,٩٥٪ لـ Selpa (Chlorfenapyr1) ، و ٩١,١ و ٩٠,٩٦٪ لـ Brometa (Chlorfenapyr2) ، و ٨٨,٩٠ و ٨٨,٨٨٪ لـ Quick في عام ٢٠٢١ و 2022 ، على التوالي ، مقابل A. tulipae. فإن الخفض العام سجل زيادة بنسبة ٣,٥ و ٤,٢٪ لـ Selpa (Chlorfenapyr1) وبنسبة ٢,٧ و ٣,٢٪ في Brometa في عامي ٢٠٢١ و ٢٠٢٢ ، على التوالي ، مقابل A. tulipae . بشكل غير متسق ، كانت الظاهرة الغريبة التي تسببها الكلورفينابير في موسمي الدراسة هي زيادة الإصابة بـ *Tetranychus urticae* في الثوم المعالج بالكلورفينابير مقارنة بـ Quick (Abamectin1.3%+Bifenthrin8.8%) أو الكنترول أيضا. كانت نسب الزيادة العامة للإصابة بالمعاملات في Selpa (Chlorfenapyr1) و Brometa (Chlorfenapyr2) ٣٣,٧١ و ٣١,٥١٪ في عام ٢٠٢١ ، على التوالي .وبالمثل ، فإن النسب المئوية للزيادة العامة في كلورفينابير ١ والكلورفينابير ٢ كانت ٣١,٨١ و ٣٠,٣١٪ في عام ٢٠٢٢ ، على التوالي .تم تفسير النتائج من خلال الاختلاف في انخفاض نشاط مستويات الإنزيمات المضادة للأكسدة بين *Aceria tulipae* و *Tetranychus urticae*.

INTRODUCTION

A halogenated pyrrole class presented in 2001 the first commercial pesticide, Chlorfenapyr, which was approved for use in the US. Chlorfenapyr is commercially used for crop protection against various pests and mites (Lovell et al.1990, Pimprale et al.1997, Sheppard and Joyce 1998). It is a pro-insecticide as an oxidative removal of the N-ethoxymethyl group of chlorfenapyr by mixed functional oxidase (MFO) results in a toxic form identified as CL 303268; its N-dealkylated metabolite functioned

to segregate mitochondrial oxidative phosphorylation and ATP (Black et al.,1994, Sparks and Nauen, 2015).

Resulting in disruption of production, loss of energy leads to cellular dysfunction and subsequent death of the organism. The molecule has low mammalian toxicity and is classified as a slightly hazardous insecticide according to WHO criteria (Tomlin,2000). Due to its novel mechanism of action, chlorfenapyr is cross-resistant to the standard neurotoxin

Chlorfenapyr is effective against *T.urticae* that infested varieties of many crops (Van Leeuwen et al.2009). Likewise, the effects of *allium sativum* extracts and concentrated organosulphur components were able to reduce mite pests' population densities (Singh et al. 2001, Prischamann et al. 2005).

Moreover, plants produce chemicals produced by plants have a certain mechanism to reduce phytophagous injury. Alongside, these chemicals have several modes of action as an antifeedant, repellent, inhibitory molting, respiratory failure element, and disrupter of the cuticle. Garlic with its volatile organosulphur represented 68.3% of the whole component and diallyl trisulphide is featured as the main garlic essential oil (Virtanen 1965), followed by allyl methyl trisulphide, diallyl sulfide, diallyl tetrasulfide, allyl methyl tetrasulfide, vinyl dithiin and others (Prowse et al. 2006).

Further, plant essential oils such as Thymol and eugenol which were present in clove bud and thyme oils at concentrations of 87.2% and 31.1%, respectively capable to alter the rate at which the central motor neurons fired. But in conjugation with pyrrole insecticide chlorfenapyr, synergistic toxicity has occurred (Yoon and Tak, 2022).

Acaricides such as abamectin and chlorfenapyr have translaminar properties and the main material resides within the

leaf epidermis and leaf tissue. This results in long-term residual activity against TSM even after the surface residue has dried (Cloyd, 2003), such compounds can be toxic by contact and ingestion to chewing and piercing-sucking arthropods. Spider mites can ingest lethal concentrations of active ingredients even after spray residues have disappeared.

This reduces the frequency of acaricide applications and decreases worker exposure. There is a possibility, however, miticides with persistent residual activity, such as stratified miticides, may increase the likelihood of resistance development in TSM populations (Clark et al., 1994). Although both pesticides have transmembrane activity but failed to control motile stages of TSM. In cotton plants, abamectin provided 85% to 96% control of TSM up to 49 days after treatment. However, residual activity can be affected by plant species and leaf age (Lasota and Dybas, 1991). Furthermore, abamectin is susceptible to UV degradation (Wislocki et al., 1989), but this is unlikely to explain the poor regulation in both his TSM larvae and adults. Some explanations were depending on the effect of UV (Wislocki et al., 1989) and others upon the gradual increase of resistance (Campos et al., 1995; Clark et al., 1994; Price et al., 2002; Stumpf and Nauen, 2002). Chlorfenapyr is a new acaricide that is active in all life stages of TSM (Dekeyser, 2005), but the decreased effect may be directly proportional with the plant type as butterfly bush vs. marigold.

Antioxidative enzymes include superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), and the combined action of these three proteins can keep up free radicals at low levels in insects to secure the cells from harmful effects (Gill and Tuteja, 2010). Protein

exercises can be utilized as a biomarker of life forms exposed to sublethal concentrations of pesticides (Rumpf et al.1997).

Therefore, the purpose of this study was to discover the reason for the noticeable attraction of *T.urticae* to garlic cultivars treated with chlorfenapyr during the control of the eriophyid mite, *Aceria tulipae* with a comparison of another pesticide (Quick (Abamectin1.3%+ Bifenthrin 8.8%)). Secondly, an explanation of gained results upon antioxidant enzymes' differences between *Tetranychus urticae* and *Aceria tulipae*.

Materials and METHODS

-Used Pesticides

Table (1) showed used pesticides to control *Aceria tulipae* infested garlic bulbs in 2021 and 2022.

There are two pesticides Selpa and Brometa, the main active ingredient is chlorfenapyr as shown

-Chlorfenapyr 36% SC (4-bromo-2-(4-chlorophenyl)-1-ethoxymethyl-5-trifluoromethyl-1H-Pyrrole-3-carbonitrile).

The other used pesticide is a mixture of Abamectin and Bifenthrin:

-Quick 10.1% EC (Abamectin1.3%+Bifenthrin8.8%).

Table (1) Used pesticides to control *Aceria tulipae* infested garlic bulbs in 2021 and 2022

Pesticides	Producer Company	a.i%	Recommended conc.
Selpa (Chlorfenapyr 1) 36% SC	Hangzhou Tianlong Biotech. Co.,ltd.China	Chlorfenapyr 36%	55ml/100L
Brometa (Chlorfenapyr 2) 36% SC	Hebei Xingbai Agricultural Technology CO.,ltd.China		
Quick 10.1% EC	Hebei Veyong Bio- Chemical CO.,ltd.China	Abamectin1.3%+Bifenthrin8.8%	75ml/100L

-Site of the Study and mite incidence

All selected garlic cultivars were in Egypt, at Aga district, mit abul-hussein village, during the 2021–2022 cropping season. They were treated as treatment variables and necessary cultivation techniques such as irrigation, weed control, and fertilization were applied equally to ensure uniform growth of all cultivars till infestation with *Aceria tulipae* occurred.

For sampling, in each field, 30 plants were randomly chosen, and the leaves were gathered, sealed in polyethylene bags with rubber bands, and transported to the laboratory where the mite population was viewed through a binocular microscope. By selecting the middle leaf from each of the 300 randomly chosen plants, a total of 300 leaves of each type were collected. Then, by using a binocular microscope the field of vision of a 1cm² area of the leaf from the basal, middle, and tip regions along the upper side of the lamina, the number of mature and immature

mites (excluding eggs) per cm² leaf area was counted (Debnath and Karmakar 2013).

-Tested Pesticides

Throughout the 2021–2022 crop season, the experiment was performed in randomized blocks of the selected garlic cultivars after the previous sampling process. Each treatment had four replicates besides the control. The number of active stages of mites per cm² of leaf area on 30 leaves from 30 randomly selected plants (i.e., one leaf per plant) of each replicate was counted. Counting was done as explained heretofore, before treatments and then after the 3rd, 7th, 10th, and 14th days of spraying certain pesticides for all mites which were found. Pesticides' reduction percentages were calculated according to Henderson and Tilton (1955). Correlated mortality percentages were calculated according to Abbott (1925).

-Antioxidant Enzymes Evaluation

Ascorbate Peroxidase (APX) kinetics were measured by evaluating the oxidation rate of ascorbate (removal factor 2.8 mM/cm). Reactions contained three 50 mM phosphate carriers (pH 7.0), 0.1 mM H₂O₂, 0.5 mM sodium ascorbate, 0.1 mM EDTA, and enzyme extract. Absorbance was observed at 290 nm and the procedure was performed according to that of Nakano and Asada (1981).

Superoxide dismutase (SOD) kinetics were measured by the photochemical method described by Beauchamp and Fridovich (1971). One unit of SOD activity was defined as the amount of enzyme required to inhibit the reduction rate of nitroblue tetrazolium (NBT) by 50% at 560 nm in the presence of riboflavin and light. The reaction mixture contained 0.17 mM NBT, 0.007 mM riboflavin, and an aliquot of enzyme in 45 mM potassium phosphate buffer, pH 7.0, ethanol containing 0.1 mM EDTA, and 13 mM methionine. Blanks were kept in the dark and the rest were illuminated for 15 minutes. One SOD unit is

the amount of extract that inhibits the NBT reduction rate by 50%.

-Data Analysis

SPSS (V.16) was used to show differences among treatments compared within the control. Mainly both parametric and non-parametric analyses were executed on gained data to determine expected the variance and significance.

RESULTS

The effect of Certain Tested Compounds against garlic mites in 2021 and 2022 was shown in both Figures 1&2.

In 2021, the initial kill percentages of chlorfenapyr 1 and chlorfenapyr 2 were 64.42 and 64%, resp. , while it was 61.95 % of abamectin and bifenthrin. Synchronously, the residual effect percentages of tested pesticides were 91.92,91.1, and 88.9% of the same arrangement. Subsequently, the general reduction percentages were 78.17,77.55 and 75.43%,resp., as shown in Fig (1).

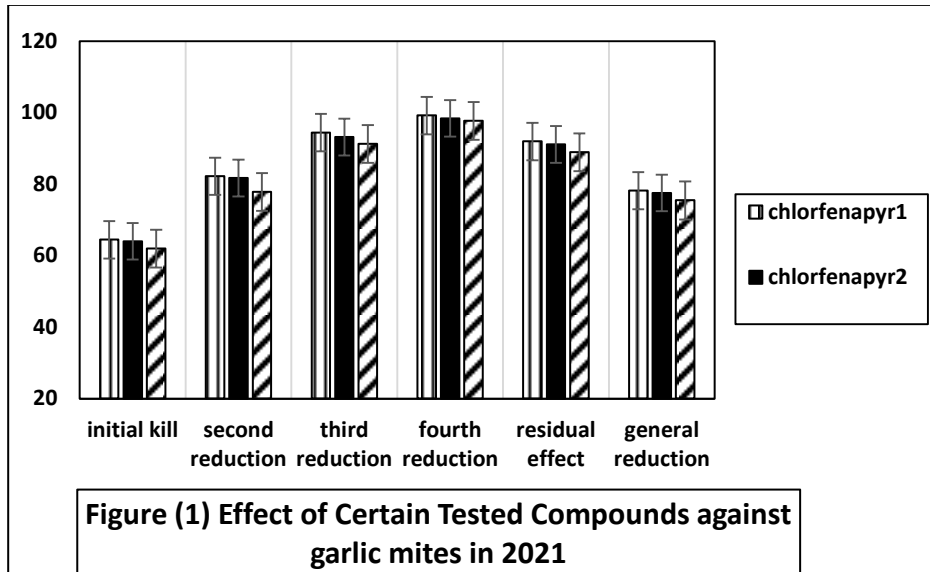
Paired Samples Correlations (.475) and Paired Samples Test ($t=2.195$) between two chlorfenapyr formulations and Quick (abamectin+bifenthrin) in comparison with control showed a significant difference at 95% (Sig. (2-tailed) =.083) which means that the main difference depended mainly on the treatments.

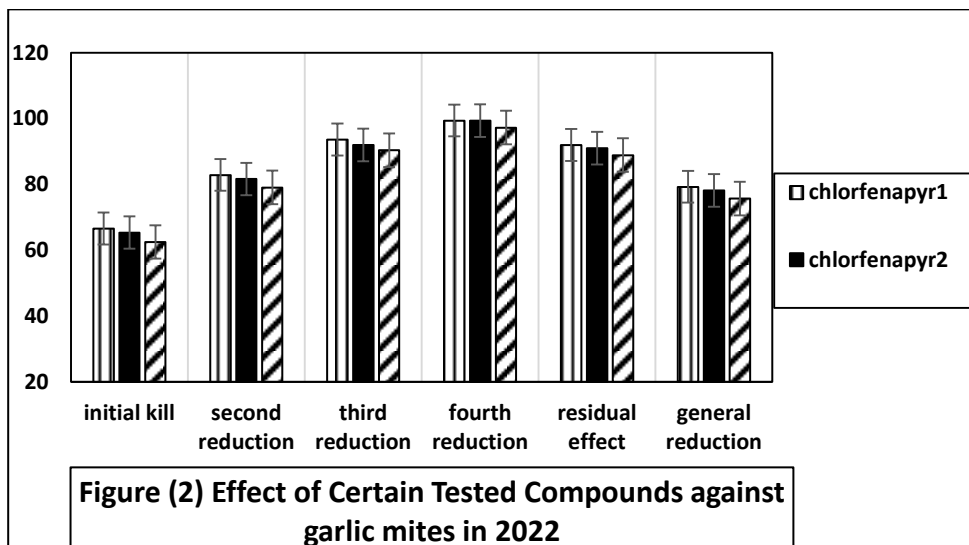
In 2022, progressively initial kill percentages of chlorfenapyr 1 and chlorfenapyr 2 were 66.55 and 65.34%, resp., while it was 62.48 % for abamectin and bifenthrin. Contemporaneously, the residual effect percentages of tested pesticides were 91.95,90.96, and 88.88% of the same arrangement. Simultaneously the general reduction percentages were 79.25,78.15, and 75.68%,resp., as shown in Fig (2).

Consequently, Paired Samples Correlations =.884 with significant difference =.033*, then among variables of chlorfenapyr formulations to control ($t=6.708^{**}$), which

resembled a significant difference at 5% between the two seasons of the study ($t=4.150^{**}$).

ANOVA showed that $F=20.984^*$ reflected significant relation between chlorfenapyr formulations and *Aceria tulipae* infestation rate. Mainly calculated Standardized Coefficient of this relation ($t= 9.977^{**}$). Among treatments, there was a significant difference at 95% depending on T Test ($t=7.663$) at Sig. (2-tailed) = .140*.

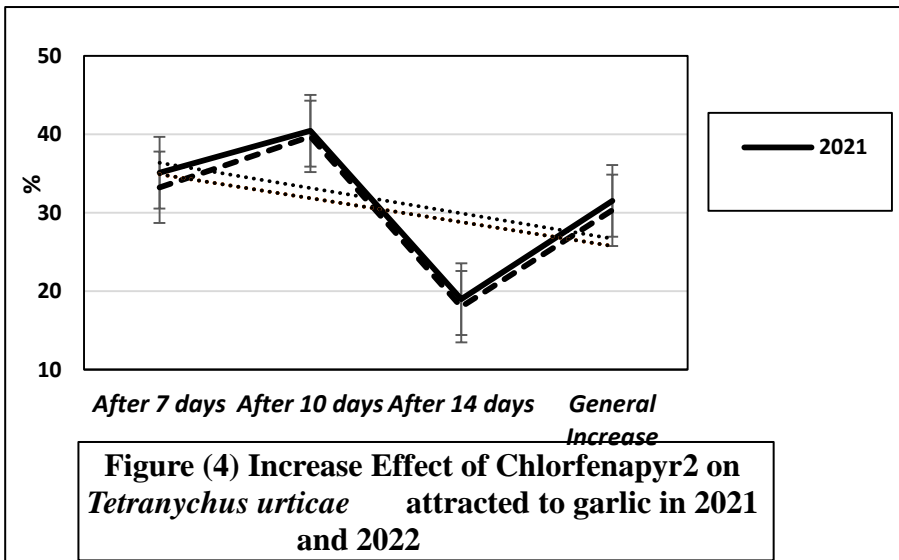
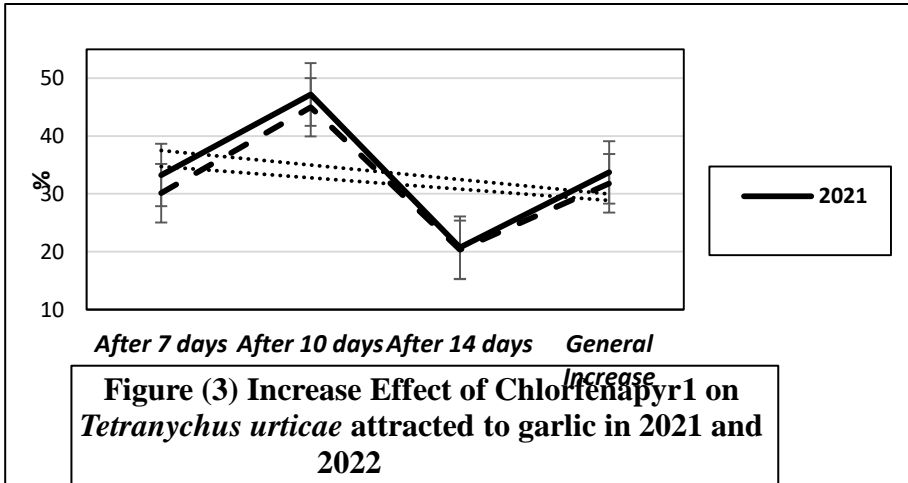




-Attraction of *Tetranychus urticae*

T. urticae alive individuals were attracted to treated cultivars with both pesticides of chlorfenapyr after spraying for 7 days but no infestation was found in others treated with abamectin+bifenthrin.

Figures (3 & 4) showed the infestation increase in 2021 and 2022, resp. General augmentation as a result of chlorfenapyr1 was recorded (33.71 and 31.8)% alongside chlorfenapyr2 (30.51 and 30.31)%, in 2021 and 2022, respectively.



There was a significant attraction of *Tetranychus* to the treated garlic with chlorfenapyr1 in the two years of the study through Kruskal Wallis (1.291**), J-T Statistic (6.000**), and Std. J-T Statistic (1.991*).

Likewise, there was a significant attraction of Tetranychus to the treated garlic with chlorfenapyr2 in the two years of the study through Kruskal Wallis (1.051**), J-T Statistic (4.033**), and Std. J-T Statistic (1.747*).

But there was no significant difference between the general increase of attraction in the two study seasons of both chlorfenapyr formulations through Kruskal Wallis (.099) depending on J-T Statistic (3.500) and Std. J-T Statistic (.313).

-Antioxidant Enzymes Activity:

Reactive Oxygen Scavengers (ROS) in chlorfenapyr treatments were significantly lower than in the control ($P < 0.05$). Figure(5) showed the effect of both chlorfenapyr formulations and quick (abamectin+bifenthrin) on *A. tulipae* mites ROS in 2021 and 2022. Superoxide dismutase (SOD) and Ascorbate Peroxidase (APX) in control were higher than in all treatments. Decreased ratio percentages of SOD than control recorded 65.11,61.74, and 34.09 % for *Aceria tulipae* mites-infested garlic in 2021 which were treated with chlorfenapyr1, chlorfenapyr2, and quick (abamectin+bifenthrin). In the same trend, decreased ratio percentages of SOD than control recorded 57.40,54.13, and 45.27 %, for *the same previous arrangement, in 2022*.

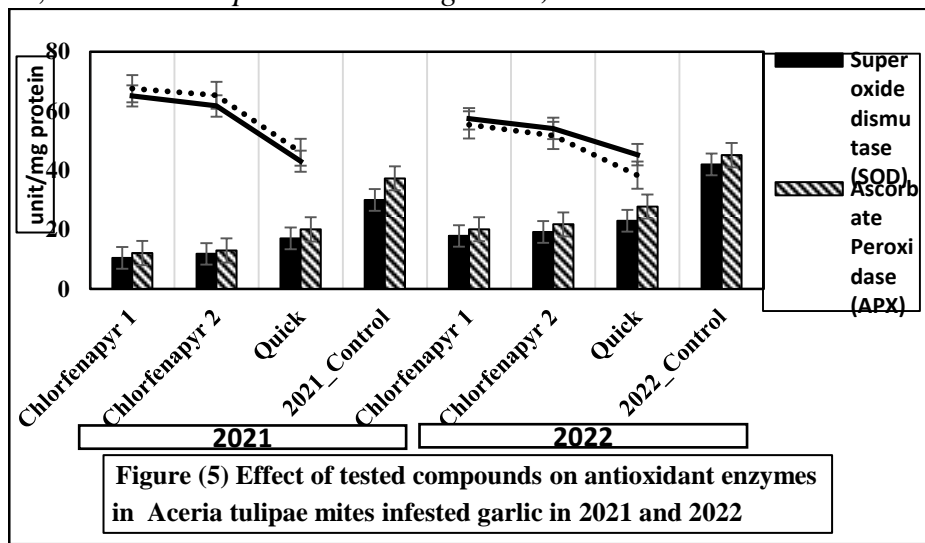
Decreased ratio percentages of APX than control recorded 67.56,65.31, and 46.09 % for *Aceria tulipae* mites infested garlic in 2021 which were treated with chlorfenapyr1, chlorfenapyr2, and quick (abamectin+bifenthrin) as shown in Fig (5). In the same trend, decreased ratio percentages of APX than control recorded 55.35,51.74, and 38.37 %, for *the same previous arrangement, in 2022*.

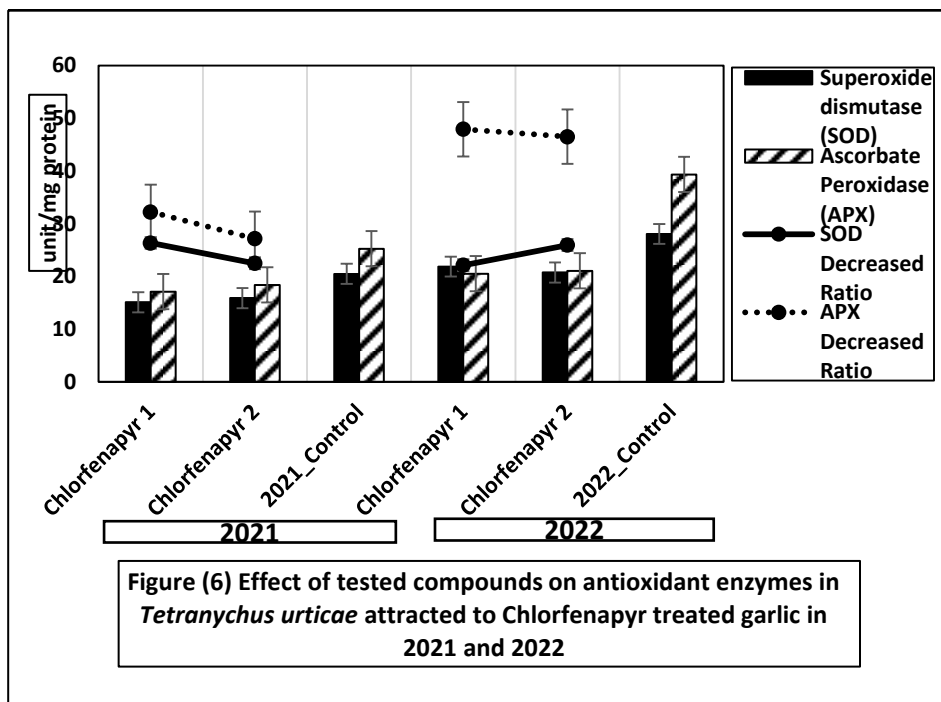
Paired samples test showed highly significant differences in the case of APX of *Aceria tulipae* with $t=2.771^{**}$ and it was $t=2.012^{*}$ in the case of SOD which was exposed to Chlorfenapyr1. Moreover, nonparametric correlations showed high significance for treatments with Chlorfenapyr1 as appeared

through Kendall's tau_b correlation coefficient=.822** and Spearman's rho=.881**.

On the other hand, the reduction of antioxidant enzymes was occurred also in the case of exposed *T.urticae* to certain pesticides but lower than *A. tulipae* which infested garlic cultivars.

Decreased ratio percentages of SOD than control recorded 26.33 and 22.47 % for *T.urticae* mites that infested garlic in 2021 and were treated with chlorfenapyr1 and chlorfenapyr2 as shown in Fig (6). In the same trend, decreased ratio percentages of SOD than control recorded at 22.11 and 25.93 % for the same previous arrangement, for the same previous arrangement, in 2022. Further, decreased ratio percentages of APX than control recorded 32.21 and 27.14 % for *T.urticae* mites that infested garlic in 2021 which were treated with chlorfenapyr1 and chlorfenapyr2 as shown in Fig (6). In the same trend, decreased ratio percentages of APX than control recorded 47.90 and 46.50 %, for the same previous arrangement, in 2022.





Paired samples test showed highly significant differences in the case of APX of *T.urticae* with $t=2.794^{**}$ and it was $t=2.11^{*}$ in the case of SOD which was exposed to Chlorfenapyr1. While nonparametric correlations showed non-significance for Chlorfenapyr treatments within control as appeared through Kendall's tau_b correlation coefficient=-.370.

DISCUSSION

There are specific acaricides against a wide spectrum of mites such as both chlorfenapyr and abamectin (Vásquez and Ceballos 2009).

Mainly chlorfenapyr exerts disruptive effects on mitochondrial respiration by inhibiting electron transportation at complex I or by perturbing the difference of proton concentration between the outer and inner membranes of mitochondria (uncoupling), respectively.

Uncouplers are the only insecticides that don't work on a protein target. They are usually weak acids that can accept a proton into the proton-rich intermembrane space, transport it through the inner mitochondrial membrane, deposit it into the matrix, and back through the membrane to pick up another proton and repeat the process cycle.

The result of this proton shuttle is that the stored energy in the proton gradient dissipated as heat without being used for ATP synthesis. In the absence of protons, Gradient spins ATP synthase backward, rapidly hydrolyzing available ATP to pump it out to no avail protons in the intermembrane space. ATP is rapidly depleted, resulting in paralysis and death.

Any weak lipophilic acid could accept and transport protons into the intermembrane space in the matrix, but to act as an uncoupler, the deprotonated form of the molecule, which has a negative charge, must re-diffuse through the inner mitochondrial membrane to pick up another proton in the intermembrane space, thus maintaining a cycle of proton transport (BASF,2014).

Most charged molecules cannot pass through the nonpolar interior of the membrane. In a polar medium like water, a charged atom in a molecule attracts polarity water molecules that shield its charge, reducing the repulsive forces between similarly charged molecules. This shielding would therefore not take place within the nonpolar lipid membrane and that would be a strong repulsion between charged molecules. Decoupling molecules can protect the charge within the molecule by relocating it to many atoms. This significantly reduces electrostatic repulsion between like molecules in a non-polar medium (BASF, 2014).

The active form of chlorfenapyr is very good at delocalizing the negative charge over a system of double bonds and is one of the strongest known decouplers.

Apparently, some pesticides were able to reduce eriophides generally and specifically *Aceria zoysiae* and *Aceria cynodonis* such as abamectin, dicofol, chlorpyrifos (Chong 2013, Boeri et al. 2018, Chong and Brown 2018), and pyrethroids (permethrin, deltamethrin, bifenthrin, and lambda-cyhalothrin). Further, many used pesticides had been withdrawn or canceled as diazinon (US EPA 2006), dicofol (US EPA 2011), and chlorpyrifos (State of California 2020). Correspondingly, even the best acaricide efficiency was inconsistent between spring and summer (Chong and Brown 2018).

Notably, antioxidant enzymes SOD, CAT, and POD protect cells to be damaged by acute lethal and sublethal effects of pesticides even in predators (Shan et al.,2020) or pests (Filipović et al.,2019). SOD can convert superoxide anion free radicals into hydrogen peroxide, and CAT and POD decompose hydrogen peroxide into water and oxygen.

Worthwhile, antioxidant enzymes played an important role in self-defense vs. exposure to pesticides such as chlorfenapyr. That was proved through the presented study and was in the same trend with assays of enzyme activity which showed that chlorfenapyr inhibited the activity of both peroxidase (POD) and catalase (CAT) while activating superoxide dismutase (SOD) of *Paracoccus marginatus* against chlorfenapyr (Li et al.2022).

In addition, plants difference has an effect to control pests depending on the interaction among the main plant, pest, and used pesticide. Further, buprofezin, chlorfenapyr, and abamectin 5% influenced significantly reduction in the population of *T. urticae* population infested pepper with 71.5, 64.16 and 59.88% in 2016, while recorded 77.8, 66.85 and 66.4%, respectively, in 2017 (Saleh et al.2019), but the efficiency of chlorfenapyr and abamectin was different in case of use against eriophyid mites infested garlic as our study.

Garlic contains at least four times more Sulphur than any other high-sulfur vegetables, including onions, broccoli, and

cauliflower (Virtanen 1965), and volatiles was able to reduce densities of mite population (Isman et al. 2001, Singh et al. 2001, Prischamann et al. 2005). Besides, released organosulphur compounds dithiins; diallyl disulfide; diallyl trisulphide, and methylallyl trisulphide (Atia et al. 2012), were represented as repellents of pests more in the case of intact garlic clove than grated garlic (Rahman and Motoyama 2000). In other words, there are specific interactions could be done between pyrroles and organic sulfur components in garlic (Marcinkowska and Henryk 2022), which were able to interact differently in the case of the presence of *A. tulipae* and contributed then in the attraction of *T.urticae* that was proved through this study by certain levels of antioxidant enzymes levels, besides unique pyrroles which were able to greenish exposed sulfur plants (Kubec et al.2017).

Finally, a solution to the problem of such attraction to use effective miticides such as chlorfenapyr to control *Aceria tulipae* mites would be to use pesticides combined with fatty acids or mineral oils holistically. Sulfur (powder) spraying is an alternative, but if dry weather persists, some spraying should be done. Chemical control becomes difficult as the culture progresses (CESAVEG, 2015). Likewise, the use of nanoparticles would increase the effect of chlorfenapyr against certain pests. Also, the association of pesticide with silica NPs upon slow release was able to elevate the effect of chlorfenapyr two folds higher than itself alone (Song et al.,2012).

Conclusion

To conclude, Chlorfenapyr is an effective miticide against a wide range of mites but at the same time the use of it against the eriophyid garlic mite, *Aceria tulipae* caused an increase of infestation with *Tetranychus urticae* after spraying it on garlic.

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Numbers of the tetranychid mite are decreased on the 10th day after application and that is explained upon the decrease of reactive oxygen scavengers.

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