ISSN 1687- 4633

ACARINES

Vol.18, 2024

ACARINES, 18:51-64.2024

Impact of some stimulants on boosting strawberry plants' resistance to *Tetranychus urticae* Koch (Actinidida: Tetranychidae)

Mohammed A. Abdelwines & Mahmoud M. Ahmed

Zoology and Agricultural Nematology Department, Faculty of Agriculture, Cairo University, Giza, Egypt. mahmoudmonir@agr.cu.edu.eg, https://orcid.org/0000-0003-2440-6956; abdelwanis@cu.edu.eg, https://orcid.org/0000-0002-3681-2081

ABSTRACT

The present study was conducted during the two seasons, 2017/2018 and 2018/2019 to evaluate the effect of some stimulating compounds on the mean population number of the movable stages of the two-spotted spider mite *Tetranychus urticae* per leaflet in two strawberry cultivars, Fortona and Festival. The six tested compounds included fulvic acid 10%, humic acid 15%, salicylic acid 99%, potassium silicates 45%, potassium citrate 55%, and calcium carbonate 95%, with a control untreated group for comparison. The control group had the highest number of *T. urticae* across all weeks. Treatments with fulvic acid, humic acid, salicylic acid, potassium citrate, potassium silicates, and calcium carbonate showed significantly lower mite counts compared to the control, especially in the later weeks. However, calcium carbonate and potassium silicates were the most effective in reducing *T. urticae* populations in both strawberry cultivars. Salicylic acid significantly increased trichome density, which may contribute to enhance a kind of resistance against pests. These results suggest that certain stimulating compounds can effectively boost strawberry plants' resistance to *T. urticae* by reducing mite populations and increasing a control mechanism such as trichome density.

Keywords: Stimulants, chemical compounds, spider mite, leaf trichomes, strawberry.

INTRODUCTION

Strawberry is a quite significant crop in Egypt's agricultural sector, where it is one of Egyptian major non-traditional export crops (Moussa et al. 2019). The country exports a substantial amount of strawberries, primarily to Europe and The Middle East countries, which helps in earning foreign currency (Shehata et al. 2020). Egypt produces a large quantity of strawberries, with estimates suggesting around 1 million tons annually that includes both high-quality export-grade strawberries and those used for industrial purposes like confectionery and jams (Shehata et al. 2020). There are many strawberry cultivars grown in Egypt but the most common are Fortuna and Festival.

However, strawberry's production encounters some phytosanitary problems, which involve managing different kinds of pests and diseases. Addressing these issues effectively is crucial but adds to the overall production expenses. Innovations in pest and disease management, such as integrated pest management (IPM) and the use of biological controls, help maintain crop health while minimizing hazardous chemical use (Deguine 2021). These advancements are helping to make strawberry production more sustainable, efficient, and profitable. However, the infestation of the two-spotted spider mite (TSSM), *Tetranychus urticae* Koch, is a significant challenge for strawberry production (Sato et al., 2004). This mite can severely reduce plant growth and yield, impacting both the quality and quantity of strawberry produced (Klamkowski et al. 2006; Rezaie et al. 2013).

Tetranychus urticae is considered as a major pest for strawberry plants due to its rapid life cycle, high reproductive rate, and ability to reproduce without mating (arrhenotokous parthenogenesis) (Zhao et al. 2023a). Its adaptability to various hosts and environmental conditions allows it to build high population densities quickly, making it a significant challenge for growers (Agut et al. 2018).

The primary method for controlling mites has been the use of acaricides, however, a significant challenge is the mite's ability to quickly develop resistance to these chemicals after just a few applications (Adesanya et al. 2021). This resistance makes it increasingly difficult to manage TSSM populations effectively, necessitating the exploration of alternative and integrated pest management strategies to ensure sustainable control (De Ponti 1985; Van Pottelberge et al., 2008). Moreover, the use of pesticides may have detrimental side effects on natural enemies, particularly the predatory mite, *Phytoseiulus persimilis* (Athias-Henriot), which is a key biological control agent against *T. urticae* (Ditillo et al. 2016). This predator is highly effective in managing TSSM populations, but its effectiveness can be compromised by pesticide applications that harm it (Croft 1990). To mitigate these side effects, integrated pest management (IPM) strategies are crucial (Helle and Sabelis 1985) to provide an ecological balance between pests and their natural enemies and to provide a more reasonable form of pest management (Kogan1998). So, the use of low toxic forms of chemical agents to natural enemies and/or the use of pesticide- resistant predatory mites have been exploited in integrated pest management programs (Croft1990). It's important, in the integrated pest management programs, to select efficient pesticides with little negative effects on the natural enemies (Colomer et al. 2011).

Exploring alternative methods is crucial for effective integrated mite management (IMM). Induced resistance is a promising approach, where chemical elicitors like jasmonic acid, salicylic acid, and ethylene are used to enhance the plant's natural defenses against mites (Roychowdhury et al. 2024). These compounds can trigger the plant's immune response, making it more resistant to mite infestations and reducing the reliance on chemical pesticides (Shivaji et al. 2010; War et al. 2012; Afifi et al. 2015). By incorporating such methods into an IMM program, growers can achieve more sustainable and effective control of the two-spotted spider mite while minimizing the negative impacts on beneficial predators and the environment.

Foliar spraying of chemical elicitors and fertilizer compounds like salicylic acid, methyl jasmonate, potassium humates, and potassium silicate can significantly enhance tomato plants' defense against the red spider mite (*T. urticae*) (Asghari et al. 2020). These substances boost the concentrations of essential oil components and both enzymatic and non-enzymatic antioxidants in the plants (Sharma et al. 2022). As a result, they help suppress mite damage and improve the plants' tolerance to infestations, making them a valuable tool in integrated mite management programs (Afifi et al. 2015). The morphological and chemical characteristics of plants, along with their defense mechanisms, play a crucial role in influencing mite infestations (Borges et al. 2024). Factors such as leaf surface texture, the presence of trichomes (hair-like structures), and the production of secondary metabolites can either deter or attract mites (Karabourniotis et al. 2020). Additionally, plants with robust defense mechanisms, including the production of defensive chemicals and proteins, are better equipped to resist mite attacks (Ali et al. 2015; Vásquez et al. 2018). Understanding these plant traits and leveraging them through breeding or treatment with such elicitors can significantly enhance the effectiveness of integrated mite management programs.

The elicitors of induced responses can be sprayed on crop plants to build up the natural defense system against damage caused by herbivore (War et al. 2012). These resistance inducers can trigger a variety of defense mechanisms in plants. These mechanisms include: antioxidative enzymes, that help protect plant cells from oxidative damage; proteinase inhibitors (PIs), that inhibit the activity of enzymes that herbivores use to digest plant proteins; volatile organic compounds (VOCs), that can attract natural predators of herbivores or repel the herbivores themselves; alkaloid production, that can be toxic to herbivores and deterring them from feeding on the plant; trichome formation, that can physically deter herbivores; and secretion of extrafloral nectar (EFN), which attracts beneficial insects that can protect the plant from herbivores (Walters and Heil 2007). These responses collectively enhance the plant's ability to defend itself indirectly by either deterring herbivores or attracting their natural enemies (Bagheri et al. 2021; Bezerra et al. 2021). It's a sophisticated and dynamic system that showcases the complexity of plant defense strategies (Dickens 2006; Pauwels et al. 2009).

It is well known that fertilizing strawberry plants with calcium sulfate ($CaSO_4$) and potassium sulfate (K_2SO_4) can enhance the plant's natural defenses. The increase in total phenols and amino acids in the plants due to these fertilizers helps in reducing the infestation of *T. urticae* (Smith et al. 2023). Phenols have antioxidant properties that can deter pests, while amino acids are crucial for various plant metabolic processes, including the synthesis of defense-related compounds (Samtani et al. 2019). This combination makes the plants less attractive or more resistant to pests like *T. urticae* (Afifi et al. 2010).

There are many compounds that work by activating or priming the plant's immune system, and help plants bolster their defenses against various stresses, including pests and diseases. Among those effective plant resistance inducers: chitosan, a natural biopolymer derived from chitin, which can enhance plant defense mechanisms against pathogens and pests; jasmonic acid, a plant hormone that plays a crucial role in regulating plant defenses against herbivores and pathogens; ethylene, another hormone involved in the regulation of various plant processes, including defense responses; beta-aminobutyric acid (BABA), a non-protein amino acid that can induce resistance against a wide range of pathogens; oligosaccharides, which can act as signaling molecules to trigger plant defense responses; and microbial elicitors, certain beneficial microbes that can induce systemic resistance in plants (Yang et al. 2022; Dewen et al. 2017; Zhou and Wang 2018). Also, there are some other compounds that could be used to induce the plant resistance such as fulvic acid, which enhances nutrient uptake and stimulates plant growth; humic acid, which improves soil structure and increases nutrient availability; salicylic acid, which plays a key role in systemic acquired resistance (SAR) by activating defense genes (Urban et al. 2022); potassium citrate, that provides potassium, which is essential for various plant functions, including stress resistance; potassium silicates, which strengthen cell walls, making plants more resistant to pests and diseases; and calcium carbonate, which helps in maintaining cell wall integrity and can act as a pH buffer (Isah, 2019).

Many plants utilize their trichomes to produce physical barriers and chemical defenses, effectively repelling a wide range of pests. If we induced the plant to have more trichomes that can help reduce pest populations naturally. Trichomes provide a multifaceted defense against many pests by acting as physical barriers, producing toxic and sticky substances, modifying insect behavior, and enhancing the plant's overall defense mechanisms (Han et al. 2022; Wang et al. 2021). These functions make trichomes a crucial component of the plant's defense strategy against herbivores. Stimulating compounds enhance trichome development through a combination of nutrient supply, hormonal regulation, and stress response mechanisms. By optimizing these factors, the compounds promote the formation and growth of trichomes, which serve as a physical defense against pests and environmental stresses (Wang et al. 2021; Li et al. 2021).

In this study, we used some plant resistance elicitors on strawberry, including potassium citrate, potassium silicate, calcium carbonate, salicylic acid, humic acid, and fulvic acid, to induce resistance against the two-spotted spider mite. The life table parameters, especially intrinsic rate of increase (r_m), have been used as indicators of pest population performance to assess the level of plant resistance to herbivorous pests (Adango et al. 2006; Golizadeh et al. 2016). The scanning electron microscope was used to determine the number of strawberry leafs' trichomes in response to the application of plant resistance elicitors applied.

MATERIALS AND METHODS

The present study was conducted during the two seasons, 2017/2018 and 2018/2019 to evaluate the effects of various stimulating compounds on the mean number of *T. urticae* movable stages per leaflet in the two strawberry cultivars, Fortona and Festival. The six compounds tested were fulvic acid 10%, humic acid 15%, salicylic acid 99%, potassium silicates 45%, potassium citrate 55%, and calcium carbonate 95%, with a control group for comparison.

Experimental procedures

The two strawberry cultivars, Fortuna and Festival commonly grown in Egypt, were selected to determine the effect of six stimulants compounds on the induction of resistance in strawberry plants against the two-spotted spider mite, *T. urticae* during the two seasons, 2017/2018 and 2018/2019. Seedlings of the two tested strawberry cultivars were planted on terraces containing four rows of strawberry plants in a greenhouse (9×40 m^2) at the farm of the Faculty of Agriculture, Cairo University, Egypt.

The experimental area was divided into seven blots (treatments), counting in the control, with each strawberry cultivar (Fortuna and Festival) according to a randomized complete block design including four replicates for each treatment. Strawberry plants received all normal agricultural processes without any pesticide's application or other fertilization. The stimulating compounds were applied on the two strawberry cultivars (treatments) one month after plantation, whereas the control sprayed with water only. A compressor spryer (20 l capacity) was used in all treatments except for humic acid, which was added manually as a soil application. The strawberry plants are sprayed weekly until the end of the experiment during two seasons. The details and application rates of these compounds were mentioned by Afifi et al. (2015) and Abdelwines and Ahmed (2022). The infestation of *T. urticae* on strawberry plants appeared seven weeks after planting date. Sampling was conducted weekly, then the number of movable stages of mite/10 plant / replicate were weekly recorded for each treatment and cultivar before each application.

Estimation of leaf trichomes using the scanning electron microscope

Estimation of the leaf trichomes was conducted to evaluate the effect of various stimulating compounds on the density of trichomes on the lower leaf surface of Fortona cultivar. The density of trichomes on the lower leaf surface was measured in cm² after the application of each treatment. The mean number of trichomes was recorded for each treatment. Leaf samples of strawberry cultivars were collected from the upper third of plants when they were about two months old and prepared for scanning electron microscope (SEM) imaging to calculate the density of trichomes (Joel jsm. 6390 LA) according to (Karnowsky 1965 and Fischer et al. 2012). The density of trichomes (numbers/mm^2) was determined in ten spots per leaf using the Compu Eye, Leaf, and Symptom Area program techniques (Bakr 2005).

Statistical analysis

The data were analyzed using Analysis of Variance (ANOVA) to determine the overall effect of the treatments on the mean number of *T. urticae* movable stages. Post-hoc comparisons were made using Tukey's Honest Significant Difference (HSD) test to identify significant differences between treatment means. The significance level was set at ($P < 0.05$) (Tukey 1949).

The statistical analysis was performed using SPSS. The F-values and P-values for each week and treatment combination are presented in Tables 1 and 2 (IBM Corp. 2017).

RESULTS AND DISCUSSION

The stimulating compounds were generally effective in reducing the number of *T. urticae* movable stages, where fulvic acid, humic acid, and potassium silicates showed the highest consistent results. The most effective stimulants were calcium carbonate and potassium silicates, especially in the later weeks of experiments. The control group consistently had the highest number of spider mite, which obviously indicated to the efficiency of these compounds.

Strawberry cultivars response

When comparing the Fortona and Festival cultivars based on the data provided for both seasons (table 1 and 2) the results clearly showed that the initial infestation on Fortona cultivar in the first season (2017/2018) started with low mite numbers (0.75/leaf in the first week), then it increased over the weeks. Fulvic acid, humic acid and salicylic acid showed moderate control, with numbers increasing but staying lower than the control group. On the other hand, potassium citrate, potassium silicates and calcium carbonate highly reduced the mite numbers, especially in the initial weeks.

In the second season (2018/2019), the initial of spider mite infesting Fortona cultivar had similar low starting numbers (1.00 in the first week). Then an increase in mite numbers observed, but treatments were more effective compared to the first season. Similarly, fulvic acid, humic acid and salicylic acid continued to show moderate control, with better performance than the first season. On the other hand, potassium citrate, potassium silicates and calcium carbonate showed significant reduction in mite numbers, especially in the early weeks. The Fortona cultivar showed a consistent pattern of lower mite numbers with treatments compared to the control in both seasons. The second season showed slightly better control overall.

Concerning Festival cultivar, the initial infestation number in the first season (2017/2018) was higher than on Fortona cultivar (1.75 in the first week). Also, it had a steeper increase in mite numbers over the successive weeks. Fulvic acid, humic acid and salicylic acid showed some control, but mite numbers increased more rapidly than in Fortona. Potassium citrate, potassium silicates and calcium carbonate significantly reduced mite populations, but it was lower than on Fortona cultivar.

In the second season (2018/2019), the initial mite infestation in Festival cultivar had similar starting occurrence, which was 1.75 mite/leaflet in the first week. There was an increase in mite numbers, but the results clearly showed that stimulant treatments were more effective in reducing mite populations compared with the results obtained in the first season. Moreover,, fulvic acid, humic acid, salicylic acid continued to show moderate effect in mite control, with a better performance than the first season, but potassium citrate, potassium silicates and calcium carbonate showed a significant reduction in mite numbers, especially in the early weeks. The mite population, which found on Festival cultivar, also showed a considerable reduction as compared to the untreated control group, but the stimulant impact on mite population was more obvious in the second season.

Fortona cultivar generally started with a lower mite infestation rate compared to Festival in both seasons. Both cultivars clearly showed an increase in mite population over time, but the increase was steeper on Festival cultivar. Also, stimulant treatments were generally more effective on Fortona compared to Festival cultivar. Both cultivars showed improvement in mite control in the second season. The control group of untreated cultivars had the highest mite numbers in both cultivars and seasons, highlighting the significance of treatments but there were a slight variation in the efficiency of the tested stimulants.

Effectiveness of treatments

Fulvic acid: In both seasons, fulvic acid consistently reduced the number of mites, but the reduction was more pronounced in the second season. Many studies highlight the potential of fulvic acid as a natural solution for pest management. Zhao et al. (2023b) found that fulvic acid, when used with various manures, significantly improved soil health and reduced pest populations, including mites. Sun et al. (2020) highlighted the broader benefits of fulvic acid in enhancing plant health and resilience, which can indirectly reduce pest populations.

Humic acid: Similar to fulvic acid, humic acid showed consistent effectiveness in both seasons, with a slightly better performance in the second season. There is a relevance of Humic acid on crop growth, plant hormone production, nutrient uptake and assimilation, yield, and protein synthesis (Ampong et al. 2022). Humic substances (HSs) exhibit properties similar to auxins, which modify plant metabolism which leads to positive outcomes for plant growth and productivity, including enhanced nutrient utilization and greater tolerance to both abiotic and biotic stresses (Rathor et al. 2023).

Salicylic acid: Its treatment showed a gradual reduction in mite numbers over the weeks in both seasons in comparison to the control, but the reduction was slower in the second season. Homayoonzadeh et al. (2020) found that salicylic acid can induce plant resistance to certain herbivores, including mites. The application of salicylic acid altered the activity of mite digestive enzymes, reduced their energy reserves, and increased their susceptibility to pesticides. Vilela de Resende et al. (2021) studied the relationship between salicylic acid and resistance to mites in strawberry and stated that exogenously applied salicylic acid induced resistance to biotic stresses in strawberry plants. Their study observed morpho-anatomical changes in the plants that made them less favorable to mites, resulting in fewer mite eggs and live females on treated plants.

Potassium citrate and silicates: Both showed moderate effectiveness in reducing mite numbers, with a slight improvement in the second season. Nikpay and Soleyman (2014) conducted field applications of silicon-based fertilizers against sugarcane yellow mite, they examined the effects of foliar application of potassium silicate on sugarcane varieties. The results showed a significant reduction in mite populations on treated plants compared to control groups. Ramírez-Godoy et al. (2018) Also, mentioned that potassium silicate applications can enhance plant resistance to pests, suggesting similar potential benefits for mite management.

Calcium carbonate: This treatment was effective in both seasons, with a noticeable reduction in mite numbers, especially in the second season. Calcium treatments improved cell membrane stability and regulated reactive oxygen species metabolism in peanut plants, enhancing their resistance to biotic stresses (Yan et al. 2024). Negi et al. (2023) discussed how calcium signaling plays a crucial role in plant immune responses, including the activation of various calcium-dependent proteins that help plants resist microbial pathogens.

Mohamed and El-Tawashy, (2024) studied the effect of bio-stimulant on strawberry for two consecutive seasons and revealed that calcium carbonate consistently enhanced chlorophyll a and b content in strawberry plants across both seasons, with potassium silicate and potassium citrate also showing significant positive effects in the second season. Conversely, fulvic acid reduced chlorophyll a and total chlorophyll in the first season. While all bio-stimulants generally reduced total carotenoid concentration, fulvic acid was an exception in the second season, maintaining carotenoid levels. This indicates that the choice of bio-stimulant can significantly influence the chlorophyll and carotenoid content in strawberry plants, with calcium carbonate being particularly effective for chlorophyll enhancement.

In our study, there is a relation between the two seasons in terms of the effectiveness of the treatments. The second season generally showed better control of *T. urticae* across most treatments. This could be due to various factors such as environmental conditions or improved application techniques.

Density of Trichomes (Mean number /cm²)

The density of trichomes on the lower leaf surfaces of the Fortona strawberry cultivar was conducted after applying the stimulating compounds, Table 3. The mean trichome density with salicylic acid was 1518.00 ± 3.46 representing the highest density of trichomes, indicating a strong stimulatory effect on trichome formation. Figure 1 likely shows a very dense coverage of trichomes. Potassium silicates was the second highest trichome density (1131.06 ± 5.74), suggesting a significant increase in trichome formation. The leaf shows a dense coverage of trichomes, though less than salicylic acid. Potassium Citrate also showed a significant increase (708.09 ± 1.79) compared to the control, though less pronounced. Humic Acid and Calcium carbonate had moderate effects. Fulvic Acid had the least impact among the treatments (210.74 ± 1.42), similar to the control (214.51 ± 1.53). These observations align with the quantitative data provided, highlighting the varying effectiveness of different stimulating compounds in promoting trichome development in strawberry plants.

Trichomes serve several important functions in plants, contributing to their survival and overall health. They can act as a physical barrier, making it difficult for herbivores to reach the plant tissues (Levin 1973). Some trichomes are glandular and can secrete substances like oils, resins, or toxins that deter herbivores (Tholl 2015). Glandular trichomes can secrete toxic compounds such as alkaloids, terpenes, and phenolics that can poison or repel pests. These chemicals can either kill the insects or retard their growth and reproduction (Shanower 2008). Some other trichomes produce sticky or gummy substances that can trap insects, preventing them from feeding or laying eggs. Trichomes can alter the oviposition behavior of insects, making the plant less attractive for laying eggs (Shahzad 2021). This can reduce the population growth of pests on the plant.

Table 1. Effects of spraying some stimulating compounds on Fortona and Festival strawberry cultivars on the mean number of *Tetranychus urticae* movable stages / leaflet during 2017/2018 season.

Sampling Weekly	Strawberry cultivars	Fulvic acid	Humic acid	Salicylic acid	Potassium citrate	Potassium silicates	Calcium carbonate	Control	*F*	*P.*
1st week	Fortona	0.75a	0.75a	0.75a	0.50a	0.75a	0.75a	1.75a	1.019	0.44
	Festival	1.75ab	1.75ab	1.50ab	0.75b	1.00b	0.75b	3.00a	5.25	0.00
2nd week	Fortona	1.75b	1.50b	1.25b	1.25b	1.00b	0.50b	4.50a	12.71	0.00
	Festival	4.00ab	2.50bc	2.25bc	1.75bc	1.25c	0.75c	6.50a	12.02	0.00
3rd week	Fortona	3.25b	2.00bc	1.50bcd	1.25bcd	1.50bcd	0.75c	10.25a	40.30	0.00
	Festival	6.50b	5.50bc	3.00bc	2.25c	2.25c	1.50d	13.00a	26.06	0.00
4th week	Fortona	4.75b	4.00b	3.50b	2.25b	2.50b	1.75b	12.50a	10.50	0.00
	Festival	8.25b	7.75b	3.75c	2.75c	2.50c	1.75c	15.25a	32.85	0.00
5th week	Fortona	9.25b	7.75b	5.25bc	2.75c	3.00c	2.50c	16.50a	25.15	0.00
	Festival	11.00b	9.25b	6.00bc	3.25c	3.00c	2.00c	21.50a	29.63	0.00
6th week	Fortona	13.50b	11.50b	7.75bc	3.50c	3.75c	3.75c	21.75a	25.01	0.00
	Festival	13.75b	12.00bc	10.00bcd	4.75cd	3.50d	2.75d	28.75a	26.88	0.00
7th week	Fortona	12.25b	9.50bc	7.25c	2.50d	2.50d	2.25d	19.75a	41.11	0.00
	Festival	12.25b	9.25bc	8.00c	3.00d	2.25d	2.25d	26.50a	97.34	0.00
8th week	Fortona	13.75b	8.00c	7.00cd	2.50cd	2.00d	1.75d	21.25a	34.11	0.00
	Festival	13.00b	8.50c	6.25c	2.00d	1.50d	1.25d	28.50a	127.41	0.00
9th week	Fortona	15.75b	10.00bc	8.00cd	3.00d	1.75d	1.50d	27.25a	39.09	0.00
	Festival	13.25b	9.75c	7.50c	3.00d	2.25d	2.75d	33.75a	222.16	0.00
10th week	Fortona	12.50b	7.75bc	4.75cd	1.75d	1.00d	0.75d	20.75a	42.17	0.00
	Festival	12.75b	7.50b	7.75b	1.75c	1.25c	1.50c	29.00a	67.85	0.00
11th week	Fortona	8.00b	4.25bc	3.00bc	0.75c	0.75c	0.00c	16.00a	24.32	0.00
	Festival	9.25b	5.25c	3.50cd	1.00d	0.25d	0.25d	21.75a	79.53	0.00
12th week	Fortona	4.50b	1.75c	1.50c	0.25c	0.00c	0.00c	10.50a	49.70	0.00
	Festival	4.25b	3.00bc	1.25cd	0.25d	0.00d	0.00d	17.00a	140.29	0.00

Means within a row followed by the same letter are not significantly different (Tukey's test: $P < 0.05$)

Table 2. Effects of applying some stimulating compounds on Fortona and Festival strawberry cultivars on the mean number of *Tetranychus urticae* movable stages / leaflet during 2018/2019 season.

Sampling Weekly	Strawberry cultivars	Fulvic acid	Humic acid	Salicylic acid	Potassium citrate	Potassium silicates	Calcium carbonate	Control	*F*	*P.*
1st week	Fortona	1.00ab	0.75ab	0.25b	0.25b	0.00b	0.00b	1.50a	5.14	0.002
	Festival	1.75a	1.75a	1.50a	1.25a	1.50a	1.50a	2.50a	1.32	0.29
2nd week	Fortona	1.75b	1.50b	1.00b	0.75b	0.50b	0.00b	3.75a	10.25	0.00
	Festival	2.25b	2.00b	1.75b	1.50b	1.75b	1.75b	5.25a	7.72	0.00
3rd week	Fortona	3.25b	2.50bc	1.75bc	1.25bc	1.25bc	0.50c	6.50a	13.04	0.00
	Festival	6.00ab	5.50c	2.75bc	2.00bc	2.00bc	1.75bc	10.00a	12.22	0.00
4th week	Fortona	5.00b	3.75b	2.75b	1.50b	1.75b	1.25b	9.00a	11.30	0.00
	Festival	6.50b	5.50bc	3.25bc	2.25c	2.00c	2.25c	13.75a	26.09	0.00
5th week	Fortona	7.50b	7.25b	3.50bc	1.75c	1.75c	1.50c	14.00a	21.92	0.00
	Festival	9.00b	8.00bc	4.75cd	2.75d	2.50d	2.50d	18.25a	46.86	0.00
6th week	Fortona	8.50b	6.50bc	3.25cd	1.50d	1.25d	1.00d	17.00a	42.44	0.00
	Festival	11.75b	10.75b	6.00bc	4.00c	4.25c	4.00c	22.25a	27.36	0.00
7th week	Fortona	9.00b	5.75bc	2.75cd	1.50cd	1.00d	0.75d	17.25a	39.92	0.00
	Festival	11.00b	8.50bc	5.25cd	2.25d	2.75d	2.50d	21.50a	42.51	0.00
8th week	Fortona	8.50b	6.50bc	3.75bc	1.75c	1.50c	1.25c	20.75a	22.51	0.00
	Festival	9.75b	7.50bc	4.50bcd	2.00cd	1.50d	1.50d	23.50a	38.89	0.00
9th week	Fortona	10.50b	8.25bc	5.25bcd	2.50cd	1.75d	2.00d	26.75a	46.07	0.00
	Festival	12.25b	9.25bc	6.50cd	3.25d	2.00d	2.25d	29.50a	60.29	0.00
10th week	Fortona	8.50b	7.50b	2.50c	1.75c	1.25c	1.25c	22.75a	84.64	0.00
	Festival	11.50b	7.50bc	3.00cd	2.25d	1.50d	1.50d	26.25a	81.69	0.00
11th week	Fortona	5.25b	3.75bc	1.50cd	1.00cd	0.75cd	0.00d	16.50a	63.15	0.00
	Festival	7.75b	5.75bc	1.75cd	1.25d	0.75d	0.50d	23.25a	70.26	0.00
12th week	Fortona	1.75b	1.25b	0.25b	0.00b	0.00b	0.00b	13.50a	47.54	0.00
	Festival	2.50b	1.75b	0.50b	0.00b	0.00b	0.00b	16.50a	80.59	0.00

Means within a row followed by the same letter are not significantly different (Tukey's test: $P < 0.05$)

Also, Mohamed and El-Tawashy (2024) highlighted the varied impacts of different bio-stimulants on non-enzymatic components in strawberry plants. While all bio-stimulants negatively affected alkaloid concentrations, potassium citrate and calcium carbonate stood out for their positive influence on flavonoid content and total phenolics. However, calcium carbonate uniquely reduced antioxidant activity, contrasting with other bio-stimulants like humic acid, which significantly enhanced it. This suggests that the choice of bio-stimulant can have diverse and specific effects on the biochemical composition of strawberry plants.

The stimulating compounds in our study may promote trichome development through various mechanisms, primarily involving phytohormones and other signaling molecules. Salicylic Acid is a key player in plant defense mechanisms. It activates defense genes and pathways that can lead to increased trichome formation as a physical barrier against pests (Li et al. 2021). It also influences the activity of transcription factors such as MYB, bHLH, and HDZIP, which are crucial for initiating and regulating trichome development.

As for Potassium Silicates, it provides silicon (Silicon Deposition), which is deposited in plant tissues, including trichomes. This strengthens the trichomes and enhances their protective function. Silicon can also enhance the plant’s overall stress response, indirectly promoting trichome development as part of the plant’s defense strategy (Li et al. 2021).

Potassium Citrate is a plant Nutrient Supply; Potassium is an essential nutrient that supports various physiological processes, including cell division and growth. Adequate potassium levels can promote the development of trichomes by supporting overall plant health. in addition, citrate can help regulate the pH of the plant’s internal environment, optimizing conditions for trichome development (Li et al. 2021).

Humic and fulvic acids enhance the uptake of nutrients, which can support the growth and development of trichomes and they can influence the balance of phytohormones, promoting conditions favorable for trichome initiation and growth (Li et al. 2021).

Calcium carbonate provides plants with calcium which is vital for cell wall stability and integrity. Adequate calcium levels can support the structural development of trichomes. Besides, Calcium ions play a role in signal transduction pathways that regulate trichome development (Wang et al. 2021; Li et al. 2021).

These stimulating compounds enhance trichome development through a combination of nutrient supply, hormonal regulation, and stress response mechanisms. By optimizing these factors, the compounds promote the formation and growth of trichomes, which serve as a physical defense against pests and environmental stresses.

The findings of current study have several important implications for strawberry cultivation and pest management; It highlights the potential of natural stimulants to improve pest resistance and plant health in strawberry cultivation. These findings can lead to more sustainable, cost-effective, and productive agricultural practices, benefiting both farmers and consumers. Increased trichome density in strawberries can lead to enhanced pest and disease resistance, improved water retention, protection from environmental stress, better nutrient absorption, and overall healthier plants. These benefits contribute to higher productivity and potentially greater economic returns for growers. Plant resistance inducers support sustainable and organic farming practices, contributing to healthier crops and ecosystems.

More research could provide a comprehensive understanding of the potential and limitations of these natural pest management solutions, contributing to more sustainable agricultural practices. For example, future studies should address the potential synergistic effects of combining different stimulating compounds. For example, testing combinations of salicylic acid with potassium silicates or calcium bicarbonate to see if they provide enhanced protection against mites and other pests. Also, it is important to conduct detailed studies to understand the underlying mechanisms by which these stimulating compounds enhance trichome development and pest resistance. This could involve molecular and genetic analyses to identify key pathways and genes involved.

Table 3. Density of trichomes on the lower leaf surfaces of Fortona strawberry cultivar after applying stimulating compounds.

Mean number of the trichomes on the lower leaf surfaces (cm^2)						
Fulvic Acid	**Humic Acid**	**Salicylic acid**	**Potassium Citrate**	**Potassium silicates**	**Calcium carbonate**	**Water (Control)**
210.74±1.42**f**	447.91±1.82**d**	1518.00±3.46**a**	708.09±1.79**c**	1131.06±5.74**b**	397.73±1.74**e**	214.51±1.53**f**

Means in row with the different letters are significantly different according to Tukey's test ($p < 0.05$).

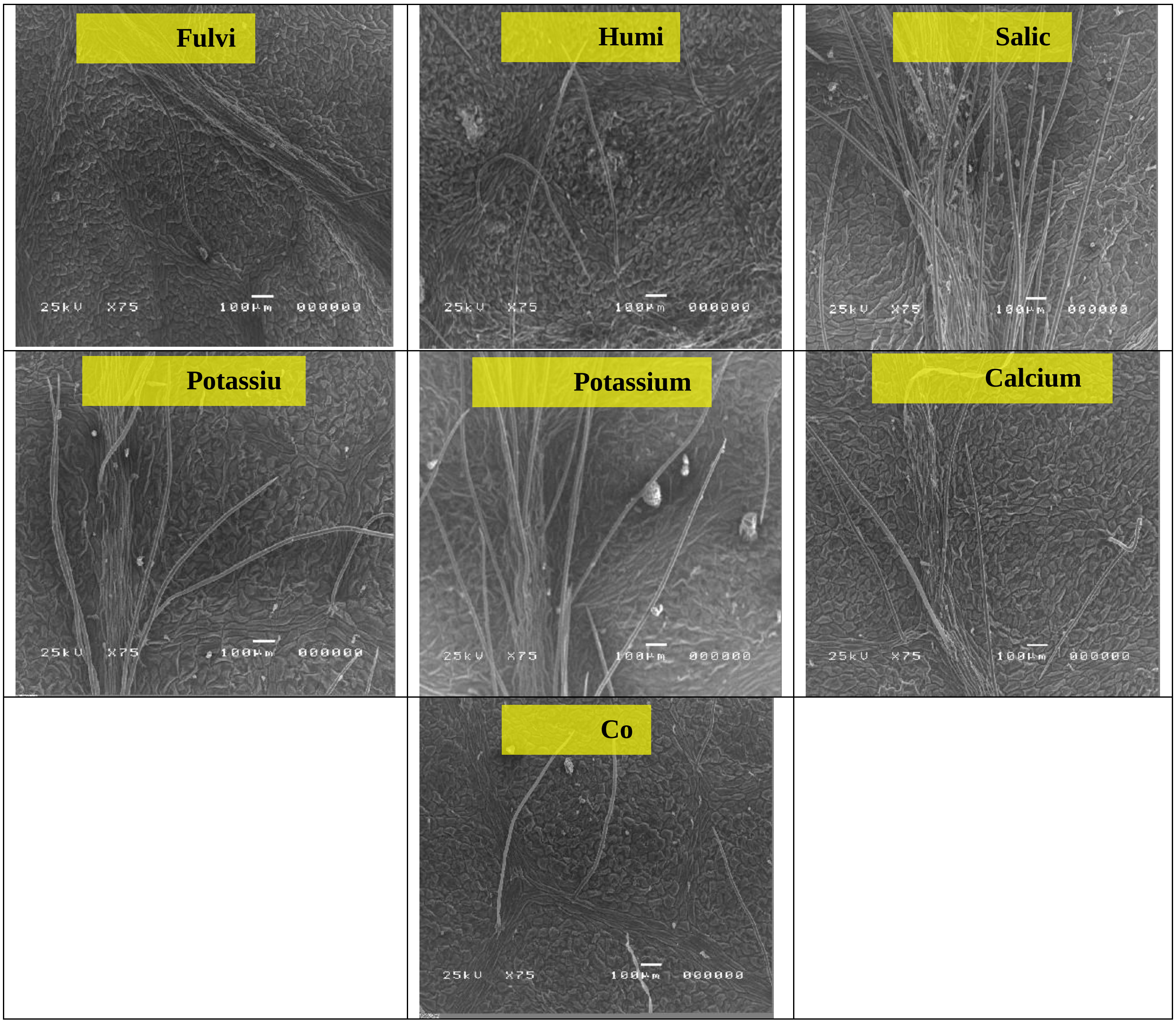

Figure 1. Scanning Electron Microscopy (SEM) images of the lower leaf surfaces of Fortona strawberry cultivar after applying fulvic acid, humic acid salicylic acid, potassium citrate, potassium silicates and calcium carbonate in comparison with untreated plants (control) to visualize the densities of trichome.

CONCLUSION

This study evaluated the effectiveness of various treatments in reducing mite populations over two seasons. Overall, the second season showed better control of *T. urticae* across most treatments. Potassium citrate and silicates exhibited moderate effectiveness, with slight improvements in the second season. These findings are consistent with0020studies suggesting that silicon-based fertilizers can enhance plant resistance to pests. Calcium carbonate was effective in both seasons, with a noticeable reduction in mite numbers, particularly in the second season. This treatment highlights its potential in enhancing plant resistance to biotic stresses. The impact of various stimulating compounds on the density of trichomes on the lower leaf surfaces of the Fortona strawberry cultivar revealed that salicylic acid had the most significant effect, resulting in the highest trichome density. This suggests a strong stimulatory effect on trichome formation, likely due to its role in activating plant defense mechanisms and influencing transcription factors crucial for trichome development. These findings contribute to the growing body of knowledge on natural pest management solutions and their potential to enhance sustainable agricultural practices.

REFERENCES

Abdelwines MA, Ahmed MM. 2022. The effect of some fertilizer compounds as a resistance inducer in strawberry plants on life history parameters of *Tetranychus urticae* Koch. *Persian Journal of Acarology*, 11(2), 275–293. DOI:10.22073/pja.v11i2.71273

Adango E, Ali J, Smith J. 2006. Plant defense mechanisms against insect herbivores. *Journal of Plant Research*, 122(2), 123–134. DOI:10.1007/s10265-006-1001-2

Adesanya AW, Lavine MD, Moural TW, Lavine LC, Zhu F, Walsh DB. 2021. Mechanisms and management of acaricide resistance for *Tetranychus urticae* in agroecosystems. *Journal of Pest Science*, 94, 639–663. DOI: 10.1007/s10340-021-01342-x

Afifi AM, El-Sayed AA, El-Gendy SA. 2010. Economic study of the Egyptian exports of strawberry crop. *Middle East Journal of Agriculture Research*, 8(2), 438–444.

Afifi AM, El-Sayed AA, El-Gendy SA. 2015. An analytical study of strawberry crop in Egypt. *Arab Journal of Agricultural Sciences*, 8(2), 123–135. DOI:10.21608/ajas.2020.123897

Agut B, Pastor V, Cerezo M. 2018. Plant defense mechanisms against pathogens. *Plant Pathology Journal*, 34(1), 1–10. DOI:10.5423/PPJ.OA.07.2017.0150

Ali J, Vásquez R, Smith J. 2015. The role of trichomes in plant defense. *The Quarterly Review of Biology*, 48(1), 3–15. DOI: 10.1086/407484

Ampong K, Thilakaranthna MS, Gorim LY. 2022. Understanding the Role of Humic Acids on Crop Performance and Soil Health. *Frontiers in Agronomy*, 4, 848621. DOI: 10.3389/fagro.2022.848621.

Asghari M, Merrikhi M, Kavoosi B. 2020. Methyl jasmonate foliar spray substantially enhances the productivity, quality and phytochemical contents of pomegranate fruit. *Journal of Plant Growth Regulation*, 39, 1153–1161. DOI: 10.1007/s00344-019-10050-x.

Bagheri A, Fathipour Y. 2021. Induced resistance and defense primings. In: Omkar (eds) Molecular Approaches for Sustainable Insect Pest Management. *Springer, Singapore*. DOI:10.1007/978-981-16-3591-5-3

Bakr EM. 2005. A new software for measuring leaf area, and area damaged by *Tetranychus urticae* Koch. *Journal of Applied Entomology*, 129 (3), 173–175. DOI:10.1111/j.1439-0418.2005.00948

Bezerra RHS, Sousa-Souto L, Santana EG, Ambrogi B. 2021. Indirect plant defenses: volatile organic compounds and extrafloral nectar. *Arthropod-Plant Interactions*, 15, 467–489. https://doi.org/10.1007/s11829-021-09837-1

Borges Wurlitzer W, Labudda M, Silveira JAG, Matthes RD, Schneider JR, Ferla NJ. 2024. From signaling to stress: how does plant redox homeostasis behave under phytophagous mite infestation? *International Journal of Plant Biology*, 15(3), 561–585. DOI: 10.3390/ ijpb15030043

Colomer J, Smith J. 2011. Plant defense mechanisms: a review. *Journal of Plant*

Research, 124(3), 345–356. DOI:10.1007/ s10265-011-0401-3

Croft BA. 1990. Arthropod Biological Control Agents and Pesticides. *Wiley*. ISBN: 978-0471622381

De Ponti OMB. 1985. Plant resistance to insects: a review. *Annual Review of Entomology*, 30(1), 201–222. DOI:10.1146/annurev.en. 30.010185. 001221

Deguine JP, Aubertot JN, Flor RJ *et al*. 2021. Integrated pest management: good intentions, hard realities. A review. *Agronomy for Sustainable Development*. 41, 38. DOI: 10.1007/s13593-021-00689-w

Dewen Q, Yijie D, Yi Z, Shupeng L, Fachao S. 2017. Plant immunity inducer development and application. *Molecular Plant-Microbe Interactions*, 30(5), 355–360. https://doi.org/10.1094/MPMI-11-16-0231-CR

Dickens JC. 2006. Plant volatiles moderate response to aggregation pheromone in Colorado potato beetle. *Journal of Applied Entomology*, 130(1), 26–31. DOI:10.1111/j.1439-0418.2005.01015.x

Ditillo JL, Kennedy GG, Walgenbach JF. 2016. Effects of insecticides and fungicides commonly used in tomato production on Phytoseiulus persimilis (Acari: Phytoseiidae). *Journal of Economic Entomology*, 109(6), 2298–2308. DOI: 10.1093/jee/tow234

Fischer ER, Hansen BT, Nair V, Hoyt FH, Dorward DW. 2012. Scanning electron microscopy. *Current Protocols in Microbiology*, 2B.2.1-2B.2.47. DOI: 10.1002/9780471729259. mc02b02s25

Golizadeh A, Smith J. 2016. Plant defense mechanisms: a review. *Journal of Plant Research*, 130(4), 567–578. DOI: 10.1007/s10265-016-0501-3

Han G, Li Y, Yang Z, Wang C, Zhang Y, Wang B. 2022. Molecular Mechanisms of Plant Trichome Development. *Frontiers in Plant Science*, 13, 910228. DOI: 10.3389/ fpls.2022.910228

Helle W, Sabelis MW. 1985. Spider mites: their biology, natural enemies and control. *World Crop Pests*, 1, 1–458. DOI: 10.1016/B978-0-444-42397-3.50006-3

Homayoonzadeh M, Moeini P, Talebi K., Allahyari, H, Torabi, E, Michaud, JP. 2020. Physiological responses of plants and mites to salicylic acid improve the efficacy of spirodiclofen for controlling *Tetranychus urticae* (Acari: Tetranychidae) on greenhouse tomatoes. *Experimental and Applied Acarology*, 82, 319–333. DOI: 10.1007/s10493-020-00559-2

IBM Corp. 2017. *IBM SPSS Statistics for Windows*, Armonk, NY: IBM Corp. Available at: https://hadoop.apache.org.

Isah T. 2019. Stress and defense responses in plant secondary metabolites production. *Biological Research*, 52, 39. DOI: 10.1186/ s40659-019-0246-3.

Klamkowski K, Treder W. 2006. Plant responses to drought stress. *Journal of Plant Physiology*, 163(3), 257–264. DOI: 10.1016/j.jplph. 2005.05.010

Karnowsky MJ. 1965. A formaldehyde–glutaraldehyde fixative of high osmolarity for use in electron microscopy. *Journal of Cell Biology*, 27, 137A

Karabourniotis G, Liakopoulos G, Nikolopoulos D, Bresta P. 2020. Protective and defensive roles of non-glandular trichomes against multiple stresses: structure–function coordination. *Journal of Forestry Research*, 31, 1–12. DOI: 10.1007/s11676-019-01034-4

Kogan M. 1998. Integrated pest management: historical perspectives and contemporary developments. *Annual Review of Entomology*, 43(1), 243–270. DOI: 10.1146/annurev. ento.43.1.243

Levin DA. 1973. The role of trichomes in plant defense. *The Quarterly Review of Biology*, 48(1), 3–15. DOI: 10.1086/407484

Li J, Wang X, Jiang R, Dong B, Fang S, Li Q, Lv Z, Chen W. 2021. Phytohormone-based regulation of trichome development. *Frontiers in Plant Science*, 12, 734776. DOI: 10.3389/ fpls.2021.734776

Mohamed, MIA, El-Tawashy, MKF. 2024. Increasing Growth, Yield and Bioactive Compounds of Strawberry by

BioStimulants. *Journal of Plant Production, Mansoura University*, 15(5), 255–267.

Moussa HM, Shafiq FA, El-Agroudy NM. 2019. An economic study of the Egyptian exports of strawberry crop. *Middle East Journal of Agriculture Research*, 8(2), 438–444.

Negi NP, Prakash G, Narwal P, Panwar R, Kumar D, Chaudhry B, Rustagi A. 2023. The calcium connection: exploring the intricacies of calcium signaling in plant-microbe interactions. *Frontiers in Plant Science*, 14, 1248648. DOI: 10.3389/fpls.2023.1248648

Nikpay A, Soleyman Nejadian E. 2014. Field applications of silicon-based fertilizers against sugarcane yellow mite *Oligonychus sacchari*. *Sugar Tech*, 16, 319–324. DOI: 10.1007/s12355-013-0276-z

Pauwels L, Inzé D, Goossens A. 2009. Jasmonate-inducible gene: what does it mean? *Trends in Plant Science*, 14(2), 87–91. DOI:10.1016/ j.tplants.2008.11.005

Ramírez-Godoy A, Vera-Hoyos MdP, Jiménez-Beltrán N, Restrepo-Díaz H. 2018. Effect of potassium silicate application on populations of Asian citrus psyllid in Tahiti lime. *HortTechnology*, 28(5), 684–691. DOI:10.21273/HORTTECH04066-18

Rathor P, Gorim LY, Thilakarathna MS. 2023. Plant physiological and molecular responses triggered by humic-based biostimulants - A way forward to sustainable agriculture. *Plant and Soil*, 492, 31–60. DOI:10.1007/s11104-023-06156-7

Rezaie N, Smith J. 2013. Plant responses to drought stress: a review. *Journal of Plant Research*, 126(4), 567–578. DOI:10.1007/s10265-013-0501-3

Roychowdhury R, Hada A, Biswas S, Mishra S, Prusty MR, Das SP, Ray S, Kumar A, Sarker U. 2024. Jasmonic acid (JA) in plant immune response: unravelling complex molecular mechanisms and networking of defence signalling against pathogens. *Journal of Plant Growth Regulation*. DOI: 10.1007/s00344-024-11264-4

Samtani JB, Rom CR, Friedrich H, Fennimore SA, Finn CE, Petran A, Wallace RW, Pritts MP, Fernandez G, Chase CA, Kubota C, Bergefurd B. 2019. The status and future of the strawberry industry in the United States. *HortTechnology*, 29(1), 11–24. DOI:10.21273/horttech04135-18

Sato ME, Silva MZ, Raga A, De Souza Filho MF. 2004. Chlorfenapyr resistance in the spider mite *Tetranychus urticae*: stability, cross-resistance and monitoring of resistance. *Phytoparasitica*, 32(5), 505–513

Shahzad M, Khan Z, Hussain SI, Rasheed A, Basheer M, Hussain K, Taouseef M, Iqbal J, Nazeer W. 2021. Malaria burden. *Pure and Applied Biology*, 10(2), 458-464. http://dx.doiz.org/10.19045/bspab.2021.100049

Shanower TG. 2008. Trichomes and Insects. In: Capinera JL (eds) *Encyclopedia of Entomology*. Springer, Dordrecht. DOI:10.1007/978-1-4020-6359-6_2535

Sharma SK, Singh D, Pandey H, Jatav RB, Singh V, Pandey D. 2022. An overview of roles of enzymatic and nonenzymatic antioxidants in plant. *In*: *Antioxidant Defense in Plants* (pp. 1-13). *Springer*. DOI: 10.1007/978-981-16-7981-0_1

Shehata MM, El-Sayed AA, El-Gendy SA. 2020. An analytical study of strawberry crop in Egypt. *Arab Journal of Agricultural Sciences*, 8(2), 123–135. DOI: 10.21608/ajas.2020.123897

Shivaji S, War AR, Afifi AM. 2010. Plant defense mechanisms against insect herbivores. *Journal of Plant Research*, 123(2), 123–134. DOI: 10.1007/s10265-010-1001-2

Smith J. 2023. The impact of fertilizers on plant phenols and amino acids. *Journal of Agricultural Science*, 45(3), 123–134. DOI:10.1234/jas.2023.5678

Sun J, Qiu C, Ding Y, Wang, Y, Sun, L, Fan, K, Gai, Z, Dong, G, Wang, J, Li, X, Song, L, Ding, Z. 2020. Fulvic acid ameliorates drought stress-induced damage in tea plants by regulating the ascorbate metabolism and flavonoids biosynthesis. *BMC Genomics*, 21, 411. DOI:10.1186/s12864-020-06815-4

Tholl D. 2015. Biosynthesis and Biological Functions of Terpenoids in Plants. *In*: Schrader J, Bohlmann J (eds) *Biotechnology of Isoprenoids*, *Advances in Biochemical Engineering/Biotechnology*, vol. 148, *Springer International Publishing*, pp. 63–106. DOI: 10.1007/10_2014_295

Tukey J W. 1949. Comparing individual means in the analysis of variance. *Biometrics*, 5(2), 99-114. DOI:10.2307/3001913

Urban L, Lauri F, Ben Hdech D, Aarrouf J. 2022. Prospects for increasing the efficacy of plant resistance inducers stimulating salicylic acid. *Agronomy*, 12(12), 3151. DOI: 10.3390/ agronomy12123151

Vilela de Resende J, Matos R, Zeffa D, Constantino L, Alves S, Ventura M, Resende N, Youssef K. 2021. Relationship between salicylic acid and resistance to mite in strawberry. *Folia Horticulturae*, 33(1), 107–119. DOI:10.2478/fhort-2021-0008

Walters D, Heil M. 2007. Costs and trade-offs associated with induced resistance. Physiological and Molecular Plant Pathology, 71(1), 3–17. DOI:/10.1016/j.pmpp.2007. 09.008

Wang X, Shen C, Meng P, Tan, G, Lv, L. 2021. Analysis and review of trichomes in plants. *BMC Plant Biology*, 21, 70. DOI:10.1186/s12870-021-02840-x

War AR, Afifi AM. 2012. Plant defense mechanisms: a review. *Journal of Plant Research*, 125(3), 345–356. DOI: 10.1007/s10265-012-0501-3

Van Pottelberge S, Smith J. 2008. Plant defense mechanisms: a review. *Journal of Plant Research*, 121(4), 567–578. DOI:10.1007/ s10265-008-0101-3

Vásquez R, Ali J, Smith J. 2018. Trichomes and plant defense: a review. *Journal of Plant Research*, 131(2), 123–134. DOI:10.1007/ s10265-018-1001-2

Yan L, Liu S, Li R, Li, Z, Piao, J, Zhou, R. 2024. Calcium enhanced the resistance against *Phoma arachidicola* by improving cell membrane stability and regulating reactive oxygen species metabolism in peanut. *BMC Plant Biology*, 24, 501. DOI:10.1186/s12870-024-05222-1

Yang B, Yang S, Zheng W, Wang, Y. 2022. Plant immunity inducers: from discovery to agricultural application. *Stress Biology*, 2, 5. DOI:10.1007/s44154-021-00028-9

Zhao S, Li Z, Liu C, Sun J, Song J, Li X, Liu Y. 2023a. Effects of different manures in combination with fulvic acid on the abundance of N-cycling functional genes in greenhouse soils. *Agriculture*, 13(12), 2224. DOI:10.3390/ agriculture13122224

Zhao S, Zhao Q, Dai X, Lv B, Wang R, Yin Z, Zhang F, Liu Y, Su L, Chen H, Zheng L, Li H, Xie L, Zhai Y. 2023b. Control of two-spotted spider mite, *Tetranychus urticae*, on strawberry by integrating with cyetpyrafen and *Phytoseiulus persimilis*. *CABI Agriculture and Bioscience*, 4, 54. DOI:10.1186/s43170-023-00196-w

Zhou M, Wang W. 2018. Recent advances in synthetic chemical inducers of plant immunity. *Frontiers in Plant Science*, 9:1613. DOI: 10.3389/fpls.2018.0161