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Evaluation of four biostimulants for the induction of systemic resistance in cucumber (*Cucumis sativus* L.) and tomato (*Solanum lycopersicum* Mill.) in monoculture and associated greenhouse cultivation

Evaluación de cuatro bioestimulantes en la inducción de la resistencia sistémica en pepino (*Cucumis sativus* L.) y tomate (*Solanum lycopersicum* Mill.) en monocultivo y cultivo asociado en invernadero

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Data of the Article

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Abstract

In order to evaluate four biostimulants in the induction of systemic resistance in cucumber (*Cucumis sativus* L.) and tomato (*Solanum lycopersicum* Mill.) in monoculture and associated greenhouse cultivation, five treatments were applied at three developmental stages (growth, flowering and fruiting): T_1 Bioremedy (2.0 g/L), T_2 Grandsil (2.0 g/L), T_3 Control (water), T_4 Fossil (5.0 g/L), T_5 L-amino (2 cm^3/L). The treatments were distributed in a 3 x 5 factorial arrangement and evaluated in a completely randomised experimental design. Each experimental unit consisted of three rows. The response variables were: plant height (cm), stem diameter (mm), number of fruits per plant, fruit volume (cm^3) and fruit weight (kg). The results, cucumber in monoculture did not present notable differences for any of the evaluated variables, with the exception of plant height, significant differences were observed at P<0.05 of probability, Bioremedy, L-amino and control were the best. In tomato monoculture, there were significant differences at P<0.05 for all the variables, with Bioremedy standing out for plant height, stem diameter, fruit weight, fruit diameter and fruit number. In the associated crop, both cucumber and tomato had significant differences at P<0.05 for all the variables evaluated, with Bioremedy being the best.

Resumen

Con el objetivo de evaluar cuatro bioestimulantes en la inducción de la resistencia sistémica en pepino (*Cucumis sativus* L.) y tomate (*Solanum lycopersicum* Mill.) en monocultivo y cultivo asociado en invernadero, fueron aplicados cinco tratamientos en tres estadios de desarrollo (crecimiento, floración y fructificación): T_1 Bioremedy (2.0 g/L), T_2 Grandsil (2.0 g/L), T_3 Testigo (agua) T_4 Fossil (5.0 g/L), T_5 L-amino (2 cm^3/L). Los tratamientos fueron distribuidos en arreglo factorial 3 x 5 y evaluados en un diseño experimental Completamente Aleatorio. Cada unidad experimental estuvo constituida por tres hileras. Las variables de respuestas fueron: altura de planta (cm), diámetro de tallo (mm), número de frutos por planta, volumen del fruto (cm^3) y peso de fruto (kg). Los resultados, pepino en monocultivo no presento diferencias notables para ninguna de la variable evaluadas, con excepción de la altura de planta, se observó diferencias significativas al P<0.05 de probabilidad, Bioremedy, L-amino y testigo fueron los mejores. En monocultivo de tomate, hubo diferencias significativas al P<0.05 de probabilidad para todas las variables, destacándose a Bioremedy para altura de planta, diámetro de tallo, peso de fruto diámetro de fruto y número de fruto. En el cultivo asociado tanto el pepino como el tomate con diferencias significativas al P<0.05 de probabilidad para todas las variables evaluadas, destacándose al Bioremedy como el mejor

Introduction

The world's food supply depends to a large extent on agriculture. To achieve this objective today, the task is complex and difficult without pesticides in the control of pests and diseases that attack crops[1]. Their application has undesirable consequences, such as adverse effects on human health, soil and the environment in general, due to their toxicity and persistence[1]. Another negative aspect is the selection of resistant individuals due to their evolution, the survival of better adapted and possibly more aggressive individuals leads to an increase in their application, thus forming a closed spiral circle that worsens the conditions of the ecosystem[1]. In order to respond to growing consumer demands, sustainable food production must develop alternatives to conventional synthetic plant protection products. At present, the long-term application of pesticides on crops has decreased and has been accepted by consumers[1].

Ross[2] first observed that plants acquire a type of non-specific systemic immunity after localized infection, recognized as systemic acquired resistance (SAR). A large number of studies, reporting plant pathological, chemical, biochemical, genetic, genomic, molecular, agronomic and molecular aspects, contributed and continue to reveal a wealth of facts and information, including the potential way to exploit phenomenological processes in crop protection[3]. In the last two decades, models[4-7] was published on how the SAR process develops, either in its innate or evolved expression, as well as in its potential chemically induced form. The various investigations carried out, in addition to its forms of action, dealt with the effectiveness of resistance induced under field conditions[8-11].

Despite this research, the molecular basis of SAR is still in the process of being clearly formulated, although it remains the most investigated model of plant induced resistance (PIR), it represents only one of its multifunctional defence mechanisms[3]. The latest research has identified SAR as one of the multifaceted defence mechanisms inducible in plants, according to different pathways, characterized by different signals, metabolites and genes[3]. SAR is defined as a process that depends on the salicylic acid (SA) content that is involved in the transduction protein to develop a defence response[12]. A second process, called systemic induced resistance (SIR), motivated by symbiont-generated hormone-dependent pathways, such as jasmonic acid (JA)[3] and ethylene[13], and a third defence mechanism, called β-aminobutyric acid (RI-BABA)-induced resistance (RI-BABA), which emerged in the last decade through the discovery that exogenous application of BABA[13,14], can activate multiple defence responses, which enhance AS-induced defences by stimulating pathogen deposition of callose, independent of AS and AJ[3,15].

These interconnected mechanisms contribute to creating a network of defences that make up the plant's own immune system, which can most appropriately be included in induced resistance, SAR being the best known, but not the only process involved in the defence response to pathogens[3,13].

After an attack by arthropod herbivores, mechanical damage, contact with some chemicals, immunizes the plant against subsequent pathogen infections, even if it does not carry cultivar-specific resistance genes. Obviously, the first infecting pathogen, or damage, induced the expression of resistance reactions against subsequent pathogen infections, irrespective of whether they are viruses, fungi or bacteria. The ability of the cells to repel subsequent attacks is dispersed throughout the whole plant, to this response called SAR[3]. Another form of resistance induced by plant growth-promoting rhizobacteria (PGPR) has also been discovered, called SIR[3,16].

Thus, several authors[1,3,17], in an extensive review of the subject, argue that the plant defence system contains a combination of physical and biochemical changes[11,18], the former including lignification, cell wall hardening, papilla formation, the latter comprising oxidative burst, accumulation of phytoalexins and activation of pathogenesis-related proteins (PRPs) such as chitinases, β-1,3-glucanases and peroxidases[1,18,19]. Activation of defence responses can be achieved by treatment with biotic agents[20], avirulent forms of pathogens, incompatible strains, in certain circumstances by virulent forms of pathogens, essential oils[17], plant extracts, fungi[21], bacteria[18,22], viruses[23], abiotic agents[24,25] and others[26].

Considering the importance and current relevance of the subject, the aim of the research was to evaluate four biostimulants in the induction of systemic resistance in cucumber (*Cucumis sativus* L.) and tomato (*Solanum lycopersicum* Mill.) in monoculture and associated greenhouse cultivation.

Materials and methods

Location. The research was carried out in a greenhouse in the Recinto Puerto la Boca belonging to the parish of Puerto Cayo in the canton of Jipijapa, which is located at latitude 1°18'20"S and longitude 80°45'42" W, at an altitude of approximately 53 m above sea level, its climate has a temperature of 24.8° C, the average annual rainfall is 298 mm, with the greatest amount of rainfall concentrated in the month of February, while the driest month is August[27].

Study factors. *Factor* A: Cropping system (A_1 monoculture of Seminis' Intimator cucumber hybrid, A_2 monoculture of Enza Zaden's Pawnee F1 tomato hybrid and A_3 cucumber+tomato combined crop). *Factor* B: Systemic Resistance inducing biostimulants (B_1 Fossil, B_2 Grandsil, B_3 L-amino, B_4 Control (water application only) and B_5 Bioremedy.

Experimental design. The treatments used in the research were distributed in a 3 x 5 factorial arrangement and analyzed in a Completely Randomized Experimental Design (CRED)[28].

Statistical analyses. In the agronomic evaluations, once the data satisfied the assumptions of normality and homogeneity of variance, the experiment was analyzed using the model of a completely randomized design[28].

Based on the defined model, analyses of variance (ANVA) were performed to test hypotheses about fixed effects, as well as comparisons of treatment means using Tukey's test Pr<0.05 probability. The ANVA was also used to estimate variance components for random effects. The above analyses were performed using INFOSTAT[28] statistical software.

A correlation analysis was carried out using the between the corresponding response variables. Pearson's correlation analysis[28] was used for the aforementioned procedures.

Response variables. Plant height AP (cm). Five plants taken at random were evaluated within each of the treatments. Stem diameter DT (mm). It was evaluated when the crop reached 50 % flowering. Number of fruits per plant (NFru). It was evaluated in all harvests. Fruit weight PFru (kg). It was evaluated in all harvests with a balance. Fruit volume VFru (cm^3). The Martel Moreno[29] formula was applied, which takes into account the length, width and height of the fruit.

Research management. Moist chambers were prepared with moistened paper towels, in which cucumber and tomato seeds were sown for germination. Four days after germination, the seedlings were transplanted into seedling trays with substrate prepared with biocompost, guava leaf and local soil in a 2:1:1 ratio. A 10 kg humus and a 10 g bag of mycorrhiza were added to prevent damping-off. The trays were irrigated twice a day to maintain humidity, and the fungicide carboxin + captan (vitavax) was applied at a rate of 3 g/L to prevent diseases[27].

Subsequently, the soil was prepared by hand, removed and broken up, then the planting beds were assembled and biocompost was added at a rate of 75 kg/33 m of row length. The beds were 0.80 m wide, 0.15 m high and 33 m long.

The definitive transplanting to the field was carried out on the assembled platforms, for this purpose holes were made with a stake at a depth of 0.15 m and a distance of 0.30 m between plants within the row, 50 g of earthworm humus was applied per hole, and the transplanting was carried out, pressing the soil well so that no air remained in the soil.

During growth at 10 ddt, preventive control for the oomycetes *Pseudoperonospora cubensis* and *Phytophtora infestans* was initiated for melon and tomato respectively[27], which recommends starting with an alternating application of a systemic fungicide based on metalaxyl and mancozeb (2.5 g/L), and a contact fungicide such as chlorothalonil (2.5 mL/L), every seven days and for six opportunities during the whole crop cycle. The systemic fungicide should not be applied more than three times to prevent the selection of resistant biotypes in the ommycetes mentioned.

For the control of insect pests[27], alternating application with a systemic insecticide Thiamethoxan+lamda cyhalothrin (0.25 mL/L) and a contact insecticide (imidacloprid 0.60 g/L) and/or Neen (organic) 4 mL/L (alternating application) instead of the contact insecticide. Do this process alternately for six weeks. This is an efficient strategy for the control of pest insects such as the blackfly (Prodiplosis longifilia), leafminer (*Liriomyza* spp.), aphid (*Myzus persicae*), moth (*Diaphania* spp.), thrips (*Frankliniella* sp.) and whitefly (*Bemisia* sp.).

An NPK soil fertilisation was applied at 30 ddt with Yaramira (2 g/plant) or solufol (100 g/20 L) every week for at least six times, and a foliar fertiliser with Chefare (25 mL/20 L), every week for at least six times.

Pruning was done on a main branch for both crops, old leaves and shoots were removed to avoid the formation of other secondary branches. Trellising was done after pruning, and then a contact fungicide (Mancozeb 0.47 g/L) was applied to avoid diseases in the wounds caused by pruning.

Irrigation of the plants was applied by drip irrigation, twice a day, for 15 to 20 min at each irrigation. Harvesting was carried out after 120 ddt for tomato and 60 ddt for cucumber.

Results

The analysis of normality for the variables evaluated by means of the Kolmogorov-Smirnov test at $P<0.05$ probability was not significant, which suggested that the variables had a normal distribution. Likewise, it was determined through the Chi-Square test at $P<0.05$ of probability that there were no significant differences between the variances, meaning that there was homogeneity of variances.

Analysis of variance. The ANVA for cultivation, with highly significant differences at P<0.01 of probability AP, TFru and DFru. Tomato in monoculture showed highly significant differences for PFru, TFru and DFru (Table 1). Likewise, the tomato in the combined system showed highly significant differences for all the variables evaluated. This would indicate that at least one of the treatments had a differential effect on the associated cucumber and tomato crops, and on the tomato monoculture. The coefficients of variation (CV) were between 2 and 33 %, which is the range allowed in this type of research (Table 1).

Table 1 Analysis of variance for biostimulant application

Var	Mean squares							
	Cucumber	CV	Associated cucumber	CV	Tomato	CV	Associated tomato	CV
AP	.01	13.14	<.0001	4.73	.06	10.76	<.0001	5.20
DT	.95	8.75	.37	14.81	.41	7.97	.00	9.06
PFru	.12	32.72	.66	31.15	<.0001	13.05	<.0001	6.93
TFru	.25	19.43	<.0001	6.07	<.0001	9.34	.00	3.20
DFru	.20	19.34	.01	6.89	<.0001	10.20	<.0001	2.63

AP Plant height, DT Stem diameter, PFr Fruit weight. TFr Fruit size, DFr Fruit diameter, CV Coefficient of variation

Analysis of means for monocultures. The analysis of comparison of means for cucumber monoculture using Tukey's multiple test at P<0.05 probability (Table 2), only with significant differences for AP, Bioremedy, Fossil, L-amino and the control stood out with respect to Grandsil. However, analysing the trend of the PFr averages, Grandsil and L-amino stood out with respect to the control, Fossil and Bioremedy, although no significant differences were observed.

Table 2 Comparison of means by Tukey's multiple probability test at P<0.05 of cucumber monoculture

Treat	Variables						
	AP (m)	DT (mm)	NFlo	PFru (Kg)	TFru (cm)	DFru (mm)	NFru
Fossil	161.57 ab	.98	4.29	.34	19.35	4.49	3.24
Grandsil	160.63 b	.98	4.60	.49	21.32	4.99	3.43
L-amino	174.00 ab	.99	4.60	.48	22.06	5.15	3.62
Bioremedy	178.83 ab	.98	4.76	.39	20.38	4.79	3.9
Testigo	180.33 a	.97	4.64	.42	21.27	4.97	3.52

AP Plant height, DT Stem diameter, NFlo Number of flowers, PFr Fruit weight. TFr Fruit size, DFr Diameter of fruit, NFr Number of fruits

Regarding the tomato monoculture, the analysis of mean comparison by Tukey's multiple test at P<0.05 probability (Table 3), with significant differences for NFlo, PFru, TFru, DFru and NFru. NFlo stood out for Bioremedy with respect to Fossil, with fewer flowers. PFru with Bioremedy stood out from the control and the other treatments. TFru with Bioremedy stood out, with the exception of the control, and NFru with Bioremedy stood out with respect to the control and the other treatments.

Analysis of means for associated crops. The analysis of comparison of means for cucumber companion planting using Tukey's multiple test at P<0.05 probability (Table 4), there were only significant differences for all the variables evaluated. In AP it was Bioremedy. L-amino and Fossil vs. the control and

Grandsil. For NFlo it was Bioremedy vs. the control. For PFru, Bioremedy stood out compared to the control. For TFru, L-amino, Bioremedy and the control stood out compared to Fossil. For DFru, L-amino stood out against Fossil and finally for NFru, Bioremedy stood out against the control and the other treatments.

Table 3 Comparison of means by Tukey's multiple probability test at P<0.05 for tomato monoculture

Treat	Response variables											
	AP (m)	DT (mm)	NFlo		PFr (Kg)		TFru (cm)		DFru (mm)		NFru	
Fossil	111.26	.99	5.62	c	.139	b	4.54	b	5.8	b	9.00	b
Grandsil	107.97	.98	6.76	abc	.132	b	4.6	b	5.31	b	9.62	b
L-amino	114.71	.99	7.50	ab	.140	b	4.93	b	5.8	b	8.90	b
Bioremedy	117.68	.98	7.86	a	.161	a	5.96	a	6.39	a	11.19	a
Testigo	116.87	.95	6.36	bc	.132	b	5.84	a	5.77	b	9.90	ab

AP Plant height, DT Stem diameter, NFlo Number of flowers, PFr Fruit weight. TFr Fruit size, DFr Diameter of fruit, NFr Number of fruits

Table 4 Comparison of means by tukey's multiple t-test at P<0.05 probability of the combined crop of cucumber

Treat	Response variables										
	AP (m)	DT (mm)	NFlo		PFru (kg)		TFru (cm)		DFru		NFru	
Fossil	130.04 a	.83	5.11	bc	.51	b	21.82	c	5.19	b	11.36	b
Grandsil	118.37 b	.78	5.20	ab	.41	b	22.64	bc	5.29	ab	11.00	b
L-amino	126.09 a	.82	5.34	ab	.57	b	23.87	a	5.57	a	12.48	ab
Bioremedy	129.47 a	.85	5.56	a	.57	a	23.69	ab	5.50	ab	14.32	a
Testigo	113.02 c	.80	4.67	c	.57	b	23.47	ab	5.49	ab	10.77	b

AP Plant height, DT Stem diameter, NFlo Number of flowers, PFr Fruit weight. TFr Fruit size, DFr Diameter of fruit, NFr Number of fruits

Table 5 Comparison of means by tukey's multiple t-test at P<0.05 probability of tomato combined crop

Treat	Response variables												
	AP (m)		DT (mm)		NFlo	PFru (kg)		TFru (cm)		DFru		NFru	
Fossil	128.21	b	.76	ab	5.5	.12	b	5.7	ab	6.04	b	25.3	b
Grandsil	130.73	b	.69	bc	5.6	.12	b	5.6	b	5.94	b	24.2	b
L-amino	130.61	b	.68	c	5.5	.13	b	5.6	b	5.57	c	25.9	ab
Bioremedy	152.58	a	.79	a	5.6	.17	a	5.9	a	6.71	a	28.2	a
Testigo	113.51	c	.71	bc	5.5	.10	c	5.5	b	5.69	c	23.2	b

AP Plant height, DT Stem diameter, NFlo Number of flowers, PFr Fruit weight. TFr Fruit size, DFr Diameter of fruit, NFr Number of fruits

The analysis of comparison of means for the associated tomato crop using Tukey's multiple test at P<0.05 probability (Table 5), with significant differences for all the variables evaluated. In AP with Bioremedy compared to the control. For DT it was distinguished with Bioremedy versus the control. For DT it stood out with Bioremedy versus L-amino. For PFru it stood out with Bioremedy versus the control. For TFru it stood out with Bioremedy versus the other treatments. For DFru it excelled with Bioremedy with respect to the control and the other treatments and finally for NFru it was observed that it excelled with Bioremedy versus the control and the other treatments.

Discussion

The morphological and agronomic behaviour of two taxonomically different crops, one belonging to the Solanaceae, tomato (*S. lycopersicum*) and the other to the Cucurbitaceae, cucumber (*C. sativus*), was evaluated in this study. Various experiences have shown that the associated cultivation with different species brings substantial benefits[30,31], in principle they do not share the same types of pests and diseases as when they are cultivated in monoculture. In addition, four types of organic biostimulants were used to observe their effect in monoculture as well as in companion planting. Our experience shows evidence of a differentiated behaviour, indicating in general that the biostimulants gave a better response in companion planting than in monoculture.

It is important to note that the inoculation of crops with biostimulants such as PGRP substantially reduces the use of synthetic fertilisers and the negative impact on the soil, increasing crop yields and contributing to the producer's economy and the population's food supply[32,33]. The interactions of PGRP with the biotic environment-plants and micro-organisms are very complex and use different mechanisms of action to promote plant growth[34].

Biostimulants have an effect on the SRI of cucumber and tomato crops, so they are involved in the plant's defence mechanisms and meet the requirements for safe application in greenhouse and field conditions, do not cause toxicity to plants, have no negative effects on plant growth, encourage plant development, improve yield, are used in low concentrations, induce a broad spectrum of defences, produce a long-lasting protective effect and are inexpensive[35].

Peterira et al.[35] observed the effect of phytomes on the induction of different enzyme systems related to defence mechanisms in rice plants infested with *Steneotarsonemus spinki* and compared it with the effect caused by BION under identical conditions. His results showed that the application of phytomes was as effective in reducing mite populations as the application of BION and that it caused the activation of enzymes such as peroxidases, polyphenol oxidases, phenylalanyl ammonium lyases and chitinases.

Ribaut & Poland[34], mention that biostimulants applied to plants develop complex and varied defence mechanisms. These can be constitutive or inducible. Inducible ones can systemically activate distant cells and tissues, and the plant acquires a physiological immunity. In this sense, the induction of SAR and with it, of a set of proteins and defence compounds that include enzymes involved in the phenylpropanoid synthesis pathway, Phenylalanine ammonium lyase (PAL), Chalcone synthase (CHS), Peroxidases (PO), among others), glycoproteins rich in hydroxyproline (Hyp), related to cell wall reinforcement, and glycanases and chitinases that hydrolyse fungal cell walls, among others.

Burbano-Figueroa[11] mentions that the contaminating effect and the possibility of pathogens developing resistance to chemical pesticides have led to their disuse in recent years. The development of varieties resistant to Fusarium rot has also been tested with less promising results in commercial tomato production36. In this sense, studies have been directed towards the use of biological control strategies for the management of tomato spot caused by Fusarium using biocontrol agents such as Pseudomonas fluorescens[16], *Trichoderma harzianum* and *Glomus intraradices*, which are arbuscular mycorrhizal fungi, which apart from the direct effect against the pathogen, have shown a systemic induction effect of plant resistance. Several species of *Trichoderma* and isolates of *Pseudomonas* have been found to be effective in controlling fusarium in tomato[16,36].

In our experiment we were able to determine that Bioremedy proved to be an alternative for use in associated cultivation, although it is also recommendable in monoculture. Bioremedy, a biostimulant based on humic acids, maltodextrin, sucrose, algae extract and total amino acids, encourages the development of rhizobacteria that promote plant growth, free-living nitrogen-fixing bacteria, bacteria that promote extracellular enzymes, and fungi that promote the decomposition, transformation and cycling of soil nutrients[37].

It should be noted that biostimulants such as L-amino and Grandsil are recommended for use, because Grandsil is a biocatalyst that stimulates the respiration function, enduces the stem, leaves and roots which makes them resistant and stimulates the defence mechanisms against fungi[16]. L-amino is an amino acid complex that is used as a foliar biostimulator and can increase the plant's resistance to adverse conditions[38] and finally Grandsil contains silicon, potassium and monosilicic acid and acts as an agricultural enhancer, increasing electrical conductivity, regenerating a higher cation exchange capacity, incorporating insoluble minerals, stimulating microbial activity in the soil, improving soil structure and thus water management, as a consequence plants will have access to more nutrients, better resist stress and significantly increase yields[16].

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Conflicts of interest

The authors declare that this research was carried out at the Universidad Estatal del Sur de Manabí (Cantón Jipijapa) and presents no conflicts of interest.

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Ethical considerations

The research was approved by the Directorate of Research and Postgraduate Studies, the Ethics Committee and the Research Committee of the Agricultural and Livestock Department of the Universidad Estatal del Sur de Manabí (UNESUM), (Cantón Jipijapa), following the guidelines established by these bodies.

Research limitations

The authors note that there were no limitations in the present research work.

Authors' contribution to the article

Julio Gabriel-Ortega, experiment planning, statistical analysis, systematisation and interpretation of data. *Pablo Chonillo Pionce*, data collection, statistical analysis, systematisation and interpretation of the information. *Washington Narváez Campana*, systematisation and interpretation of information, revision of the document. *Tomas Fuentes Figueroa*, systematisation and interpretation of the information, revision of the document. *Fernando Ayón Villao*, systematisation and interpretation of the information, revision of the document.

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