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INDUCED SYSTEMIC RESISTANCE OF OKRA (*ABELMOSCHUS ESCULENTUS* L. *MOENCH*) AGAINST *OKRA YELLOW VEIN MOSAIC VIRUS* USING AMINO ACIDS AND ALGAE EXTRACTS

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A B S T R A C T

Abelmoschus esculentus L. Moench, more often known as okra, is a popular and widely consumed vegetable that belongs to the Malvaceae family. It is rich in a variety of essential nutrients, including carbohydrates, proteins, and vitamins. The okra yellow vein mosaic disease (OYVMD) is one of numerous biotic and abiotic variables that pose a danger to okra productivity. In order to investigate the impact of amino acid treatments and algal extracts on inducing systemic resistance in okra against the Okra yellow vein mosaic virus, an experiment was carried out in the fields in the western areas of Samawa City-Muthanna Governorate, Iraq (OYVMV). Research on the impact of the virus found that amino acid and algal extract treatments were most effective, leading to the fewest number of infected okra plants. Peroxidase enzyme, superoxide dismutase, and catalase levels were highest in those who received a combination of amino acids and algal extract. The duration of 14 days was also longer than the spans of 7 and 21 days. While amino acid and algal extract therapy for 21 days had the highest total phenolic concentration, it also had the most negative effects.

Keywords: *Okra yellow vein mosaic virus*, Algal extract, Amino acids, DAS-ELISA**.**

INTRODUCTION

Okra (*Abelmoschus esculentus* L. *Moench*) is one of the most important and well-liked vegetables in the world and is a member of the *Malvaceae* family (Mubeen *et al*., 2021). One of the important vegetable crops grown in Iraq is okra (Al-Shammary, 2018). The production has substantially decreased over the past few years as a result of a variety of problems, including biotic pressures from various insects and viral infections (Fekrat and Shishehbor, 2007; Hussain *et al.,* 2011, 2012, 2014, 2016; Hussain and Mukhtar, 2019; Kassi *et al.,* 2018, 2019; Mukhtar and Hussain, 2019; Mukhtar *et al.,* 2013a b, 2014, 2017). The whitefly *Bemisia tabaci* (Genn.), which feeds on okra, is responsible for the propagation of Phyto-viruses such okra yellow vein mosaic virus and okra leaf curl. Significant crop losses due to whitefly infestations and viral transmission affect the okra industry. The family Geminiviridae includes the genus Begomovirus, which includes the Okra Yellow Vein Mosaic Virus (OYVMV) (Mubeen *et al.,* 2021). There is a sizable viral family called OYVMV that is responsible for widespread crop failures around the world. Caused by a virus, symptoms include chlorosis, stunting, chlorosis of different degrees, and yellowing of veins and veinlets (Venkataravanappa *et al*., 2012). However, the OYVMV has a detrimental effect on okra plants, the virus is widespread throughout the world's okra-growing regions, and the virus infects okra plants at every stage of development and is to blame for 80–90% of yield losses (Mubeen *et al.,* 2017; Mohanta *et al.*, 2020). Since there is currently no viricide, viral infections are only indirectly treated by using insecticides against their vectors, the widespread use of insecticides puts the environment's safety at risk by contaminating the air, water, and soil (Ali *et al.,* 2014). It can be tricky to detect a virus through the morphological symptoms of the host plant, as the symptoms of each viral strain differ from host to host, and as the symptoms of a plant virus

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infection can manifest in a wide range of ways depending on the plant (Lacroix *et al*., 2016). Thus, bioassays based on polymerase chain reaction (PCR) and enzyme-linked immune-sorbent assays (ELISA) have greatly improved virus detection (Diaz-Lara *et al*., 2020). The creation and application of new alternative inputs for agricultural production are in high demand. Researchers have recently been investigating the use of organic biostimulants to improve plant growth and defensive mechanisms (yakhin *et al.,* 2017). Seaweed extracts, which have been demonstrated to exhibit both phytostimulatory and phytoelicitor qualities, are a prospective source of biostimulants (Ramkissoon *et al.,* 2017; Rayorath *et al.,* 2008). Although extracts from a number of marine algae species have been shown to have potential as plant biostimulants, *Ascophyllum nodosum*, brown seaweed, is the most significant species used internationally for commercial extraction (Ali *et al.,* 2016; Jayaraj *et al.*, 2008). Inducible defense systems allow plants to fend off a wide range of pathogens, but they are only activated in response to specific signaling molecules released by pathogens or by plants subjected to environmental challenges. Seaweed extracts containing elicitor molecules can boost several inducible defensive reactions in plants. Induced resistance occurs in plants after a sequence of events in which the elicitor attaches to specific receptor sites on the membrane. After binding, secondary chemical messengers further amplify a signal, which triggers subsequent defensive mechanisms (Jayaraj *et al.,* 2008). This starts a series of chemical processes in the plant that help it become more resistant to pathogen invasion. The plant can defend itself from a wide range of pathogens and pests, including as bacteria, fungus, parasites, viruses, nematodes and insects, thanks to this broad-spectrum resistance (Jayaraj *et al.,* 2008). Induced resistance is the outcome of chemical stimulation, which triggers a cascade of events including the phenylpropanoid pathway, the assembly of defense signaling molecules, and the accumulation of antimicrobials including pathogenesis-related (PR) proteins and phytoalexins (Ramkissoon *et al*., 2017).

As for amino acids, In order to improve growth and development, they work by enhancing the inherent tolerance and resistance of plants to stress and by stimulating genetic potential (Kumar *et al.,* 2020). As a result, biostimulants can increase the effectiveness of conventional fertilizers and can replace synthetic chemicals that are used to protect plants. The European Biostimulants Industry Council estimates that biostimulants are used on more than four million hectares of land in Europe, however other sources place the number at more than six million hectares (EBIC Biostimulants, 2003; Calvo *et al.,* 2014). Infection problem was reduced after amino acid spraying was implemented, with reports of a 16% decrease in Fusarium patch (*Microdochium nivale*) infection and a 20% decrease in Dreschlera leaf spot (*Drechslera siccans*) (Radkowski *et al*., 2020). Biostimulant treatments had varying effects on all test parameters, but they were most effective in reducing the imbalance caused by PepMV infection, which was practically halved in the two virus-resistant pepper cultivars (Betti *et al*., 1992).

MATERIALS AND METHODS

Experiment layout: The effectiveness of inducing resistance in okra to the okra yellow vein mosaic virus was assessed in an experiment (OYVMV). We used the Local-Hussainawya type of okra, and we planted the seeds in 8–10 cm deep holes in plastic dishes with a mixture of one part peat moss to two parts sand.

Spraying the plants with an algae extract (Quelafert quelgreen) and amino acid (Aminoprim) solution at a rate of 0.5 milliliters per liter of water was done after the plants had grown to the size of three complete leaves. The whitefly was then sprayed with Mospilan (acetamiprid 20%) after being artificially inoculated with the virus by pre-feeding on an infected plant. The plants had their soil replaced with plastic pots that were 22 cm by 24 cm, and they were placed in a tiny area (hidden by a boring cloth tent) to prevent pests from getting to them. Every day and in accordance with the plants' requirements, the pots are watered, following that, tests were taken 7, 14 and 21 days after the virus inoculation. Young symptomatic leaves were gathered and examined by DAS-ELISA to confirm that the virus was the source of the symptoms (Clark and Adams, 1977). These were the treatments.

Table 1. Treatments Applied and details.

Treatment	Detail
T0	Healthy control
T1	Inoculated control
T2	OYVMV + Amino acids
T3	OYVMV + Algal extract
T4	OYVMV + Amino acids + Algal extract

Screening for OYVMV under natural conditions: The plants were grown in an open field where they could be exposed to the virus freely, and the severity of the disease was measured using a standardized scoring system (Wasala *et al*., 2019). Because of this, the following grades have been given out (Table 2).

Table 2. OYVMV disease symptoms are described for the score.

Symptoms description	Severity scale	Severity range (%)
Absence of symptoms	0	0
Very mild symptoms	1	1-20
Leaf veins turn yellow, but interveinal areas remain green or normal.	2	20-40
Curly leaves and the entire leaf become yellow.	3	40-60
The entire leaf turns yellow. The margin begins to dry.	4	60-80
Yellowish, malformed pods with completely yellow leaves.	5	80-100

Assays of antioxidant enzymes: Enzyme Extraction: According to the procedure listed below, the POD, SOD and CAT activities in okra leaves were measured. With the aid of a pestle and mortar, three 0.2 g FW leaf samples were collected, using the aid of a pestle and mortar, and ground in liquid nitrogen. After adding and homogenizing 2ml of 50mm ice-cold phosphate buffer (pH 7.7) and 1mM ethylene diamine teraacetic acid (EDTA) was added. Centrifuged for 15 minutes at 4°C at 1500 rpm. The enzyme extract was made from the supernatant.

Assay for peroxidase (POD) (U/mg): For the assay of the peroxidase enzyme, the method described by Pitotti *et al*. (1994).

Assay for superoxide dismutase (SOD) (U/mg): For the assay of the superoxide dismutase enzyme, the method described by Magnani *et al.* (2000).

Assay for catalase (CAT) (U/mg): For the assay of the catalase enzyme, the method described by Hadwan and kadhum (2018).

Assay for Total phenolic (µg/ml): For the assay of the Total phenolic, the method described by Cl and Indira (2016).

STATISTICAL ANALYSIS

The experiment was designed to be carried out using a randomized complete block design (RCBD). The results were put through a series of statistical analyses using GenStat version 18. (LSD, 0.05).

RESULTS

Disease Severity of OYVMV: Disease Severity was measured and the results were displayed graphically in Figure 1. The inoculation plants (control) comparison plants gave the highest infection severity by 41%, while the treatment (OYVMV + amino acids + algae extract) had the lowest disease severity with the virus, amounting to 13%. Disease Severity was at (26% and 18%, respectively) for the two treatments (OYVMV + algal extract and OYVMV + amino acids).

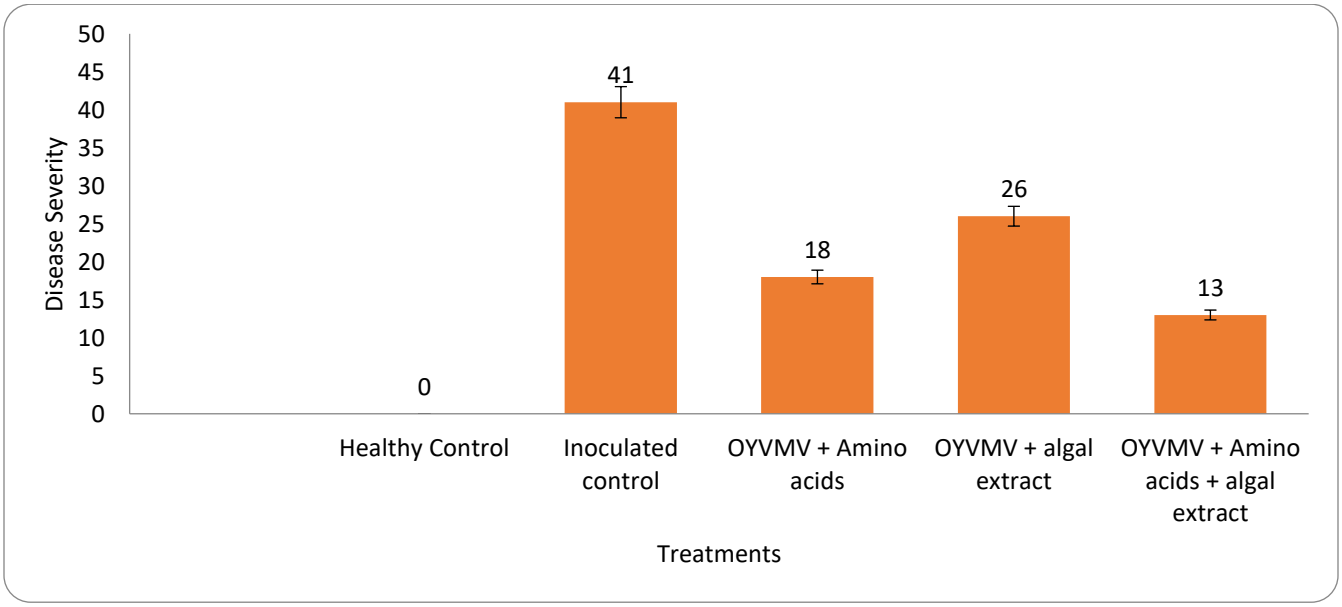
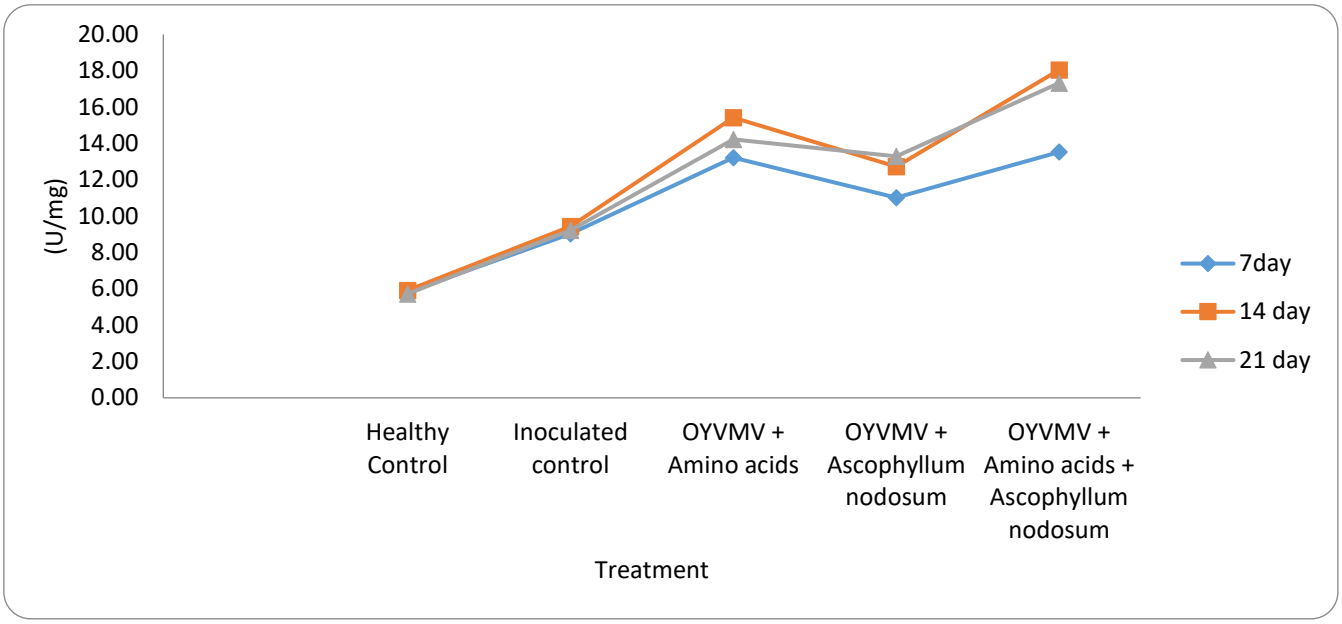


Figure 1. Disease Severity of OYVMV in a Glass House Regarding Treatments

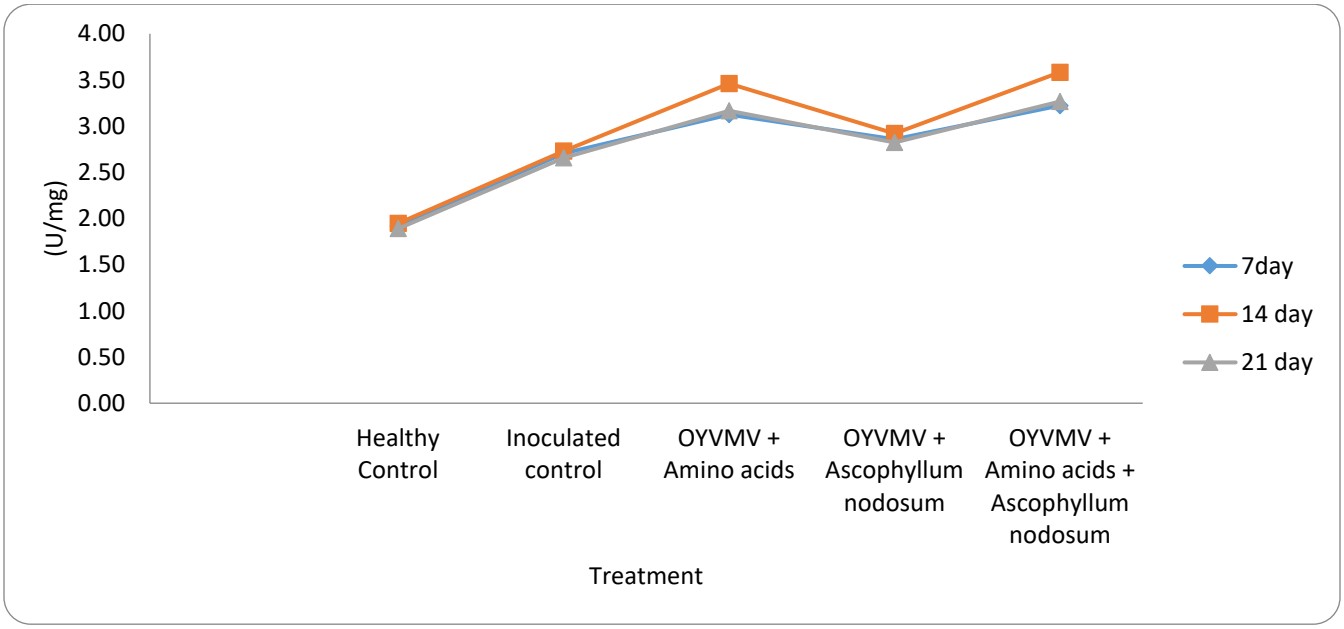
Peroxidase (POD) (U/mg): It also showed the peroxidase enzyme (POD) concentration in okra leaves (Figure 2), When compared to the other treatments, the treatment (OYVMV + Amino acids + algal extract) had the greatest concentration of the enzyme after 14 and 21 days (18.05 and 17.32 U/mg). Then followed the (OYVMV + Amino Acids) treatment, which resulted in the lowest concentration of the enzyme after 21 days of all treatments.



LSD $_{(0.05)}$ Treat. = 0.023, Times= 0.018 and for Intersection= 0.04

Figure 2. Okra leaf content of peroxidase (POD) (U/mg)

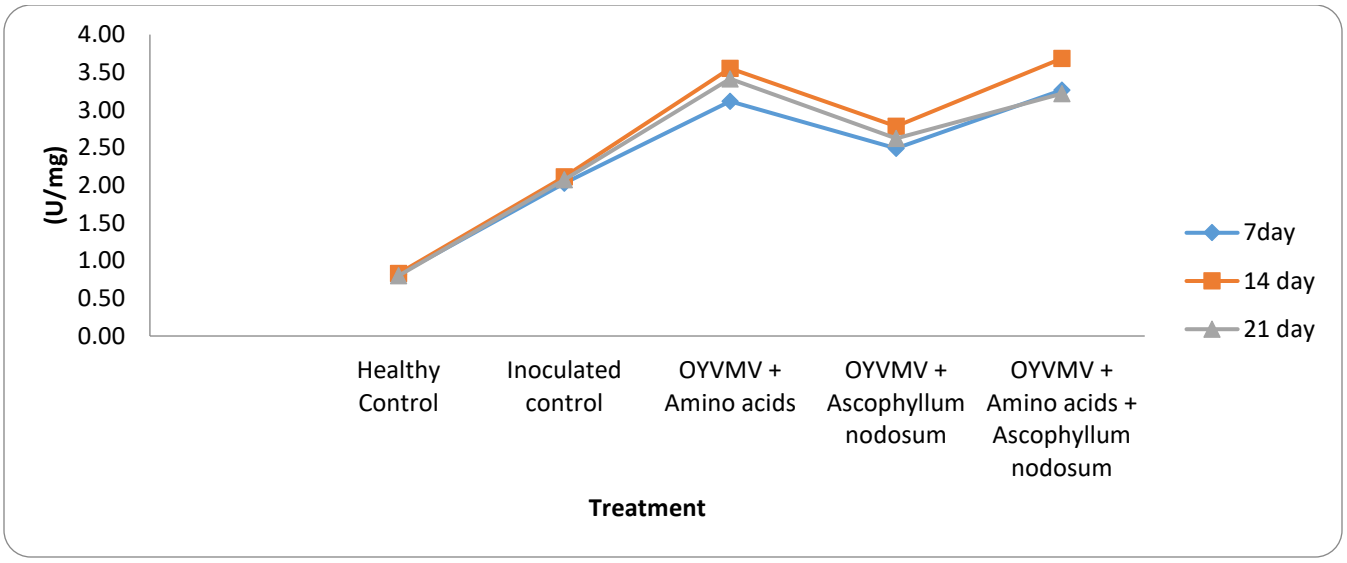
Superoxide dismutase (SOD) (U/mg): During the 14 days, the highest concentration of the enzyme superoxide dismutase (SOD) was also given to the treatment (OYVMV + Amino acids + algal extract) (3.58 U/mg), there was no significant variation in enzyme concentration between 7 and 21 days (3.22 and 3.27 U/mg) (Figure 3).



LSD $_{(0.05)}$ Treat. = 0.015, Times= 0.012 and for Intersection= 0.026

Figure 3. Okra leaf content of superoxide dismutase (SOD) (U/mg)

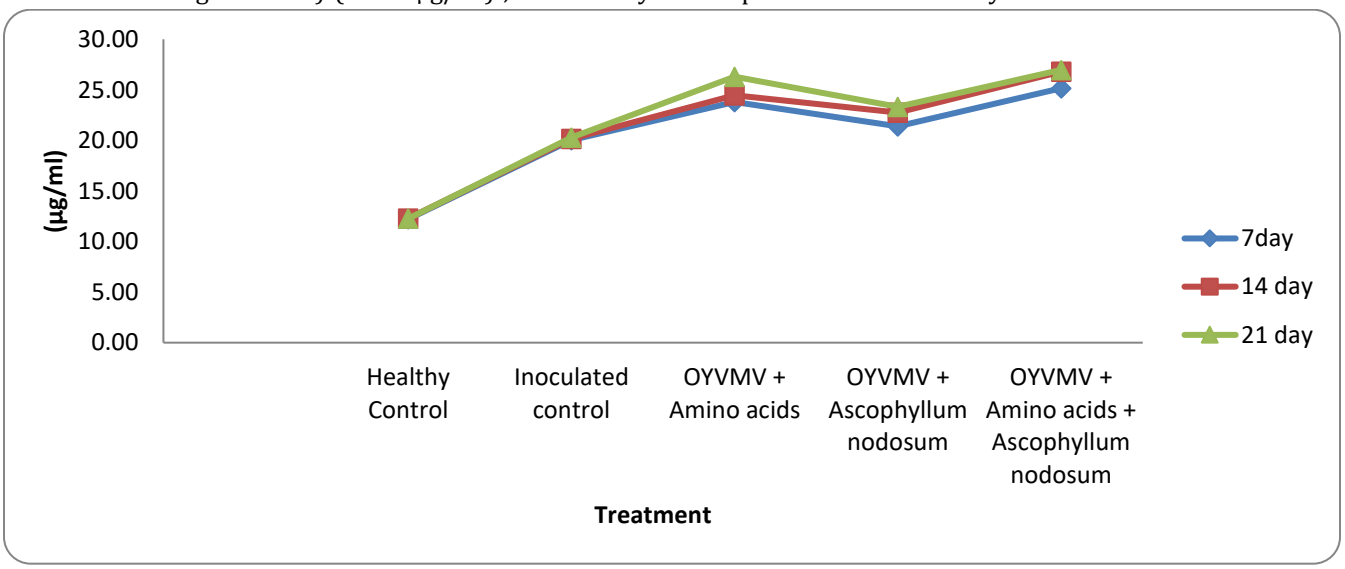
Catalase (CAT) (U/mg): Figure 4 shows that after 14 days, there was no statistically significant difference between the OYVMV + Amino acids + algal extract and OYVMV + Amino acids treatments in terms of enzyme concentration (3.68 and 3.55 U/mg). However, there was little change in enzyme content between 7 and 21 days in the leaves.



LSD $_{(0.05)}$ Treat. = 0.148, Times= 0.115 and for Intersection= 0.257

Figure 4. Okra leaf content of catalase (CAT) (U/mg)

Total phenolic (µg/ml): Regarding the concentration of total phenolic in okra leaves (Figure 5), The highest concentration after 21 days of treatment was (OYVMV + Amino acids + algal extract) (26.97 µg/ml) , followed by the enzyme concentration for the treatment (OYVMV + Amino acids) (26.29 µg/ml). There was no significant difference between the concentration of total phenols for the periods of 7 and 14 days for most of the treatments.



LSD $_{(0.05)}$ Treat. = 0.72, Times= 0.557 and for Intersection= 1.246

Figure 5. Okra leaf content of Total phenolic (µg/ml)

DISCUSSION

The severity of symptoms caused by Okra yellow vein mosaic virus (OYVMV) varies from plant to plant, season to season, whitefly population to whitefly population, and nutritional status of the plant. With no effective viricide against plant viruses, researchers have focused on developing resistant plant varieties through selective breeding and the introduction of resistance genes, or on controlling insect vectors through the use of pesticides (Lacroix *et al*., 2016).

Pesticides are harmful to the environment, so scientists are increasingly looking to chemical and natural materials, such as amino acids (Betti *et al*., 1992; Jayaraman *et al*., 2011) to induce systemic resistance in plants. It was determined that a commercially available *Ascophyllum nodosum* alkaline extract effectively stimulated plant growth and elicited a plant's defense mechanism against foliar diseases caused by

Xanthomonas campestris pv tomato and sweet pepper. The highest levels of illness were reduced by 60%, and the highest yield was increased by 57% (Ali *et al*., 2019). Applications of *A. nodosum* extracts to the leaves and roots of carrot, cucumber, and tomato plants significantly reduced the incidence of leaf and soil-borne diseases (Jayaraj *et al*., 2011).

In our study, we utilized (amino acids and algal extracts) obtained in local markets to see how they affected the virus-infected okra plant. The findings revealed that all of these treatments decreased the virus's effects on the plant. And it gave the highest level of peroxidase enzyme, superoxide dismutase, and catalase, especially for the period after 14 days, while phenols gave the highest level during the period after 21 days.

CONCLUSIONS

Coefficients were used in this experiment (Healthy Control, Inoculated control, OYVMV + Amino acids, OYVMV + algal extract and OYVMV + Amino acids + algal extract). The treatment (OYVMV + Amino acids + algal extract) gave the highest results compared to the rest of the treatments, which also gave good results. The 14-day duration performed the best for peroxidase, superoxide dismutase, and catalase. Except for total phenolic, period 21, was the best.

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Malik H. Karem	:	Designed the layout and performed experiments and wrote the manuscript.
Ali A. A. Haidery	:	Editing of manuscript