



# Effect of Poultry Manure (litter) and Seagrass (Posidonia oceanic) Extract on Enhancing Pepper Plant Resistance to Cucumber Mosaic Virus (CMV)

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## A B S T R A C T

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This study was conducted in the area of 27 East Sirte in 2022 to investigate the impact of different levels of poultry manure (poultry litter) extract (50 ml, 100 ml, 150 ml per irrigation process) and levels of Posidonia oceanic seagrass extract (50 ml, 100 ml, 150 ml) on enhancing resistance of pepper plants grown in pots to Cucumber Mosaic Virus (CMV). The results indicated a decrease in the severity of infection compared to the control. The infection rate in the control sample was 55%, while the lowest infection rate was observed with the treatment of 150 ml of poultry manure, showing a 13% infection rate. Seagrass extract treatments also demonstrated a significant impact on infection severity, with a decrease of 47%, 40%, and 36% for 50 ml, 100 ml, and 150 ml of seagrass extract, respectively. Overall, the addition of organic fertilizer to the soil led to improved resistance against CMV infection and stimulated growth in pepper plants. Application of organic fertilizer in agricultural soil is considered a practical method to mitigate the impact of climate change, improve plant productivity, and enhance disease tolerance for sustainable agriculture

## 1 Introduction

Crop production has declined by 36% worldwide due to plant diseases, weeds, and insects, with diseases alone causing a 14% decrease in crop yield (Agrios, 2005). While chemical substances against these diseases have mostly shown good results, their misuse has raised concerns due to damaging the soil's natural fertility, killing beneficial soil organisms, reducing crop natural resistance, and causing environmental pollution (Adhikary, 2012; Keswani et al., 2014; Bisen et al., 2015). In this context, Pottorff (2004) proposed using organic fertilizers as a means of cultural control to manage viral diseases in most horticultural crops. Organic fertilizers encourage beneficial microorganisms

to live symbiotically with plants, improving their fertility and disease resistance. Organic soil relies on this cycle to produce organic solutions aimed at being preventive by improving the garden's natural balance to build healthy soil (Robert et al., 2001). Organic fertilizers act as a slow-release source of essential nutrients and micronutrients crucial for plant health, avoiding high nitrogen fertilizers resulting from non-organic fertilizers, such as NPK, which can make plants highly susceptible to viral diseases (Pottorff, 2004). Vermicompost tea, known as organic biofertilizers, contains microbes, nutrients, and plant growth stimulants, showing improvements in seed germination, growth, increased yield, and suppression of plant diseases (Khattiyaphutthimet et al., 2020; Arancon et al.,

2020). Several researchers have studied biological control factors using Plant Growth Promoting Rhizobacteria (PGPR) with various pathogens in several crops such as tomatoes, cucumbers, peppers, wheat, etc. These bacteria enhance plant growth by stimulating both quantitative and qualitative aspects, aiding plant nutrient uptake from the soil (Saharan & Nehra, Singh, 2013). These bacteria, either free-living or root-associated, reduce plant disease severity and increase plant growth. They can trigger self-defense mechanisms in plants known as Induced Systemic Resistance (ISR) against a wide range of diseases including viruses, fungi, nematodes, and insects. Viral diseases and their control remain a significant goal for plant virologists. Due to the nature of viruses and their complete association with infected plant cells, there is no direct method for their control. Therefore, combating any viral disease is expected to involve an integrated approach relying on preventive measures and protection (Hadidi et al., 1998). Considering the multiple means of virus transmission and its survival during inter-seasonal periods, the appropriate control methods also vary. Some control methods that might not entirely eliminate the disease can still reduce losses (Albrechtsen, 1997). Peppers are among the high nutritional value crops grown in many countries worldwide, used as a vegetable crop, medicinal plant, or ornamental plant (Govindarajan & Salzer, 1985). Pepper crops, like other Solanaceae crops, are susceptible to various viral diseases, with Cucumber Mosaic Virus (CMV) being one of the most important. CMV infects several crops including cucumber, pepper, squash, and tomato (Chabbouh & Cherif, 1990). The economic loss caused by CMV infection depends on the strain, variety, timing of infection, and environmental conditions (Sutic et al., 1999). Infected plants may display leaf, flower, and fruit deformities (Laemmlen, 2004). Some strains of CMV cause abnormal elongation of the flower cup, and sensitive varieties may show partial yellowing along stems and branches (Zitter & Floroni, 1984). To protect pepper plants from various viral diseases, different control methods have been used, including chemical control methods against virus vectors, different agricultural practices, and the application of biological control agents (Chung et al., 2006; Lee et al., 2005). This study aimed to assess the efficacy and impact of fermented poultry manure and seagrass as a source of organic nutrients in reducing the occurrence and severity of CMV and its impact on cultivated pepper plants.

## 2 Materials and Methods.

This study was conducted to determine the effect of adding fermented chicken litter and fermented seagrass on the vegetative measurements of pepper plants and the severity of Cucumber Mosaic Virus (CMV) infection. The experiment took place in the 27th area east of Sirte, approximately 27 km north of the Great Man-Made River reservoir.

### Materials Used:

**Pepper plants:** Seedlings were obtained from a nursery in Sirte, using local varieties.

**Poultry litter:** Sourced from a poultry farmer in the 27th area east of Sirte.

**Sea grass:** Extracted from the shores of Sirte, washed with distilled water, dried, and ground at the Faculty of Agriculture, Sirte University.

The acidity (pH) and electrical conductivity (EC) of both poultry litter and sea grass were measured (Table 1).

Table 1: pH and EC Values for Poultry Manure and seagrass

poultry manure	7.9 pH	6.1 EC
seagrass	8.9 pH	8.5 EC

### Soil Used

The agricultural soil used in the experiment was obtained from the 27th area east of Sirte. Various chemical and physical properties were measured before planting (Table 2).

Table 2: Chemical and Physical Properties of Soil before Planting

properties	Value
Mechanical analysis	Sand Loamy
phosphorous is a facilitator	1.26 mg/kg
Potassium is a facilitator	90.2 mg/kg
pH	8.16
EC	1.2

### Fermentation Process

Two kilograms of poultry litter were fermented in 10 liters of irrigation water for 20 days with agitation every 3 days. Similarly, 500 grams of seagrass were fermented in a closed container with 2.5 liters of water and 20 grams of poultry litter to aid decomposition. This fermentation process also lasted for 20 days.

### Preparation of Application Levels

Application levels of 50 ml, 100 ml, and 150 ml were prepared for both fermented poultry litter and sea grass.

### Planting

Planting took place on 4/9/2022, with 5 kg of soil placed in each container and two seedlings planted to ensure growth.

### Treatments

Control group: Three containers received only regular irrigation water.

Different treatment groups received 50 ml, 100 ml, and 150 ml of either seagrass or fermented poultry litter along with irrigation water.

### Irrigation

Plants were watered every 4 days or as required. In the last 40 days of plant growth, treatments were applied with irrigation water.

### Thinning Process

After 10 days of planting, weaker plants were removed from the containers.

### Virus Isolation

Virus isolation was conducted at the Faculty of Agriculture's plant production laboratory. Infected plant material was ground, and the extract was separated using a centrifuge (Figure 1A).

### Injection Process

On 31/10/2022, leaf wounds were made using a scalpel, and the extracted virus solution was applied to the wounds (Figure 1B).

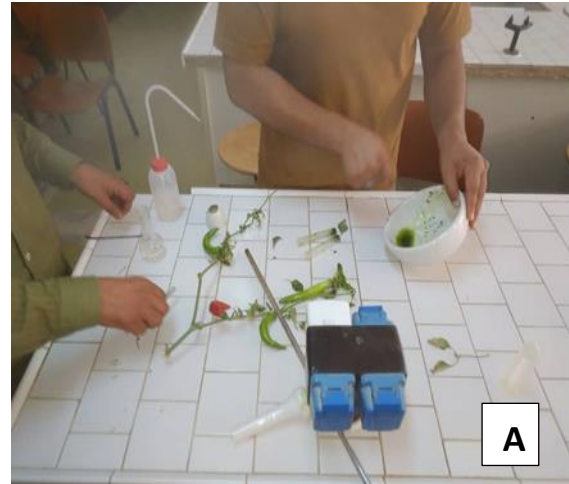


Figure 1 shows the virus isolation process from an infected sample (A) and the virus injection into a healthy plant (B).

### Plant Characteristics Studied:

- Plant height and fruit length: Measured using a measuring ruler.
- Number of leaves.
- Fresh fruit weight: Weighed using a digital scale with a sensitivity of 0.1 gram.
- Infection severity: Calculated as a percentage by counting infected leaves out of the total leaf count on the plant.

### Statistical Analysis:

Statistical analysis of the obtained data was conducted using Analysis of Variance (ANOVA) via the SPSS program. Mean comparisons were performed using the Least Significant Difference (LSD) test at a probability level of  $\geq 5\%$ .

### 3 Results

#### Effect of Treatments on Infection Severity and Vegetative Measurements of Pepper Plants:

The statistical analysis results presented in Table (3) indicate the impact of treatments on infection severity concerning fruit length, number of leaves, fruit weight, and plant height.

Table (3): Effect of Treatments on Infection Severity in Relation to Fruit Length, Number of Leaves, Fruit Weight, and Plant Height

Treatment	Fruit Length (cm)	Number of Leaves	Plant Length (cm)	Fruit Weight (g)	Infection Severity %
Control	4	16	21	4	55
seagrass 50 ml	5	19	18	4	46
seagrass 100 ml	7	18	21	6	47
seagrass 150 ml	7	18	19	6	40
Poultry litter 50 ml	8	22	18	7	36
Poultry litter 100 ml	9	21	15	8	23
Poultry litter 150 ml	9	33	19	11	13
Average	7.12	21	18.67	6.40	37.29
Moral level	<.0001	0.0059	----	0.0008	<.0001
The least significant difference is LSD	1.27	8	----	2.60	10.24

Regression analysis (significance level) showed values (P) for fruit length (0.0001 = P, R-Square = 0.90), explaining 90% of infection severity variance. This was followed by fruit weight (0.0008 = P, R-Square = 0.82), explaining 82%, and then the number of leaves (0.005 = P, R-Square = 0.77), explaining 77% of infection severity variance. No significant value was found for plant height. When considering the unified model for infection severity and vegetative measurements of pepper plants, the significant value (0.0001 = P, R-Square = 0.907298) indicates a high positive impact of treatments on pepper plants, explaining 91% of the significant variance in infection severity.

#### Effect of Treatments on Fruit Length of Pepper Plants:

The impact of different ratios of seagrass and poultry litter additives on fruit length is shown in Figure (1). There is minimal impact at the 50 ml ratio of seagrass, whereas at the 100 ml and 150 ml ratios, there is a higher impact on infection severity compared to the 50 ml ratio.



Figure 1 demonstrates the effect of treatments on fruit length (cm).

#### Effect of Treatments on Number of Leaves of Pepper Plants:

Figure (2) illustrates the comparison of seagrass and poultry litter at different ratios with the control to measure infection severity based on the number of leaves.

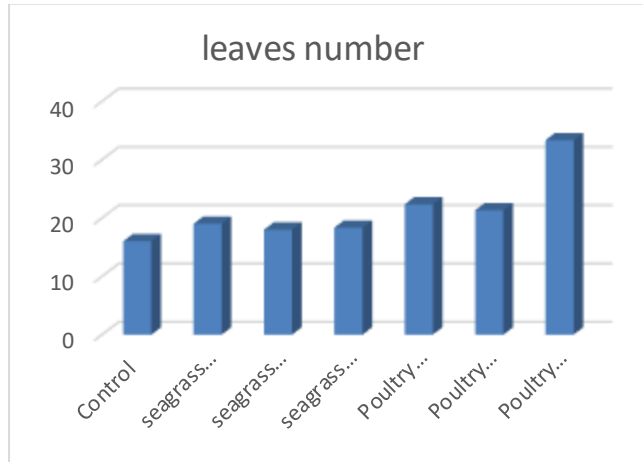


Figure 2 illustrates the effect of treatments on the number of leaves (leave).

Minimal impact, especially at the 100 ml & 150 ml ratios, is observed. However, at the 50 ml ratio of seagrass, there is a greater effect on infection severity compared to the first ratios (100 ml and 150 ml), albeit minimal. Regarding poultry litter, a significant impact is also noticed, particularly with greater variance at the 150 ml ratio, indicating a statistically significant effect of poultry litter over seagrass on the number of leaves.

**Effect of Treatments on Plant Height for Pepper Plants:**

Comparing seagrass at all the added ratios with the control to measure infection severity and also poultry litter shows no variation in the effect on plant height at all the ratios indicated in the figure, i.e., no statistical significance that could have an impact, as depicted in Figure 3.

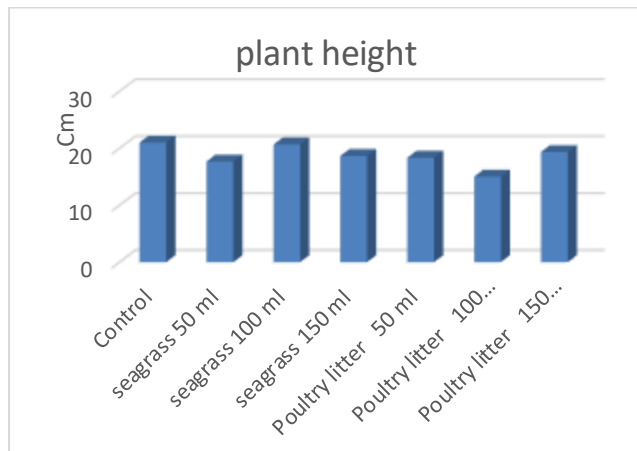


Figure 3 illustrates the effect of treatments on plant height (cm).

**Effect of Treatments on Fruit Weight for Pepper Plants:**

Through Figure 4, comparing seagrass at all the added ratios with the control to measure infection severity concerning fruit weight shows minimal impact, especially at the 50 ml ratio. However, at ratios (100 ml, 150 ml) of seagrass, there is a more substantial effect on infection severity compared to the lower ratio (50 ml), but the difference between them is minimal. Looking at poultry litter, a significant variance in impact is also noticed, and as the addition ratio decreases, it limits the infection severity, indicating statistically significant superiority of poultry litter over seagrass.

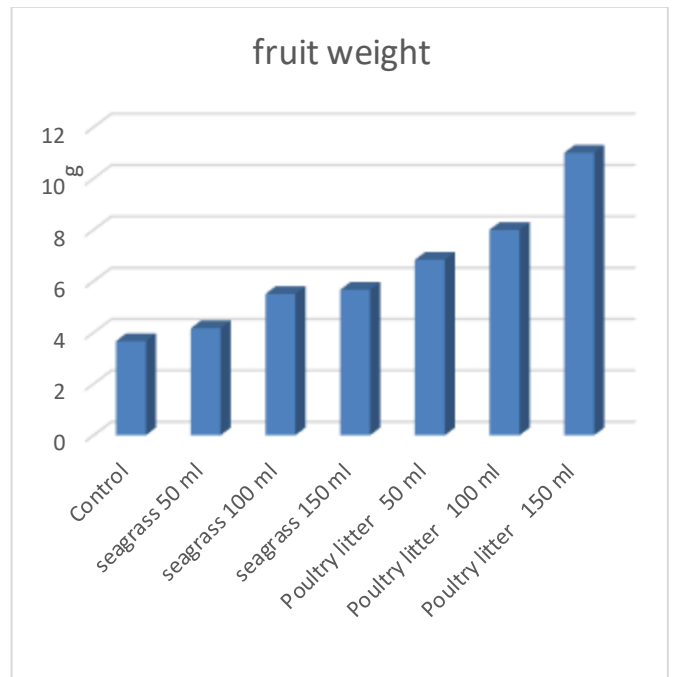


Figure 4 demonstrates the effect of treatments on fruit weight (g).

**Effect of Treatments on Disease Severity in Pepper Plants:**

Figure 5 compares seagrass at all the added ratios with the control to measure infection severity.

### Effect of Treatments on Disease Severity in Pepper Plants:

Figure 5 compares seagrass at all the added ratios with the control to measure infection severity.

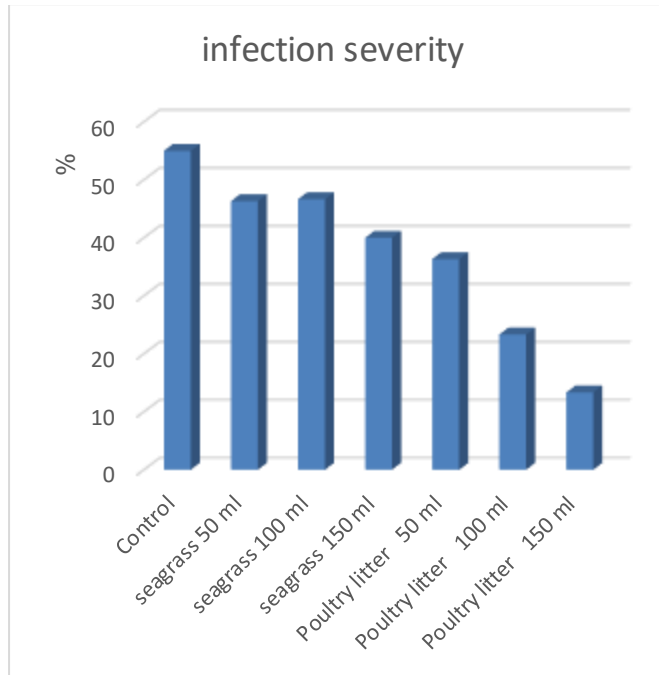


Figure 5 illustrates the effect of treatments on infection severity.

It is noted that there is no significant impact, especially at ratios (50 ml and 100 ml). However, at the ratio (150 ml) of seagrass, there is a greater effect on infection severity compared to the lower ratios (50 ml and 100 ml), although it is minimal.

Considering poultry litter, a significant impact is also observed, as depicted in Figure 5. This result aligns with what Fagimi and Odebode (2007) found. As the addition ratio decreases, the infection severity diminishes, indicating that statistically, poultry litter has a more significant effect than seagrass.

From the statistical analysis (SPSS) interpretation, it can be concluded that overall, treatments have a significant impact on disease severity and vegetative measurements in pepper plants. This aligns with observations from Robert et al. (2001) and Beth (2005) that organic nutrients originating from living organisms decompose bacteria in the soil, transforming them into soluble forms to modify and adapt the soil. They contain essential nutrients like nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca), manganese (Mn),

copper (Cu), and zinc (Zn), gradually releasing iron (Fe) and boron (Br) in small amounts for prolonged plant utilization, enhancing cation exchange capacity to retain and release more nutrients for healthy plant growth over time. This model can be applied to other plant types, especially using poultry litter, which has shown significant and positive effects on this plant type. Additionally, the current model can be developed further, focusing on seagrass in future studies, utilizing other statistical analysis techniques.

## 4 Conclusions

The study results revealed that using poultry litter extract and *Posidonia oceanica* seagrass reduced the severity of the disease caused by the CMV virus. The poultry litter extract was more effective in suppressing the virus-induced disease severity due to its distinct fermentation rate and breakdown of primary compounds compared to the seagrass extract. The poultry litter extract led to increased plant height, overall plant weight, fruit quantity, and fruit weight. The seagrass extract followed in terms of its impact on plant growth. Using seagrass as an alternative organic fertilizer due to its availability in the Mediterranean basin and cost-effectiveness is recommended. Therefore, it is recommended to use it and allow more time for its fermentation and breakdown.

**Conflict of interest:** The authors declare that there are no conflicts of interest

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