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Phytochemical and Anti-Fungal Activity of *Eucalyptus globulus* Against Late Blight (*Phytophthora infestans*) Disease of Tomato (*Lycopersicon esculentum* Mill.)

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Abstract: *Phytophthora infestans (P. infestans) is a pathogenic fungus known for its destructive characteristic against tomato and potato. The pathogen is notorious for its mutative ability against very effective antifungal drugs. This research, therefore, focused on the possible effects of methanol leaf extract of Eucalyptus globulus (E. globulus) against P. infestans of tomato. Crude extract of E. globulus was obtained through percolation eucalyptus leaf sample with methanol. Tomato seeds were planted in a nursery bed and later transplanted into pots. The plants were inoculated with P. infestans pathogen then treated with different concentrations of E. globulus extract. There are 6 experimental treatments consisting of T_0 (negative control without pathogen inoculation), T_1 (inoculated with P. infestans without treatment), T_2, T_3 and T_4 were inoculated with pathogen and treated with 0.2, 0.4 and 0.6 mg/ ml E. globulus extract respectively, while, T_5 was inoculated with pathogen and treated with 0.2 mg/100 ml Mancozeb solution. Data were collected on plant height, leaf count, leaf area, stem girth, number of flowers, fruit count and yield weight. The phytochemical analysis showed the presence of alkaloids, flavonoids, tannins, saponins, phenols and cardiac glycosides, while phlobatanins, terpenoids and amino acids were absent. The effects of the E. globulus extract showed significant increase ($p < 0.05$) on all growth parameters with the highest mean leaf count (218.53), leaf area (29.11 cm^2) and stem girth (8.45 cm) occurring in T_0, while the highest mean plant height (39.43 cm) occurred in T_5. The yield parameter showed significant increase ($p < 0.05$) in flower count, fruit count and yield weight of T_0 compared to T_1, with mean values of 67.56, 24.32 and 2.50 kg respectively. Methanol leaf extract of E. globulus expressed promising effect against P. infestans of tomato, especially at 0.4 mg/ml application rate and therefore, 0.4 mg/ml could be a promising application rate for the management of this disease.*

Keywords: Antifungal, Eucalyptus globulus, Late blight, Lycopersicon esculentum, Phytochemical, Phytophthora infestans, Tomato.

Introduction: Tomato (*Lycopersicon esculentum* Mill.) is an important vegetable crop from *Solanaceae* family (Jehani, Mohamed, Cheemala, Nath, Chonzik and Srivastava, 2025), grown worldwide for its high nutritional, antioxidant and medicinal constituents (Amr and Raie, 2022). It is native to South America, from Southern Ecuador to Northern Chile and the Galapagos Island (Okafor and Izuakor, 2023). Tomato is among the most popular vegetables grown globally for its fresh nutritional value and its economic importance (Araujo, Telhado, Sakai, Lebo and Melo, 2016). Tomato serves as a source of income as most varieties can be harvested up to four times in a year. Tomatoes are multifaceted in culinary applications, which can be consumed raw in salads, cooked in making sauces and even integrated into various dishes (Jehani *et al*., 2025). In its industrial application, it can be processed to make canned tomatoes, purees, juices, ketchup, and dehydrated pulp, making them one of the most utilized fruits globally due to their nutritional versatility (Motamedzadegan and Tabarestani, 2018). In Nigeria, cultivation of tomato is practiced throughout the country (Abraham, Banwo, Kashina and Alegbejo, 2019), however, the major production centers are located between latitudes 7.5 °N and 13 °N with a temperature range of 25 – 34 °C (GEMSA4, 2016). Tomato provides the major nutrients needed by the body including vitamins and minerals like calcium, niacin,

vitamin C, lycopene, alpha and beta tocopherol, flavonoids etc. Cultivation of tomato is, however, threatened with diseases attack, amongst which include late blight disease.
Nigeria is estimated to produce 4.1 million tonnes of tomatoes and has been ranked as the 14th largest producing country in the world, placing her as the 2nd largest producer in Africa after Egypt (Ajenifujah-Solebo, Akin-Idowu, Aduloju, Adedeji, Akinyode, Ibitoye, Arogundade, Oke, Adesegun, Ntui, Akinbo, Adetunji, Falana, Joseph and Bello, 2025). Tomato production in Nigeria is dominated by poor resource peasant farmers as their major means of livelihood. Yield losses occur due to the menace of biotic stress and abiotic factors posing serious threat to the profitable production of tomato. Among the biotic constraints facing tomato production are fungal diseases which constitute major factors affecting the production and productivity as well as quality of the crop (Keskse, Hailu and Belete, 2019). Tomato is susceptible to a wide range of diseases such as bacteria (Ally, Neetoo, Ranghoo-Sanmukhiya and Coutinho, 2023), fungi (Alsudani, 2025), viruses (Keskse *et al*., 2019) and nematodes (Elhadi, Mohammed, Asid, Meshari, Ayshah and Norah, 2025) among others which adversely affect quality, quantity and profitability (Ndala, Mbega and Ndakidemi, 2019).

Phytophthora infestans is a plant pathogen that causes late blight disease in tomato plants, leading to significant yield losses and economic damage (Kesho and Tadesse, 2023). *Phytophthora infestans* disease contributes to decreased yield and deterioration of crop quality (Gonzalez-Jimenez *et al.*, 2023). Yield losses due to the disease are attributed to premature death of foliage, stems and fruits of tomato. The disease is of economic importance due to the great losses it possesses to tomato production which can be as high as 100 % loss (Kamoun *et al*., 2015). The use of chemical method for the control of the pathogen is common among farmers. Contact and systemic fungicides are employed, however, despite the wide arsenal of methods involved, most attempts have not been sufficiently effective (Ivanov *et al*., 2021). The most common tool for the control of the disease (the use of chemicals) has certain drawbacks such as high price, a prohibited use in organic farming, potential risk to the ecosystem and human health (Pacilly, Groot, Hofstede, Schaap and van Bueren, 2016) and the possibility for resistant strains to emerge (Gonzalez-Jimenez, Andersson, Wiik and Zhan, 2019; Schepers, Kessel, Lucca, Förch, van den Bosch, Topper and Evenhuis, 2018).

P. infestans is a plant pathogen that causes late blight disease, especially in potato and tomato of the *Solanaceae* family, resulting in significant yield loss and economic damage (Kesho and Tadesse, 2023). The use of chemical fungicides for the control of the pathogen is common among farmers, however, chemical usage poses environmental threat and health risk (Pacilly *et al*., 2016). In addition, chemical fungicides are not often available to rural farmers and could induce toxicity to plants and animals, while chemical usage is not sufficiently effective (Ivanov *et al*., 2021). This could be due to the reported resistance strains of the pathogen to antifungal drugs (González-Tobón *et al*., 2019). Therefore, the best alternative method for the control of *P. infestans* could be the use of natural products which are eco-friendly, biodegradable and less expensive (Zaker, 2016). There is dearth of information as to the use of *Eucalyptus globulus* (*E. globulus*) against *P. infestans* of tomato.

Materials and Method: Materials: The materials used for this study include tomatoes seeds, top soil, loamy soil and manure, polythene bag (20 by 20 cm size), watering can, wheelbarrow, shovel, *E. globulus* leaf, waterbath (Model: DK 420, China), deep freezer (Model: PV-CF322GL), electric blender (Model: Century CB-8231-R2, China), Sabouraud Dextrose Agar (SDA), Potato Dextrose Agar (PDA), syringe, inoculating loop autoclave, petri-dish etc.

Method: Extraction of Plant Material: Fresh leaf sample of *E. globulus* was harvested from Federal College of Forestry, Jos and washed under running tap water until it was free of dirt. The sample was drained off water and air dried at room temperature on the slab in Chemistry Laboratory. The dried sample was blended using electric blender until evenly distributed finely divided powder was obtained. About 500 g of the powdered sample was percolated in 500 mL of methanol (ratio 1:1) for 24 hours. It was then filtered with muslin clothe to collect the liquid filtrate which was concentrated using waterbath (Model: Model: DK 420, China) at 35 ^{o}C to obtain the crude extract.

Phytochemical Analysis: Phytochemical analysis of the plant extract was carried out using the methods of Trease and Evans (1989); Durodola (1977). Phytochemical analysis carried out on the sample include flavonoids, tannins, terpenoids, saponins, cardiac glycosides, phlobatannins, amino acids and phenols.

Isolation, Culture and Pathogen Inoculation: The pure culture of *P. infestans* pathogen was obtained from the Department of Parasitology, National Veterinary Research Institute (NVRI, Vom). The pathogen was frozen in the laboratory at a temperature of -4^{0}C. Inoculation of the fungi on the tomato plant occurred with the method described by Subhani *et al*. (2014). Exactly 1 g of the pathogen was dissolved in 100 mL of normal saline. About 5 mL of the dissolved pathogen was sprayed and injected at the leaf-petiole and stem of the plant, except for the negative control. This was done 3 weeks after transplanting the tomato plant.

Experimental Design and Treatment: The experiment was carried out in a screen house laid out in a Completely Randomized Design (CRD) consisting of six (6) treatments replicated three (3). The treatments were negative control (T_0) which was not inoculated with the pathogen, T_1 (plants inoculated with *P. infestans* without treatment), T_2 (plants inoculated with pathogen and treated with 0.2 mg/ ml extract), T_3 (plants inoculated with *P. infestans* and treated with 0.4 mg/ ml extract), T_4 (plants inoculated with *P. infestans* and treated with 0.6 mg/ ml extract) and T_5 (plants inoculated with *P. infestans* and treated with 0.2 mg/ 100 ml mancozeb standard drug).

Planting Method: Tomato seeds were broadcasted on the nursery bed and seedlings were transplanted (3 seedling per

bag). Parameters assessed include leaf count, leaf area, plant height, number of flowers, fruit count and yield weight (Yield). Briefly, the leaf count was collected by counting the number of leaves on the sample plants and their replicates for five consecutive weeks. Leaf area was measured by drawing the circumference and 1 cm^3 of the leaf on a paper and then counting the number of complete 1 cm^3, while two incomplete 1 cm^3 was taken as one. This was done on all sample plants and their replicates. The height of the plant from the base to the top-most part of the plant was measured with a thread and extrapolated on a meter rule on each sample plant. The number of flowers were counted at week 7, 8 and 9 weeks after transplanting and the average data taken. Fruit count was taken by counting the number of fruits that emanated from each treatment plant and their replicates. The yield data was taken by weighing the fruits realized from each experimental plant and their replicates. The mean value of all data collected was calculated and used for statistical analysis.

Statistical Analysis: The data collected from each treatment were subjected to analysis of variance (ANOVA) using One Way ANOVA with the aid of Statistical Package for Social Sciences (IBM SPSS) Version 25 (SPSS, Chicago, IL., USA). The means were separated for their level of significant difference using Duncan Multiple Range Test (DMRT) at 95% confidence interval. The bar chart was drawn using GraphPad Prism, Version 9.1 (GraphPad Software, California, United States).

Results and Discussion: Results: Phytochemical Constituents: The phytochemicals analysis result of methanol leaf extract of *E. globulus* is presented in Table 1. The result showed that saponins, tannins, alkaloids, flavonoids, cardiac glycosides and phenols were present, while phlobatanins, terpenoids and amino acids were absent in the plant sample. The result showed that saponins, alkaloids, flavonoids and cardiac glycosides were moderately present, phenols and tannins were observed to be highly present.

Growth Parameter: Leaf count result of methanol leaf extract of *E. globulus* against *P. infestans* of tomato is presented in Table 2. The result showed significant reduction ($p < 0.05$) occurred in the untreated control (104.86±24.57) compared to the normal control (218.53±26.30) due to the non-treatment of *P. infestans* infection in the untreated control. However, treatment with *E. globulus* expressed significant increase ($p > 0.05$) in 0.4 mg/ml (158.27±26.67), 0.6 mg/ml (167.80±24.27) *E. globulus* extract and 0.2 mg/100 ml (155.20±25.98) mancozeb in comparison with untreated control. The treatment effectively reverted the low leaf count observed in the untreated group, especially in the 0.4 and 0.6 mg/ml of the plant extract used (Table 2). The leaf area (cm^2) result expressed significant decrease ($p < 0.05$) in the untreated control (22.23±1.18) in comparison with normal control (29.11±1.63) at $p < 0.05$ level of significance (Table 2). The Table also showed that treatment of *P. infestans* of tomato brought about significant increase ($p > 0.05$) in the leaf count of tomato in the 0.4 mg/ml, 0.6 mg/ml and 0.2 mg/ml mancozeb standard chemical compared to the untreated group (Table 2). Similar trend of result was observed in the plant height and stem girth in which significant decrease ($p < 0.05$) occurred in the height of tomatoes and stem girth between the untreated (28.27±1.51, 5.62±1.05) and normal control (38.62±4.28, 8.45±0.99) respectively. Also, significant increase ($p > 0.05$) was observed to occur in the plant height and stem girth of the treated infected tomatoes in comparison to the untreated plants, especially in the 0.4 mg/ml, 0.6 mg/ *E. globulus* extract and 0.2 mg/100 ml mancozeb standard drug. The treatment effect by of the plant extract, also, restored the plant height and stem girth to the range of the normal control.

Yield Parameter: Figure 1 showed the effect of *P. infestans* on the flower count of tomatoes. It was observed that significant decrease ($p < 0.05$) occurred between the normal control and untreated control. Also, the treatment effect expressed significant increase ($p > 0.05$), with the normal control giving the highest mean value (67.56±0.84), followed by 0.4 mg/ml extract group (60.78±0.84) (Figure 1). The result of the yield parameter on the fruit count and yield weight of tomatoes is presented in Figure 2. The fruit count expressed significant decrease ($p < 0.05$) in the untreated control, in comparison to the normal control due to the effect of the disease. Treatment with *E. globulus* extract showed significant increase ($p > 0.05$) in the fruit count of the 0.2 mg/ml extract, 0.4 mg/ml extract, 0.6 mg/ml extract and 0.2 mg/100 ml mancozeb, in comparison to the positive control. Significant decrease ($p < 0.05$) was observed in the yield weight of the untreated control in comparison with the normal control after infestation with *P. infestans.* Various concentrations of *E. globulus* showed a dose dependent manner in the treatment of tomatoes with *P. infestans,* which expressed significant increase ($p > 0.05$) in the yield weight of the tomatoes in the 0.2 mg/ml extract, 0.4 mg/ml extract, 0.6 mg/ml extract and 0.2 mg/100 ml mancozeb, in comparison with the negative control.

Discussion: Tomato is a widely cultivated vegetable crop in Nigeria which provides economic and nutritional benefits to both peasant farmers and commercial farmers (Ajenifujah-Solebo *et al*., 2025). In Sub-Saharan Africa, tomato is extensively grown as a food and cash crop, and contributes significantly to nutrition, employment, and income generation (Mcebisi and Ddamulira, 2020). According to Shabiu, Dangora, Kutama, Bello, Zakari, Musa and Dahiru, (2023), fungal diseases are the major threat and limiting factors in the production of economic crops, leading to tremendous yield loss annually. The presence of some phytochemicals in the methanol leaf extract of *E. globulus* might have contributed to the various bioactivity and medicinal functions expressed by this plant. Saponins, alkaloids, terpenoids and flavonoids have been reported for their insecticidal activity (Gindaba, Negeri, Abdisa, Nemo and Kitila, 2024). This result is similar to the previous research works that stated that terpenoids (Oyelakin, Ganiyu, Oloyede, Idehen, Agboola and Popoola, 2024), phenols, alkaloids (Chaudhary, Sharma, Singh & Nagpal, 2018) and flavonoids and phenols could serve as antifungal

activity against *P. infestans*. This might be due to the presence of chlorogenic acid, a component of phenols, an abundant phytoconstituent in the extract of *E. globulus*, which has been reported to inhibit lignin formation thereby degrading the cell wall component of the *P. infestans* (Ndala *et al*., 2019).

The bioactivity of the extract of *E. globulus* as expressed on the leaf count could be as a result of medicinal properties of this plant that was exploited in treating the effect of *P. infestans*, thereby improving the leaf count, in comparison with the non-treated group. This bioactivity effect was more pronounced at 0.4 and 0.6 mg/ml extract of the plant. This result is similar to the previous published research work of Yadav, Lal, Kakraliya, Bajiya and Sheshma, (2017) stating that foliar spray of tomato infested *P. infestans* with extract of garlic reduced the disease and resulted in significant increase in the plant height and yield of tomato. Similarly, Mavuze, Birgen and Akwa, (2021) reported a significant improvement in the number of leaves of tomatoes infected with root-knot nematode and treated using various plant extracts. The effect of both the plant extract and the standard drug did gave positive effect on the flower count of tomato. This might be a result of the extract applied as the result was much more pronounced at higher concentration of the *E. globulus* extract, especially at the rate of 0.4 and 0.6 mg/ml. Therefore, higher concentration could produce a more interesting result. Furthermore, the statistical similarities observed in the fruit count between 0.4 mg/ml and 0.6 mg/ml extract of *E. globulus* might be due to inactivation of the bioactive components in 0.6 mg/ml extract of *E. globulus*, leading to reduced bioactivity of the treatment when compared with 0.4 mg/ml *E. globulus* extract. The better performance exhibited by the plant extract, at higher doses, in comparison with mancozeb might be as a result of the non-resistant ability of the extract to the pathogen, being a natural substance. *P. infestans* has been implicated in its resistance ability to many antifungal drugs due to continuous mass application of fungicides (Wang, Tyler and Wang, 2019). The obtained results further showed that the extract of *E. globulus* did not only control the effect of the *P. infestans*, it also served as a bio-stimulant thereby enhancing the growth and yield parameters of the tomato plant. This is similar to the previous published work by Jena and Topno (2023) who stated that the extract of seed weed at the rate of 4 ml/L produced higher plant height, number of leaves, number of flowers, seed yield per plant and reduced days taken for first flowering of pansy plant. Also, Al-Samaraee and Al-Showily (2020) reported that aqueous extract of ginger at 5 g/L rate of application gave a good result on plant height, number of branches, percentage dry matter of the vegetative total, flowering traits as well as beta-carotene and lutein content of *Tagetes erecta* (Mexican marigold) plant. Furthermore, Marigold flower was reported to express significant increase in the shoot height, number of leaves, number of flowers and number of fruits of tomato plant, while reducing the disease of canker by 62.82%, early blight disease by 61.53%, fruit spot of tomato by 27.41%. (Nahak and Kanta, 2017).

Conclusion & Recommendation: Conclusively, the methanol leaf extract of *E. globulus* expressed insightful information about its potential efficacy against late blight (*P. infestans*) disease of tomato. The extract of *E. globulus* was able to successfully ameliorate the effect of stunting growth and enhanced growth and yield parameters of tomato. This was so due to the presence of the various phytochemicals present in the plant extract. The application rate of 0.6 mg/ml *E. globulus* reversed the stunted growth and improved the yield parameters of tomato. The extract of *E. globulus* also, indirectly served as bio-stimulant in its disease control mission. Further studies can be carried out to identify the bioactive compound responsible for its antifungal activity. Based on the outcome of this research work, the following recommendations will be submitted. Methanol leaf extract of *Eucalyptus globulus* expressed possible anti-fungal effect against *Phytopthora infestans* on tomato (*Solanum lycopersicum*) in a dose dependent manner. The least application rate of *Eucalyptus globulus* for the treatment of *Phytophthora infestans* in tomato should be 40 g/40 ml (1 g/ml) solution. The effect of *Eucalyptus globulus* against *Phytophthora infestans* was not enough to completely redeem the growth and yield parameters of tomato back to their normal states. The effects of other antifungal plants, in their single entities and their possible synergistic effects against *Phytophthora infestans* should be investigated.

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Table 1: Phytochemical Constituents of Methanol Leaf Extract of *E. globulus*

Phytochemicals	Result
Saponins	++
Alkaloids	++
Phlobatanins	N.D
Flavonoids	++
Terpenoids	N.D
Cardiac glycosides	++
Phenols	+++
Tannins	+++
Amino acids	N.D

Key: "++", "+++" represents moderately present and highly present; N.D means not detected

Table 2: Treatment Effect of *E. globulus* on Leaf Count, Leaf Area, Plant Height and Stem Girth of *P. aeruginosa* Infested Tomatoes

Treatment/Leaf Count	Leaf Count	Leaf Area (cm^2)	Plant Height (cm)	Stem Girth (cm)
Normal control	218.53 ± 26.30^{a}	29.11 ± 1.63^{a}	38.62 ± 4.28^{a}	8.45 ± 0.99^{a}
Untreated control	104.86 ± 24.57^{c}	22.23 ± 1.18^{b}	28.27 ± 1.51^{b}	5.62 ± 1.05^{b}
0.2 mg/ml extract	136.67 ± 24.32^{bc}	24.23 ± 1.65^{b}	32.69 ± 3.30^{ab}	8.44 ± 1.51^{a}
0.4 mg/ml extract	158.27 ± 26.67^{b}	26.86 ± 0.58^{a}	37.33 ± 2.51^{a}	8.20 ± 1.15^{a}
0.6 mg/ml extract	167.80 ± 24.27^{b}	27.83 ± 0.63^{a}	38.51 ± 2.51^{a}	8.25 ± 1.27^{a}
0.2 mg/100 ml mancozeb	155.20 ± 25.98^{b}	29.02 ± 1.36^{a}	39.43 ± 5.43^{a}	8.05 ± 1.21^{a}

All means are expressed as mean (±) standard deviation of 5 replicates. Means within the same column, having different superscripts are significantly different ($p < 0.05$)

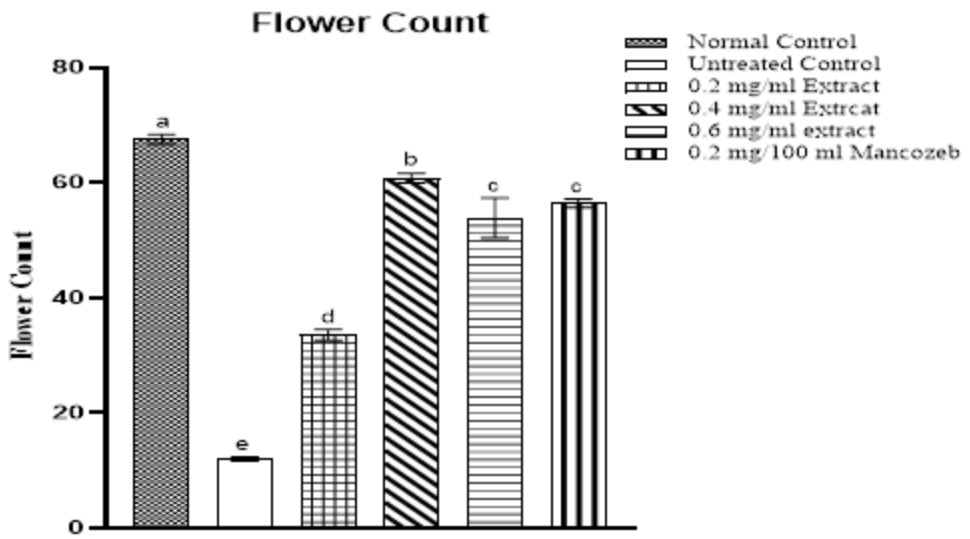

Figure 1: Treatment Effect of *E. globulus* on *P. infestans* Disease of Tomato (*Solanum lycopersicum*) on Flower Count Parameter

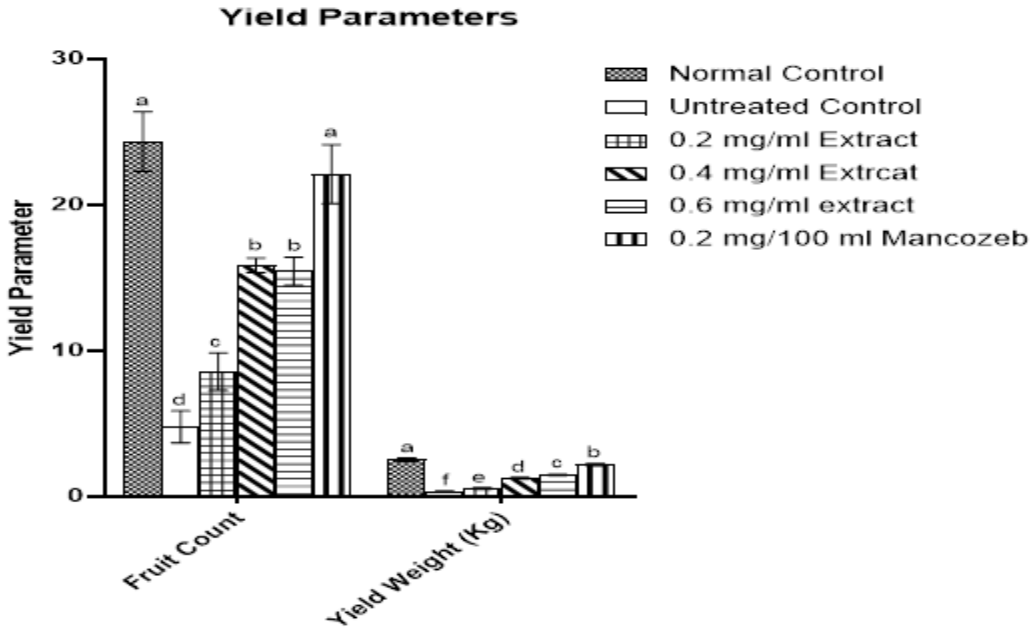

Figure 2: Treatment Effect of *E. globulus* on *P. infestans* Disease of Tomato (*Solanum lycopersicum*) on Fruit Count & Yield Weight (kg)