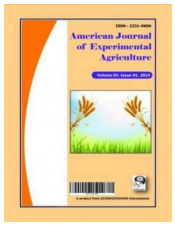

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Bio-control Effect of *Trichoderma asperellum* (Samuels) Lieckf. and *Glomus intraradices* Schenk on Okra Seedlings Infected with *Pythium aphanidermatum* (Edson) Fitzp and *Erwinia carotovora* (Jones)

O. O. Idowu[1*], O. I. Olawole[1], O. O. Idumu[1] and A. O. Salami[1]

[1]Department of Crop Production and Protection, Faculty of Agriculture, Obafemi Awolowo University, Ile-Ife, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author AOS designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors OIO, O. O. Idowu and O. O. Idumu reviewed the experimental design and all drafts of the manuscript. Author AOS managed the analyses of the study and identified the plants. Authors OIO, O. O. Idowu and O. O. Idumu performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Biological control agents are known to reduce the effect of plant pathogens and also reduce the environmental hazard caused by the persistent use of synthetic chemicals. In this study the effect of *Trichoderma asperellum* and the arbuscular mycorrhizal fungus *Glomus intraradices* were observed on young okra seedlings infected with the same concentration of disease causing microorganisms; *Pythium aphanidermatum* and *Erwinia carotovora.* This study was designed to evaluate the effect of biocontrol agents against okra seedlings infected with *Erwinia carotovora* and *Pythium aphanidermatum.* The experiment was conducted in the green house of the faculty of Agriculture, Ile- Ife, Nigeria. Different combinations of these microorganisms were observed on the

**Corresponding author: E-mail: oluyemi.idowu@yahoo.com;*

growth performance of Okra seedlings. Okra seedlings were planted in the nursery for 2 weeks and 3 weeks respectively before they were transplanted into pot of 17 cm by 17 cm filled with sterilized soil. Inoculums concentration of 10^{-8}CFU/ml of each micro- organism and 30 g of AM were introduced to the root zone of the young seedlings during transplanting from nursery tray to the growing pots according to the designated treatments and each treatment was replicated thrice. The same procedures were used for 3 weeks okra seedlings. The effects of micro-organisms were observed using plant growth parameters such as; stem girth, number of leaf, stem height and leaf area. The result shows that the bio-control agents reduced the negative effect of the pathogen on the young seedlings and *Glomus intraradices* enhanced the development of plant parameters. The organisms had a lesser synergistic effect on each other due to their high requirements for metabolic product of plant but produced more antagonistic effect on the pathogenic micro-organisms. In conclusion, *Glomus intraradices* and *Trichoderma asperellum* could be effectively used as bio-control agents to reduce the effect of *Erwinia carotovora* and *Pythium aphanidermatum* on young Okra seedlings.

Keywords: Glomus intraradices; Trichoderma asperellum; Erwinia carotovora; Pythium aphanidermatum; Bio-controls; Okra seedlings; Arbuscular Mycorrhiza.

1. INTRODUCTION

Okra (*Abelmoschus esculentus* (L) Moench) belongs to the family Malvaceae. Okra is in the same family as cotton and hibiscus. The crop is native to Africa and is still found growing wild around River Nile as well as Ethiopia [1]. Alternative names are lady's finger or gumbo and bhindi. They are widely grown in India, Nigeria, Costa Rica and Ghana on a large scale; however some cultivars are found in the wild. In Nigeria it is principally used in soup preparation, where the pods are the most edible part because they provide flavor when boiled or fried [2]. Many cultivars have been selected for local condition, but there are two main types: the long and short duration cultivars. Pod characteristics vary widely among cultivars, reflecting local preferences for the food crop.

Vegetables are the cheapest and commonest source of minerals and vitamins supply in human nutritional intake, especially in the tropical and sub-tropical world. Generally vegetable, contribute to the nutrient requirement of the low income group as a source of amino acids in comparison to expensive soybean and egg consumption. In support of this, fresh okra has been indicated as a good source of many nutrients, including fiber, vitamin A, B6, C and folic acid [3]. Significant levels of carbohydrates, potassium and magnesium are present in okra seed and mucilaginous preparation from its pod can be used as a plasma replacement or blood volume expander. Oil extracted from the pressed seed of okra is high in unsaturated fatty acids such as oleic acid and linoleic acid and it has a pleasant taste and colour. The oil is about 40% of the total composition of the seed oil can also be extracted from the okra fresh pod [3].

Production of these vegetables which greatly support and guarantee the availability of essential nutritional components for human existence is mainly affected by many soil borne pathogens, causing damages such as: root-rot, soft-rot, damping off of seedlings, or other diseases in plants [4].

Enterobacteriaceae bacteria containing mostly plant pathogenic species which was named by the first phytobacteriologist, Erwin Frink Smith. It is a gram negative, primarily rod-shaped bacterium. *Erwinia carotovora* (also known as *Pectobacterium carotovorum*) is a species, which causes diseases in many plants. These species produce pectolytic enzymes that hydrolyze pectin between individual plant cells. This causes the cells to separate, a disease plant pathologists termed bacterial soft rot. Soft rot, caused by several types of bacteria, but primarily subspecies and pathovars of *E. carotovora* and *E. chrysanthemi*, is a widespread and destructive disease of fleshy fruits, vegetables, and ornamentals throughout the world. Soft rot losses may occur in the field, garden, greenhouse, or after harvest during transit storage, or marketing.

Damping off is a very common disease problem in fields and greenhouses, where the organism kills young seedlings, caused by a genus of organisms called *Pythium* species [5]. This disease complex usually involves other pathogens such as *Phytophthora* and *Rhizoctonia*. *Pythium* wilt is caused by zoospore infection of older plants leading to biotrophic infections that become necrotrophic in response

to colonization/re-infection pressures or environmental stress [5-7], leading to minor or severe wilting caused by impeded root functioning [5,8]. Many *Pythium* species, along with their close relatives, *Phytophthora* species are plant pathogens of economic importance in agriculture. *Pythium* species tend to be very generalistic and unspecific in their host range [9], while *Phytophthora* species are generally more host-specific. For this reason, *Pythium* species are more devastating in the root rot they cause in crops, because crop rotation alone will often not eradicate the pathogen neither will field starvation, as *Pythium* species are also good saprotrophs, and will survive for a long time on decaying plant matter.

Disease problems and control are by no means peculiar to modern agriculture. Also, use of chemical pesticides increase production cost, thus making the end products to be more expensive even for the masses to obtain, in addition, chemical control poses more risk to human lives and environments [10]. Furthermore, the replacement of chemical pesticides by biological antagonists has perceived safety advantages for consumers and the environment. Biological control of plant diseases is becoming better established especially against soil-borne pathogens where preparations of antagonistic fungi such as *Trichoderma spp.*

Trichoderma asperellum is a fungus and a biofungicide. It is used for seed and soil treatment for suppression of various diseases caused by fungal pathogens. It is also a pathogen in its own right, causing green mould rot of onion. *T. asperellum* produces spores asexually, by mitosis. The mycelium of *T. asperellum* can produce a variety of enzymes, including cellulases and chitinases which can degrade cellulose and chitin respectively. It parasitizes the mycelia and fruiting bodies of other fungi and pathogens. The fungicidal activity makes *T. asperellum* useful as a biological control against plant pathogenic fungi. It has been shown to provide protection against such pathogens *as Rhizoctonia, Pythium* and even *Armillaria.* It is found naturally in soil and is effective as a seed dressing in the control of seed and soil-borne diseases including *Rhizoctonia solani*, *Macrophomina phaseolina* and *Fusarium* species. When it is applied at the same time as the seed, it colonizes the seed surface and kills not only the pa3thogens present on the cuticle, but also provides protection against soil-borne pathogens.

In recent development, mycorrhiza (AM fungi), due to its antagonist behavior was being used to achieved a mean of inhibiting the growth and ingress of pathogens, thereby increase yield. So, one of the benefits of mycorrhiza infection is that of crop protection or management against soil-borne pathogen, although this interaction may be affected by environmental condition, which as a single factor determines the success of the pathogen to incite infection or mycorrhizal to suppress the infection process [11]. However, gains from the symbiosis; occur through in the improved nutrition, and increase in the above ground biomass are notable benefit of mycorrhizal infection [12]. The positive impact of mycorrhizal infection on plant productivity via improved plant nutrition had also been extensively reviewed [13]. Species are available commercially in some countries. [14] defined biological control as the reduction of inoculums density or parasite in its active or dormant state.

The use of synthetic chemicals for pathogen control on vegetable plants has been found to be hazardous to both human and plant health, and therefore the possibility of using naturally occurring, non hazardous micro-organisms in controlling pathogen is encouraged, thus provide the need to evaluate the effect of biological control agents on *Abelmoschus esculentus* infected with *Erwinia carotovora and Pythium aphanidermatum.*

Therefore, the objective of the study is to evaluate the interaction effect of biological control agent; *Glomus intraradices* and *Trichoderma asperellum* on pathogenic organism *Erwinia carotovora* and *Pythium aphanidermatum* on the growth performance of okra plant seedlings.

2. REVIEW OF LITERATURE

Okra (*Abelmoschus esculentus* (L) Moench) ranks first on the consumption preferences among the other vegetables and this is due to the fact that it contributes to adequate nutritional requirement of the low income earners. Consumption of young immature okra pods is important as fresh fruits, and it can be consumed in different forms [15]. Fruits can be boiled, fried or cooked [16]. Test conducted in China suggest that an alcohol extract of Okra leaves can eliminate oxygen free radicals, alleviate renal tubular interstitial diseases, reduce proteinuirra and improve renal function [17].

A traditional food plant in Africa, this vegetable has potential to improve nutrition, boost food security, foster rural development and support sustainable land care. In western part of India okra is one of the most popular vegetables of all and is often cooked in daily meals. It is used as a thickening agent in gumbo. Breaded, deep fried okra is served in the southern United States. The immature pods may also be pickled [3]. Okra has found medical application as a plasma replacement or blood volume expander [18,19] Sowing typically takes place at the beginning of rainy season. Seeding rate is 2-3 seeds per hole with row spacing of 50-70 cm and 30-40 cm between plant stand. More vigorous cultivars require wider spacing. Irrigation supply and fertilizer application may be required at when appropriate. Young pods may be harvested 50-90 days from sowing, yields up to 500 kg/ha of pods may be produced over a harvesting period of 30-40 days.

The plant-pathogenic enterobacterium *Erwinia carotovora* subsp. carotovora and related *Erwinia* spp. are capable of infecting a number of different plant species, including several economically important crops [20]. The species has been listed as the major cause of potato blackleg and soft rot in Zimbabwe [21]. Soft rot caused an economic losses estimated to be between 40% to 80% depending on the climatic conditions [22,23]. Soft rot bacteria can be selected from other type of plant pathogenic enterobacteria by their pectolytic activity and colony characteristics on crystal violet pectate (CVP) medium [24]. Decay of seed tuber before emergence or infection of emerging sprout, results in non emergence, poor stands, stunting and missing hill [20]. It was recorded that Pathogen contamination during fruit handling is best prevented with an effective disinfectant. Once a load of fruit is contaminated with pathogens, even a proven disinfectant such as ClO_2 cannot completely eliminate such contaminants, particularly when they are in a dehydrated state on fruit [25]. The incidence of *E. carotovora* is two and three times greater for both stolon end and peel samples, respectively, after harvest and storage of seed lots compared with tubers hand dug just before commercial harvesting, which suggests that the process whereby tubers become contaminated has both field and postharvest components [26]. Recently, however, [24] examined stolon tissue on plants grown tubers and found the bacterium in almost all stolon samples and suggested that stolons were also an important contamination pathway. Soft rot erwinia is said to be highly successful pathogen when infecting potato, and they are also viewed as an opportunistic bacteria.

Pythium species causes serious disease problems, for example, *P aphanidermatum* cause severe root rot in bell pepper with 42% plant mortality [27]. It was stated by [28], that vegetable crops are highly susceptible to various number of soil pathogens which cause losses in the quality and yield of the produce. These pathogens cause diseases such as root rot, damping off of seedlings and other diseases in crops [4]. In addition, it has been noted that in field crops, damage by *Pythium* species is often limited to the area affected, as the motile zoospores require ample surface water to travel long distances. Additionally, the capillaries formed by soil particles act as a natural filter and effectively trap many zoospores. *Pythium* species cause extensive and devastating root rot and is often difficult to prevent or control [29,8]. The association of soil-borne pathogens with plant is parasitic, because pathogens destroy the living tissue of roots of plants and also make them as their main source of food. Rot diseases of the root and stem are known to directly cause reduction in the yield and quality of food crops grown [30,31]. The level of pathogenesis of a pathogen is the ability to secrete cell wall degrading enzymes. These enzymes are: Cellulases and Pectinases [32]. Cell wall degrading enzymes produced by pathogens are subject to catabolite repression brought about by the accumulation of sugar in cells of plants or organisms synthesizing the enzyme [33]. Pathogens are known to cause extensive damage to crops by greatly reducing the growth, yield of crops or total loss of crop in severe cases, for example, damping off of tomato seedling at Nsukka, Nigeria, caused by *Pythium aphanidermatum* [34], *Pythium* wet-rot of cowpea caused by the same pathogen [35] and root-rot of carrot when its canopy has fully developed caused by *Sclerotium rolfsii* [36].

Plants responded to pathogenic infection and those of mechanical damages by accumulation of phenolic compounds [37], this is characteristic of host-pathogen interaction in diseased plants. However, disease protection against soil borne pathogens is one of the benefits of mycorrhiza, for example, inoculated pepper and tomato seedlings with mycorrhiza spores prevents the root rot infection by *P. aphanidermatum* [38,39]. In a similar case, tomato seedling stem cause by

Phytophthora infestans has been reduced by the application of *Trichodermal viride* and *Glomus etunicatum* [40].

The genus, *Trichoderma*, is a common filamentous imperfect fungi (Deuteromycetes, Dematiaceae), the most common saprophytic fungi in the rhizosphere and found in almost any soil. Possible mechanisms of antagonism employed by *Trichoderma spp*. includes nutrient and niche competitions, antibiosis by producing volatile components and non volatile antibiotics that are inhibitory against a range of soil borne fungi, as well as parasitism. Also, synergism between different forms of action modes occurs as the natural condition for the biocontrol of fungal pathogens. Many researchers have demonstrated the potential of *Trichoderma spp*. in control of damping-off diseases of crop plants caused by *Pythium spp*. [41,39], *Macrophomina phaseolina* [42] *and Rhizoctonia solani* [43].

Fungal species belonging to the genus Trichoderma are easily isolated from soil, decaying wood and other forms of plant organic matter [44]. Biological control of plant disease especially soil borne plant pathogens and nematodes by microorganisms has been considered a more natural and environmentally acceptable alternative to the existing chemical treatment methods [45] over 75 years ago, demonstrated the antagonistic nature of fungal species from the genus Trichoderma. The interaction of the antagonists and the pathogen and occurrence of inhibition zone on agar media could be commonly considered as a result of the production of the antibiotics and competition for nutrients and space observed by [46]. [47] explained that inhibition zone in dual cultures is formed due to the production of volatile and non-volatile metabolites as well as the production of extracellular hydrolytic enzymes by Trichoderma species.

The antagonistic behavior of the mycorrhiza (AM fungi inclusive) in suppressing diseases caused by various pathogens has been reported by [14]. And this is through the mechanisms of action of the organism, where none is mutually exclusive of the other, though one may appear to dominate [48]. These mechanisms involve: competition; antibiosis and mycoparasitism [14]. During colonization, VAM fungi can prevent root infections by reducing the access sites and stimulating host defense [49]. Various mechanisms also allow VAM fungi to increase a plant's stress tolerance. This includes the intricate network of fungal hyphae around the roots which block pathogen ingress. The mechanisms involved in these interactions include physical protection, chemical interactions and indirect effects [50]. The other mechanisms employed by VAM fungi to indirectly suppress plant pathogens include enhanced nutrition to plants, which illustrated the reason why mycorrhiza infected plant contain high level of Phosphorus, Nitrogen and quick chlorophyll formation. Morphological changes in the root include increased lignifications, root hairiness and rhizospore acidification; changes in the chemical composition of the plant tissues like antifungal chitinase, isoflavonoids, among others [51]. Others are alleviation of abiotic stress and changes in the microbial composition in the mycorrhizosphere [49]. Other benefits of mycorrhizal associations that are of interest to plant pathologists include biological control and the various growth promotion effects that enhance establishment of plants in the field. The general vigour of mycorrhizal plants makes them more tolerant to root loss due to diseases. These mechanisms by which mycorrhizae protect the plant against soil-borne diseases are largely influenced by the environmental factors.

It has been shown in researches that mycorrhiza infection is measurably beneficial to plants by increasing their growth through the greater uptake of mineral [52] as well as by morphological alterations. Mycorrhiza and antagonists are technologically bio-deterrent agents to root-infecting pathogens of fungi like: *Fusarium*; *Pythium*; *Phytophthora* or *Rhizoctonia* species with beneficial contribution to plant to plant growth, health and yield [12]. Therefore the plant growth parameters is the function of both the action of the microorganisms involved in the antagonistic interaction.

3. MATERIALS AND METHODS

3.1 Experimental Site

The studies were conducted at the Greenhouse of the Faculty of Agriculture, Obafemi Awolowo University, Ile- Ife, Nigeria. The site is located on latitude 07° 28'N and longitude 04° 33'E and on the altitude of 244m above sea level. The period of the study was between late September and mid December 2013. Certified seeds of okra *Abelmoschus esculentus* (Clemson Spineless) was obtained from National Institute of Horticultural Research and Training (NIHORT), Ibadan, Nigeria. The okra seeds were planted in

the nursery seed tray. Watering was done every other day, until the seedlings emerge 4-5 days after planting; seedlings of okra were examined for uniformity in height and were transplanted after 2 weeks for the first experiment and 3 weeks for the second experiment into pot (17 cm X 17 cm). The difference in week of transplanting show the variation in terms of time of application of treatments to assess the effect of early application of biological control agent on the performance of Okra seedlings. Mycorrhiza used (*Glomus intraradices*) inoculum was supplied by the Pathology Laboratory of the Department. The isolation and sub-culturing of the microorganism was done at the Crop Production and Protection departmental laboratory.

3.2 Soil Sterilization

The soil used was collected from the greenhouse premises. Top soil of less than 15 cm depth was collected and sieved to remove root fragments, bigger stone and other debris. The soil was mixed with river sand in the ratio of 20:1 respectively. The mixture was put into a sterilizing chamber and steam heated continuously for 3.5 hours. The mixture was frequently turned to ensure even heat distribution and allowed to cool before the soil was used to fill the pots.

3.3 Preparation of Sabouraud Dextrose Medium (SDA)

62 g of SDA powder was diluted with little distilled water and dissolve thoroughly through heating. The solution was made to the volume of 1000 ml and sterilized in the autoclave at 121℃ for 15minutes. The medium prepared in section above, was poured into one-fourth of a Petri dish and acidified with 25% lactic acid solution. The acidified medium in the Petri dish was allowed to cool and solidify.

3.4 Preparation of Nutrient agar medium (NA)

28 grams of NA powder was diluted with little distilled water and dissolve thoroughly through heating. The solution was made to the volume of 1000 ml and sterilized in the autoclave at 121℃ for 15minutes. The medium prepared in section 3.3 or 3.4 above, was poured into one-fourth of a Petri dish and acidified with 25% lactic acid solution. The acidified medium in the Petri dish was allowed to cool and solidify.

3.5 Isolation and Identification of the Microorganism

Solanum lycopersicum seedling infected with damping off organism was collected from the Teaching and Research farm. The infected part especially the lower part of the stem and fruit were cut into small pieces. These pieces were washed in 1:1 solution of parazone for 1 min, so as to sterilize the surface and it was immediately rinsed in sterilized water 3 times consecutively. Each piece was taken from the washing plate and water drained on a filter paper. Two to three pieces were placed on solidified medium inside the Petri dish. The plates were put on a pyrofoam sheet and placed in such a way to intercept optimum light beam. The growth became conspicuous 24hrs after, this served as the primary plate. Secondary plates were obtained by sub-culturing from matured primary mycelium plate.

Brassica oleracea fruit infected with soft rot was collected from a fruit shop in Mayfair. The infected part was cut and placed into a mccartney bottle missed with distilled water, Some portion of mycelium was taken from 5-day old culture and placed on a slice and stained with 2 drops of lacto-phenol. This was observed under low and high magnification power lens of microscope, to ascertain if the microorganism is truly *E. carotovora*.

3.6 Preparation of *Erwinia carotovora* Inoculum

The inoculums solution was prepared from a day old culture of Erwina carotovora. The milky growth part was picked using the wired loop and place inside distilled water to loosen the mass of spore. The solution was constantly modified using distilled water. The spore was counted using a colorimeter to measure a concentration of 10^{-8} CFU/ml of inoculum.

3.7 Preparation of *Pythium aphanidermatum* Inoculums

The inoculum solution was prepared from 5-day old culture of *Pythium asperellum*. The part covered with the white fluffy growth was washed thoroughly with water to loosen the mycelia growth, the solution was compensated with distilled water using dilution factor of 10, that is, 1 ml of the mycelium with 10 ml of distilled water. Spore count was done using colorimeter to get a concentration of 10^{-8} CFU/ml of inoculum.

3.8 Preparation of *Trichoderma asperellum* Inoculums

The inoculum solution was prepared from 5-day old culture of *Trichoderma asperellum*. The part covered with the green mold growth was washed thoroughly with water to loosen the mycelia growth, the solution was compensated with distilled water using dilution factor of 10, i.e 1 ml of the mycelium with 10 ml of distilled water, and spores was done using a colorimeter to get a concentration of 10^{-8} CFU/ml of inoculums.

3.9 Inoculation Procedure

The soil around the root zone was carefully removed to avoid damage to the root hair. 10 ml of the inocula solution was poured very close to the root to ensure contact with it and immediately covered with the dug-out soil with adequate watering. 30 g of *Glomus intraradices* soil inoculum was introduced into the rhizosphere area of the seedlings to be inoculated with it and immediately watered. This activity was carried out on 2 and 3-week old okra seedlings.

3.10 Experimental Design

The experimental lay-out of the study was Randomized Complete Block Design (RCBD), with 14 treatments and each treatment replicated 3 times. These treatments are;

(a) *Erwinia carotovora* + Okra plant (EO)
(b) *G. intraradices* + Okra plant (GO)
(c) *Pythium aphinodermatum* + Okra plant (PO)
(d) *Trichoderma asperellum* + Okra plant (TO)
(e) *T. asperellum* + *E. carotovora*+ Okra plant (TEO)
(f) *T. asperellum* + *Glomus intraradices* + Okra plant (TGO)
(g) *T. asperellum* + *P. aphinodermatum* + Okra plant (TPO)
(h) *T. asperellum* + *G. intraradices*+ *P. aphinodermatum* + Okra plant (TGPO)
(i) *T. asperellum* +*G. intraradices* + *E. carotovora* + Okra plant (TGEO)
(j) *T. asperellum* + *G. intraradices*+ *P. aphinodermatum* + *E. carotovora* + Okra plant (TGPEO)
(k) *P. aphinodermatum* + *E. carotovora* + Okra plant (PEO)
(l) *G. intraradices* + *E. carotovora* + Okra plant (GEO)
(m) *G. intraradices* + *P. aphinodermatum* + Okra plant (GPO)
(n) Okra plant alone which serve as the control experiment (OA)

3.11 Data Collection

Data were collected on the growth parameters of the seedling at different ages. These include:

- Stem height (cm)-SH
- Leaf surface area (cm^2)-LSA
- Stem girth (cm)-SG
- Number of leaf per plant-NL.

3.12 Statistical Analysis

Collected data were subjected to Analysis of variance using Statistical Analysis System (SAS) package version 9.1. Mean values were separated using Least Significant Difference (LSD) at 5%.

4. RESULTS

The results show that there is significant effect of the treatments applied weeks after inoculation and interaction between treatment, trials and weeks after inoculation on the growth parameters of okra, indicating that each of the treatment affected the growth of the seedlings either negatively (poor growth due to the effect *Pythium aphanidermatum and Erwinia carotovora*) or positively (improved growth derived from *Glomus mosseae and Trichoderma asperellum*) as the weeks progresses when compared with the control (as shown in Table 1). There is no significant difference in the interaction between the treatments and weeks after inoculation on SH and LSA and trials and weeks after inoculation on LSA for okra seedlings, which means they perform uniformly.

The result from Table 2 shows that there is significant difference between the trials. Trail1 performed differently from Trial 2 for all the growth parameters except for the NL, where trial 1 performs was quite different from trial 2. The result does not indicate the better performance of trial 2 but show the implication of the presence of disease causing organism at different growth stages of plant life. The SH, SG and LSA in trial 1 seems to be higher because of the absence of microorganism which inhibit growth and utilized food stored for metabolism by the growing plant, however the implication of

delayed control measure was seen on the NL, where most of the plant in trial 2 was affected by the presence *of Pythium aphanidermatum* which causes most of the plant leaves to fall. Control measures, growth enhancing substances should be introduced early in plant life so as to make necessary corrections before the plant is too old for adaptation.

The result shown in Table 3 indicated that the stem height of the Okra seedling inoculated with *Glomus intraradices* recorded the highest value of (14.42) compared with that of *Pythium aphanidermatum* which recorded the lowest of value of (13.66), this shows the negative impact of the presence of *Pythium aphanidermatum* on the young seedlings of inoculated plant. The presence of *Glomus intraradices* and *Trichoderma asperellum* in each of the treatments reduce the effect of *Pythium aphanidermatum* and *Erwinia carotovora* on the young Okra seedlings, this can be seen in the GPO, TPO, GEO and TEO where ranking is lower and growth parameters improved, compared to those treatments that has the pathogenic micro-organisms acting alone or in combination with one another on the young Okra seedlings. The effectiveness of control of the biological control agents are of higher significance when present alone with the one pathogenic organism than when several combination of these biological agents are acting on one or more pathogenic organisms. These are evidence in all growth parameters where the combinations on young Okra seedlings are in 1:1 ratio than 2:1 or 2:2, 2:3 combination ratios. The pathogen to pathogen relationship recorded better performance than some combination due to competition that reduced their negative effect on some growth parameters.

Weeks after inoculation point to the time the effects of *Erwinia carotovora, Pythium aphanidermatum*, *Glomus intraradices* and *Trichoderma asperellum,* become conspicuous on the performance of the Okra seedlings and the appropriate time of introducing biological control organisms into the soil, in order to increase the above ground biomass and productivity of the crop. The level of significant effects of the treatments and weeks after inoculation is very high for all plant growth parameters at age of 4-5 weeks. The rate of increase in the growth parameters with weeks after inoculation is indicated as shown Table 3 and 4. This explains when the greatest

Table 1. Mean square values for growth parameters of okra seedling

Source of variation	Degree of freedom	Stem height (cm)	Number of leaves	Leaf surface area (cm^2)	Stem girth (cm)
Rep	2	0.419**	0.2757	648.82	0.00098
Treatmentt	13	6.836**	4.570**	1204.37**	0.016**
Trial_1	1	1169.98**	5.365**	49668.10**	0.7208**
Week	5	68.17**	15.236**	17667.55**	0.0956**
Trt*trial_1	13	2.089**	0.963*	1412.71**	0.0052**
Trt*week	65	0.178	1.318**	143.39	0.0013**
Trial_1*week	5	1.683**	8.226**	142.19	0.0063**
Trt*trial1*week	65	0.0601	0.415	148.5	0.00038
Error	334	0.9513	0.6625	232.29	0.00036

*** Significant at less than 0.01 level of probability.*
**Significant at 0.01-0.05 level of probability.*
Rep= Replicate; Trt= Treatment; Trial=Number of time the experiment was conducted
Week= Weeks after inoculation; R^2= co-efficiency of variation.

Table 2. Mean comparisons of the effect of trials on the growth parameters of okra seedlings

Trials	Stem height (cm)	Number of leaves	Leaf surface area (cm^2)	Stem girth (cm)
Trial 1	12.1	5.206	49.069	0.577
Trial 2	15.15	5	68.924	0.652
LSD(0.05)	0.088	0.12	2.671	0.003

*** Significant at less than 0.01 level of probability*
**Significant at 0.01-0.05 level of probability*

Table 3. Mean comparison of the effect of the treatments on the growth parameters of okra seedlings

Treatments	Stem height (cm)	Number of leaves	Leaf surface area (cm^2)	Stem Girth (cm)	Ranking
EO	13.74	5.000	64.05	0.635	4
PO	13.66	4.778	65.72	0.597	14
GO	14.42	5.583	55.44	0.652	1
TO	13.24	5.417	60.60	0.613	6
PEO	12.69	4.333	64.47	0.573	12
TEO	13.55	5.056	59.86	0.619	6
GEO	14.16	5.194	65.80	0.605	3
TPO	13.70	4.639	64.54	0.592	9
GPO	13.66	4.889	58.25	0.613	8
TGO	14.00	5.528	60.29	0.649	2
TPGO	13.55	5.194	48.15	0.610	13
TGEO	13.61	5.139	55.23	0.610	9
TPGEO	13.60	5.222	52.93	0.606	11
CO	13.83	5.472	50.61	0.625	5
LSD (0.05)	0.233	0.318	7.067	0.09	

EO=Erwinia carotovora + Okra plant ; GO=G. intraradices + Okra plant
PO=Pythium aphinodermatum + Okra plant ; TO=Trichoderma asperellum + Okra plant
PEO=P. aphinodermatum + E. carotovora + Okra plant
TEO=T. asperellum + E. carotovora+ Okra plant; GEO=G. intraradices + E. carotovora + Okra plant
TPO=T. asperellum + P. aphinodermatum + Okra plant ; GPO=G.intraradices + P. aphinodermatum + Okra plant
TGO=T. asperellum + Glomus intraradices + Okra plant
TGPO=T. asperellum + G. intraradices+ P. aphinodermatum + Okra plant
TGEO=T. asperellum +G. intraradices + E. carotovora + Okra plant
TGPEO=T. asperellum + G. intraradices+ P. aphinodermatum + E.carotovora + Okra plant
OA=Okra plant alone which serve as the control experiment

Table 4. Mean comparison of the effect of weeks after inoculation on the growth parameters of okra seedlings

Weeks after inoculation	Stem height (cm)	Number of leaves	Leaf surface area (cm^2)	Stem girth (cm)
6	14.75357	4.5476	73.19	0.652381
5	14.37738	5.1905	68.336	0.643929
4	13.96548	5.2976	67.867	0.627976
3	13.39405	5.6071	61.451	0.609286
2	12.85952	5.3571	47.512	0.58881
1	12.41548	4.619	35.623	0.564286
LSD (0.05)	0.1527	0.208	4.6262	0.0058

6---- 6 weeks after the first inoculation, 5---- 5 weeks after the first inoculation
4---- 4 weeks after the first inoculation, 3---- 3 weeks after the first inoculation
2---- 2 weeks after the first inoculation/ second week of dual inoculation, 1---- 1 week after the first inoculation.

changes occur and relatively best time to apply biological control into the seedling rhizosphere. For stem height, the greatest increase occurs between the 3rd and 4th week, for leaf surface area, it occurs between 2nd and 3rd week, for number of leaves it occurs between 1th and 2nd week and decreased rapidly between 5th and 6th week, while for stem girth, it was increasing all through with greatest increase occurring between 3rd and 4th week. This shows that the effect of biological agents in suppressing diseases caused by *Pythium aphanidermatum and Erwinia carotovora* is established about 2 weeks to 4 weeks after inoculation while the negative effect is observed at the later stage of growth if preventive measure is not applied quickly. The interaction between these organisms with the seedling root is highest between these periods.

5. CONCLUSION

Glomus intraradices and *Trichoderma asperellum* suppressed the penetration, colonization and establishment of *Pythium aphanidermatum and Erwinia carotovora* in the root of *Abelmoschus esculentus* young seedlings. The control of pathogen depends on the stages of development of plant root system, the population, selection and combination of control measures. The control of pathogenic microorganism which was done using only one antagonistic microorganism were more effective as it limit the number of organisms utilizing the products of plant metabolism which has a negative effect on the growth parameters of young plant. *Glomus intraradices* and *Trichoderma asperellum* also increased the vegetative biomass of okra seedlings. And for effective control of pathogen, the measures should be applied at the early stage of plant growth when the plant physiology is still adaptable to control measure.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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