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DITROPHIC INTERACTION BETWEEN *GLOMUS MOSSEAE* AND *PHYTOPHTHORA INFESTANS I*N JUTE MALLOW (*CORCHORUS OLITORIUS*) SEEDLINGS AT DIFFERENT AGES

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ABSTRACT

Corchorus olitorius is one of the cheapest sources of minerals and vitamins being rich in folic acids used for removal of folacin deficiency in pregnant women in Africa. Proper supply of this vegetable is affected by soil-borne pathogens, causing root-rot, damping off of seedlings, or other diseases in plants. This study, ditrophic interaction between *Glomus mosseae* fungus and *Phytophthora infestans* and their subsequent effects on the growth of jute mallow plants at different ages was carried out both in the laboratory and greenhouse. Six different treatment factors were used with three replicates for each treatment in Randomized Complete Block Design (RCBD). At different ages of the seedlings, different measurements of the growth parameters were taken as agronomic data. The collected data were subjected to the Analysis of variance (ANOVA) using the Statistical Analysis System (SAS).
At different ages (4, 6, and 8 weeks), inoculation with *P. infestans* significantly reduced the growth parameters of jute mallow, in contrast to the growth response and biomass of jute mallow seedlings inoculated with *G. mosseae*. This was found to be significantly higher than that of non-mycorrhizal plants, both in the presence and absence of the pathogen in the greenhouse. Regression analysis of this study shows that there is significant difference in the number of weeks among the growth parameters; it reveals a coefficient of determination (R^2) that is near perfect fit while treatments show significant difference based on the increase in ages of the jute mallow seedlings. It can then be concluded that, *Glomus mosseae* fungus acting as a bio-protective agent was able to suppress the incidence and severity of *Phytophthora infestans* in their ditrophic interaction. It also enhanced the growth parameters of jute mallow seedlings with respect to the age of the seedlings and time of inoculations.

Keywords: Ditrophic, Vegetable, Minerals, Vitamins, Pathogen, Mycorrhiza.

INTRODUCTION

In the tropical nations of the world, vegetables are the primary and cheapest source of minerals and vitamins (Lengsfeld Christian, 2004). Jute mallow *(Corchorus olitorius*) is one of the over 50 species of the genus *Corchorus*. About 30 of these species are however found in Africa including *C. olitorius* which originated from southern China and India/Myanmar. However, *C. olitorius* is highly variable in size and the leaves are generally dark-green with glossy appearance (Akoroda, 1988). It thrives well in areas with rainfall of about 600 mm and 2,000 mm per annum and a moist climate with a temperature range of 25-32°C (Akoroda, 1988). *Corchorus olitorius* is known for its fiber produce, “jute”, and for its leafy vegetable. Nutritionally, the leaves are of high values, being rich sources of folic acids used for the treatment of folacin deficiency which results in megaloblastic anaemia, which is prevalent among pregnant women in many developing countries (Tulio *et al*., 2002). Jute mallow is cultivated to provide bark for the production of fibres (Jute) and its mucilaginous leaves are useful in food vegetables (Abou Zeid, 2000). *Corchorus olitorius* has several names given by different ethnic groups and nations, this shows that it is widely used and accepted in the world. It is commonly known as long-fruited jute, tossa, jute mallow, bush okra, and West African sorrel. It is also called *Moroheiya* in Japan; *Molehiya* in Cyprus and *Sahiyot* in Philippines (Tulio *et al*., 2002) and *Ewedu* in Nigeria.

Production of this vegetable is affected by soil-borne pathogens causing root-rot, damping-off of seedlings, or other diseases in plants. This also affects the levels of food chain as well as the direction of energy flow which could be ditrophic, tritrophic or multitrophic interactions (Luckinbill, 1974; Salami, 2002; Thilo *et al*., 2004). Mukerji *et al.* (2006) related that vegetable crops are highly prone to a number of root and soil-borne diseases causing great losses in yield and quality. He further stated that indiscriminate use of fungicides and pesticides in controlling the diseases has polluted the environment and produce. Thus, there is a need for proper management of these diseases at reduced doses of pesticides or non-use of pollutant to sustain the vegetable production and still maintain the sanity of the environment. Disease problems and disease control are by no means peculiar to modern agriculture (Semra *et al*., 2007). Also, use of chemical pesticides increase production cost, thus, making the end products to be more expensive even for the masses to procure. Furthermore, chemical control poses risk to human lives and environments (FAO, 1989). Arbuscular Mycorrhizae (AM) fungi are recognized as high potential agents in plant protection and pest management (Yolande and Marcia, 2004). Various mycorrhiza fungi also help protect the associated plants against pathogenic fungi, a number of soil microbes, heavy metals and toxic compounds (Heino Lepp, 2002). Ilori *et al.* (1994) defined biological control as the reduction of inoculum density or parasite in its active or dormant state. However, the effect of arbuscular mycorrhiza (AM) infection on plants has been found gainful in terms of nutrient uptake and of inhibitory effect against ingress of pathogens into the plants (Salami, 2008). AM are symbiotic associations formed between plants and soil fungi that benefit both partners, one of the major benefits of mycorrhiza infection is protection against soil-borne pathogens (Yolande and Marcia, 2004).

Biological control of plant diseases by arbuscular mycorrhizal fungi varies with the combination of host- arbuscular mycorrhiza-environmental conditions (Mukerji *et al.,* 2006). It is one of the visible alternatives in sustainable agriculture as these fungi are associated with most agricultural crops and provide protection against soil-borne diseases. However, the degree of reduction in disease development is one of the benefits of mycorrhizal infection but this depends on the aggressiveness of the pathogens as well as the environmental condition (Adeoti, 1992; Salami *et al*., 2005). Mycorrhizae are important components of intensively managed vegetables because mycorrhizal colonization increases growth and yield, especially in soils with low fertility where the symbiont increases efficiency (Salami, 2007). Efficient mycorrhizal association for nutrient uptake particularly P and improved growth in low P available soil have been found important and beneficial by Habte and Osorio (2003). The implication of these results is that, crops need VAM inoculation for better performance and that indigenous mycorrhizae are not always very effective (Salami and Osonubi, 2006). The effectiveness of vesicular arbuscular mycorrhiza (VAM) in alley cropping in improving agricultural products' economic yield supports the importance of agro-forestry. VAM fungus has been found to act as link between the soil and crop; they also serve as mediator of nutrient fluxes between legume and non-legume associations (Salami and Osonubi, 2003). This makes the practice an efficient technology for crop production both in short and long term cultivations. It is however against this background that this study aims at, investigating the ditrophic interaction (ditrophic food chain) between *Glomus mosseae*, an arbuscular mycorrhiza fungus and *Phytophthora infestans, a* pathogenic fungus and their subsequent effects on the growth of jute mallow plants at different ages.

MATERIALS AND METHODS

Certified seeds of *Corchorus olitorius* were obtained from Institute of Agricultural Research and Training (IAR&T), Moor plantation Ibadan, Oyo State, Nigeria. The seeds were raised in the nursery seed trays filled with sterilized loamy soil in the Greenhouse of Obafemi Awolowo University, Ile-Ife, Osun state, Nigeria. Seedlings of Jute mallow were transplanted four weeks after planting for the ditrophic interaction study. Isolation and sub-culturing procedures of the microorganism (*Phytophthora infestans*) were carried out at the Crop Production and Protection Departmental laboratory of the same University. The subsequent inoculation of transplanted seedlings was also done in the Greenhouse. The arbuscular mycorrhiza inoculum (*Glomus mosseae),* which contains mycorrhiza spores and root fragments were obtained from the Pathology Laboratory of the same Department. This was propagated in pot culture with *Zea mays* (L) for four months. The soil used was the top soil collected from the greenhouse premises, this top soil containing essential mineral nutrients, was sieved to remove root fragments, bigger stones and other debris from the soil. The component mixtures of the sterilized soil are the top soil, weathered poultry manure, and rivers sand which were mixed together in the ratio 20:1:1 respectively. The seedlings were inoculated with the pathogen by uprooting the number required from the seed box and immersed inside the inoculum as spore suspension of $4x10^6$ obtained from 7-day old culture of *P. infestans*. The surface of the culture was scrapped with a sterile surgical blade into 1ml of distilled water in a beaker and mixed together by shaking it vigorously to allow the content to mix thoroughly. The seedlings were immersed for about 30 minutes after which they were transplanted into the plastic pot. The seedlings were inoculated at different ages; 4, 6 and 8 weeks. Mycorrhizal inoculation was done by weighing 30g of the mycorrhizal soil directly below each seedling needed to be inoculated and then drenched with water and covered back with sterile soil in the plastic pot at 3 different ages 4, 6 and 8 weeks respectively.

Six different treatment factors were used in this Randomized Complete Block Design

(RCBD) in this factorial experiment. These include: (i) the control; (ii) *Phytophthora infestans* alone; (iii) *Phytophthora infestans* (pathogen) and *Glomus mosseae* (mycorrhiza) (i.e. simultaneous inoculation); (iv) *Glomus mosseae* (mycorrhiza) alone; (v) *Phytophthora infestans* inoculation for 2 weeks before *Glomus mosseae* (dual inoculation) and also; (vi) *Glomus mosseae* inoculation for 2 weeks before *Phytophthora infestans* inoculation (dual inoculation). 3 replicates were used for each treatment while seedlings used for control treatment were left without inoculating them with either mycorrhiza or pathogen. At different ages of the seedlings, different growth parameters were taken as agronomic data. These include; stem height (cm), leaf surface area (cm^2), girth of stem (cm), and number of leaf per plant. These parameters were determined by the growth of inoculated seedlings in relation with the control seedlings and they were taken in centimeters (cm) every week after inoculation. The data collected were subjected to the Analysis of variance (ANOVA) using the Statistical Analysis System (SAS).

RESULTS

The results obtained revealed observable differences in the various treatments in relation to the different growth parameters. Plant samples showed above ground symptoms and these include: necrosis, as dark brownish spots on leaf surfaces, die back from leaf tips backwards, wilting of whole plant, shedding of leaves from the base of the stem upwards (defoliation), thinner stem in some of the treatments.

Also, the results from this study showed the ditrophic interactions (second level of food chain in predator-prey interaction) between *Glomus mosseae* (Arbuscular Mycorrhiza) fungus and *Phytophthora infestans* (Pathogenic fungus) and the subsequent effects on the growth of jute mallow seedlings (Fig. 1). Here, the error bars between weeks 1, 2, &3 indicate that there was no significance difference within them but between weeks 4, 5 & 6 which within themselves too, have no significant difference. This indicates that treatments 1, 2, 3 form a group while treatments 4, 5, & 6 form another group, these two groups of treatments are statistically different from one another. At the different weeks after inoculation, jute mallow seedlings inoculated with *P. infestans* alone or before inoculating with mycorrhiza significantly reduced the growth parameters of the plant compared to simultaneous and dual inoculations with mycorrhiza before pathogen (Fig. 2). Here, the R^2 (co-efficient of determination) in the regression analysis carried out explains the variation observed on the different growth parameters (i.e. stem height, leave surface area, number of leaves and stem girth) as near perfect fit in response of age of jute mallow seedlings to the treatments. In contrast, the growth and biomass of the seedlings inoculated with *G. mosseae* was significantly higher than that of non-mycorrhizal seedlings both in the presence and absence of the pathogens (Fig. 4). Seedlings inoculated with mycorrhiza 2 weeks before the pathogens had a lower incidence of the above ground symptoms and the growth parameters were able to compare well with that of the control (Fig 3). Susceptibility of the jute mallow seedlings to the effect of the pathogen was age mediated as it was found to reduce as the age of plants increased (Fig 5). However, the difference between the mean values for the pathogen-alone inoculated jute mallow seedlings and the mycorrhiza-alone inoculated seedlings reduced as their ages increased from 4 to 6 weeks old of the seedlings and was found very close at 6 weeks (Figs. 3 & 4).

The stem height of jute mallow plants treated with *P. infestans* alone had the lowest mean value compared to other treatments in jute mallow. This trend cuts across all the ages in weeks of the plant (i.e. 4, 6 and 8 weeks). The mean values for leaf surface area, number of leaves and stem girth also followed the same trend while jute mallow plants inoculated with *Glomus mosseae* had the highest value followed by the dual treatment 2 weeks inoculated with *G. mosseae* before *P. infestans* inoculation and then the control (Fig. 6). However, the mean values for the jute mallow seedlings inoculated with pathogen alone at older week of age increased (Fig. 3). For instance, at 8 weeks old, the stem height for pathogen alone was higher than its counterpart in age 4 week old (i.e. at 8 weeks compared to that of 4 weeks) (Figs. 5 &. 6). Regression analysis on the result of this study showed that there is significant difference in the number of weeks among the growth parameters, it revealed a coefficient of determination (R^2) that is near perfect fit (Figs. 2, 4, & 6) while treatments had significant difference based on the increase in the age of jute mallow seedlings (Figs. 1, 3, & 5).

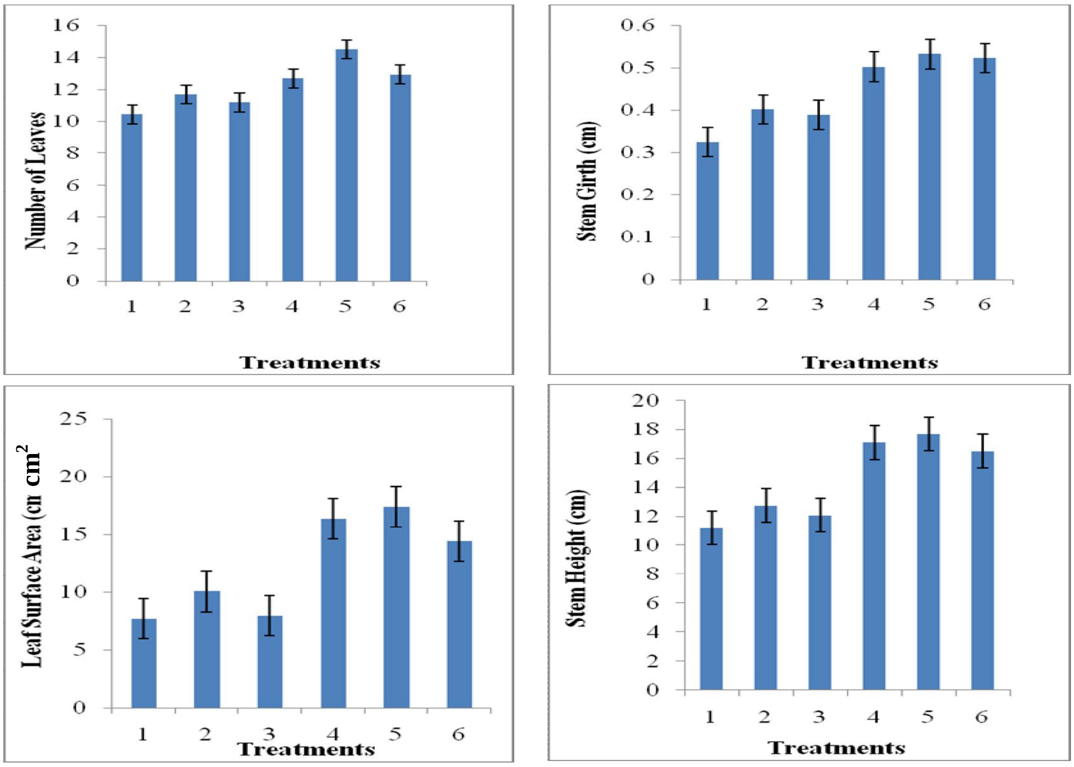

Fig. 1: Effect of treatments on growth parameters of four weeks old Jute mallow seedlings

TREATMENTS:

1……………… pathogen alone
2……………… pathogen and mycorrhizal
3……………… pathogen 2 weeks before mycorrhizal
4……………… mycorrhizal 2weeks before pathogen
5……………… mycorrhizal alone

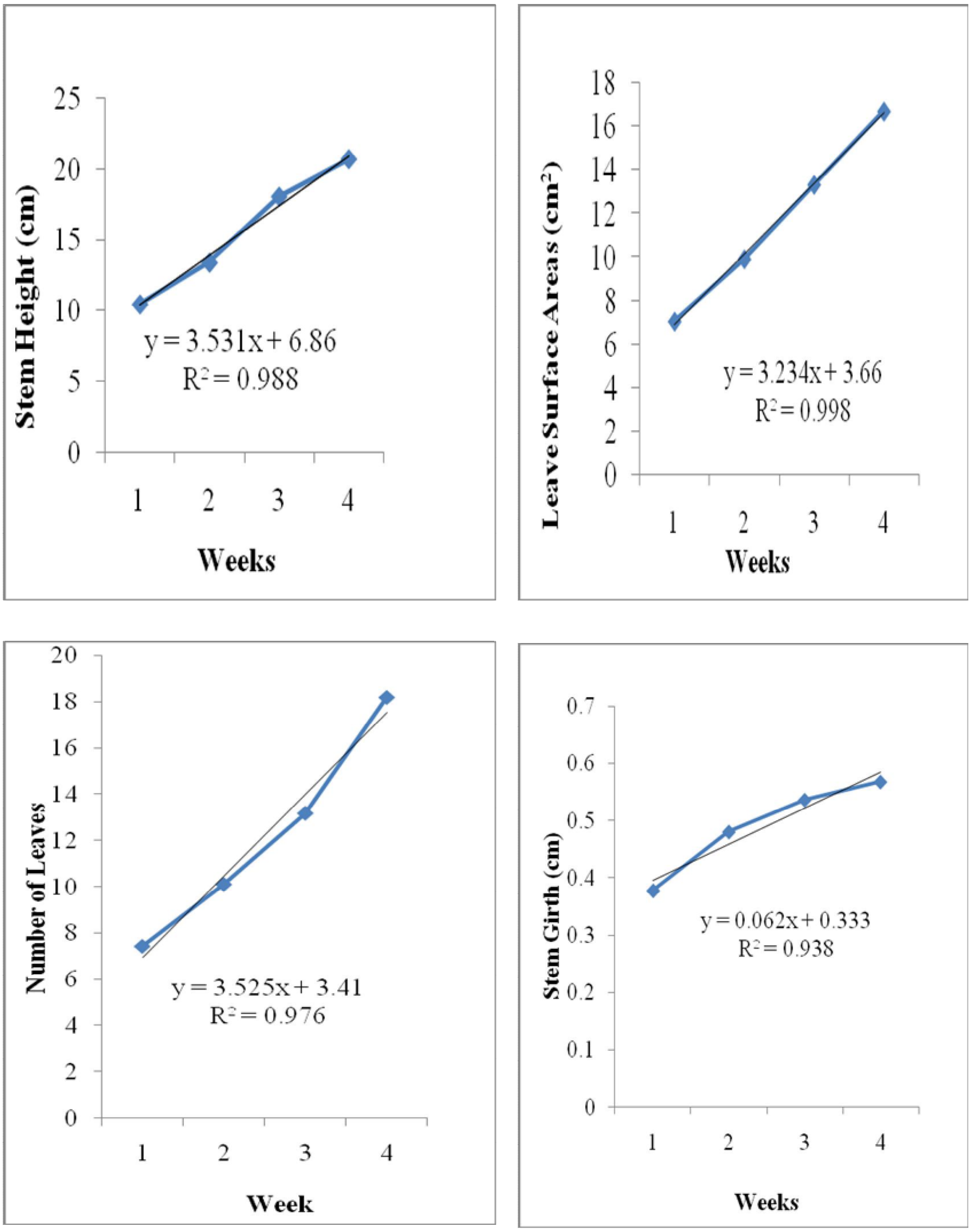

Fig. 2: Regression analysis, showing the effect of age on the response of Jute mallow seedlings to the ditrophic interaction (4 weeks old seedlings)

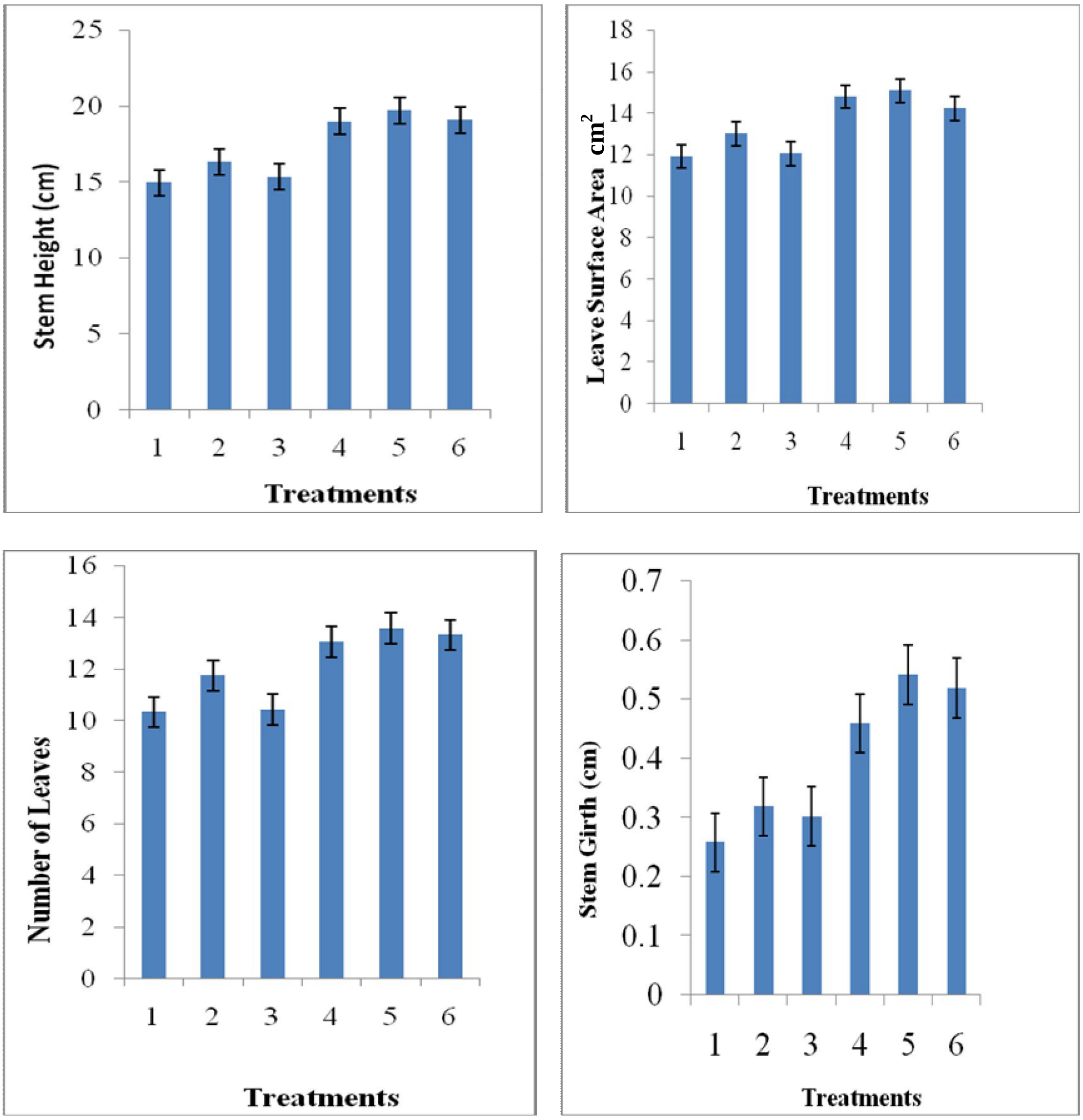

Fig.3: Effect of treatments on growth parameters of six weeks old Jute mallow seedlings.

TREATMENT :

1………………	pathogen alone
2………………	pathogen and mycorrhizal
3………………	pathogen 2 weeks before mycorrhizal
4………………	mycorrhizal 2weeks before pathogen
5………………	mycorrhizal alone
6………………	control

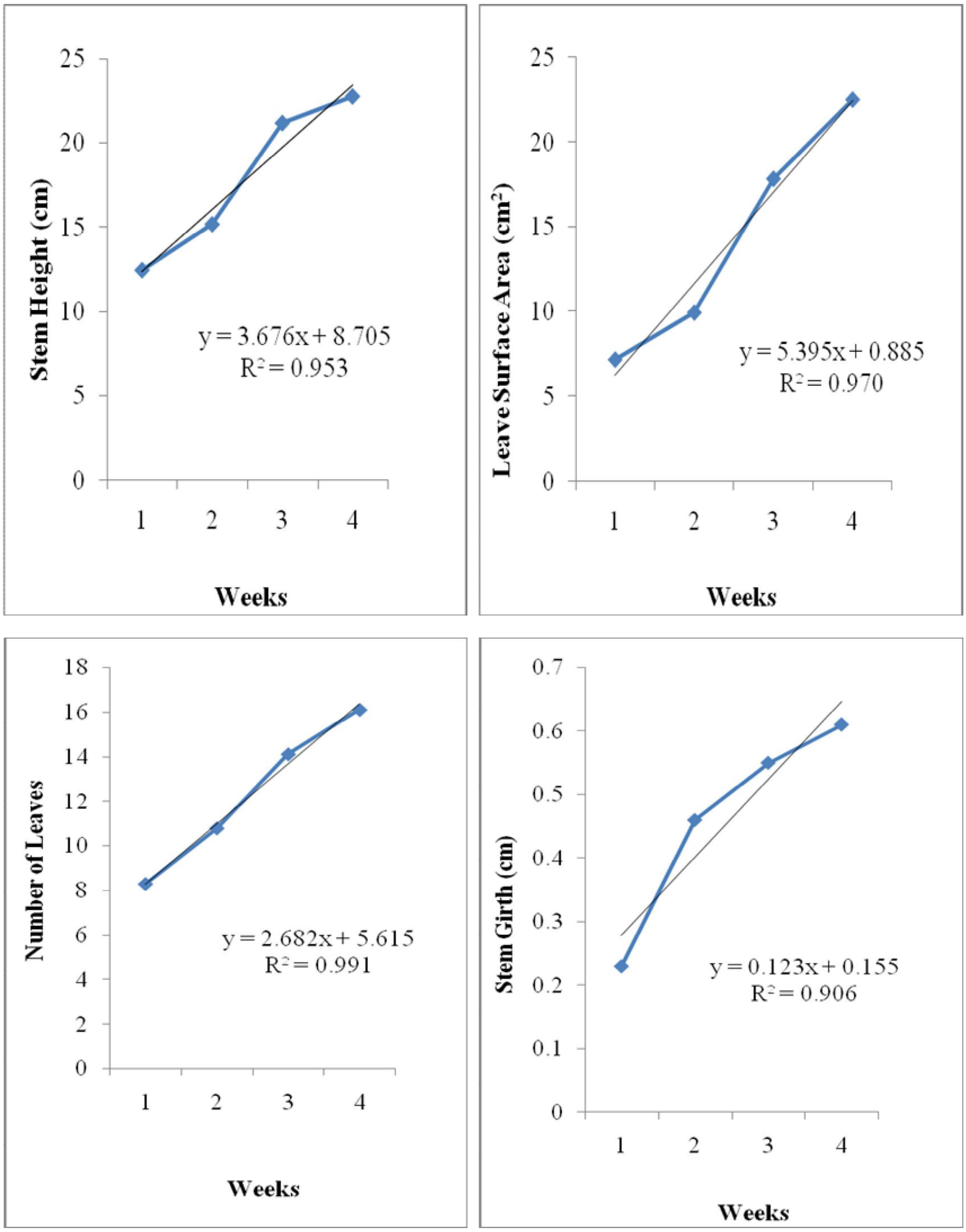

Fig.4: Regression analysis, showing the effect of 6 weeks of age on the response of Jute mallow seedlings to the ditrophic interaction

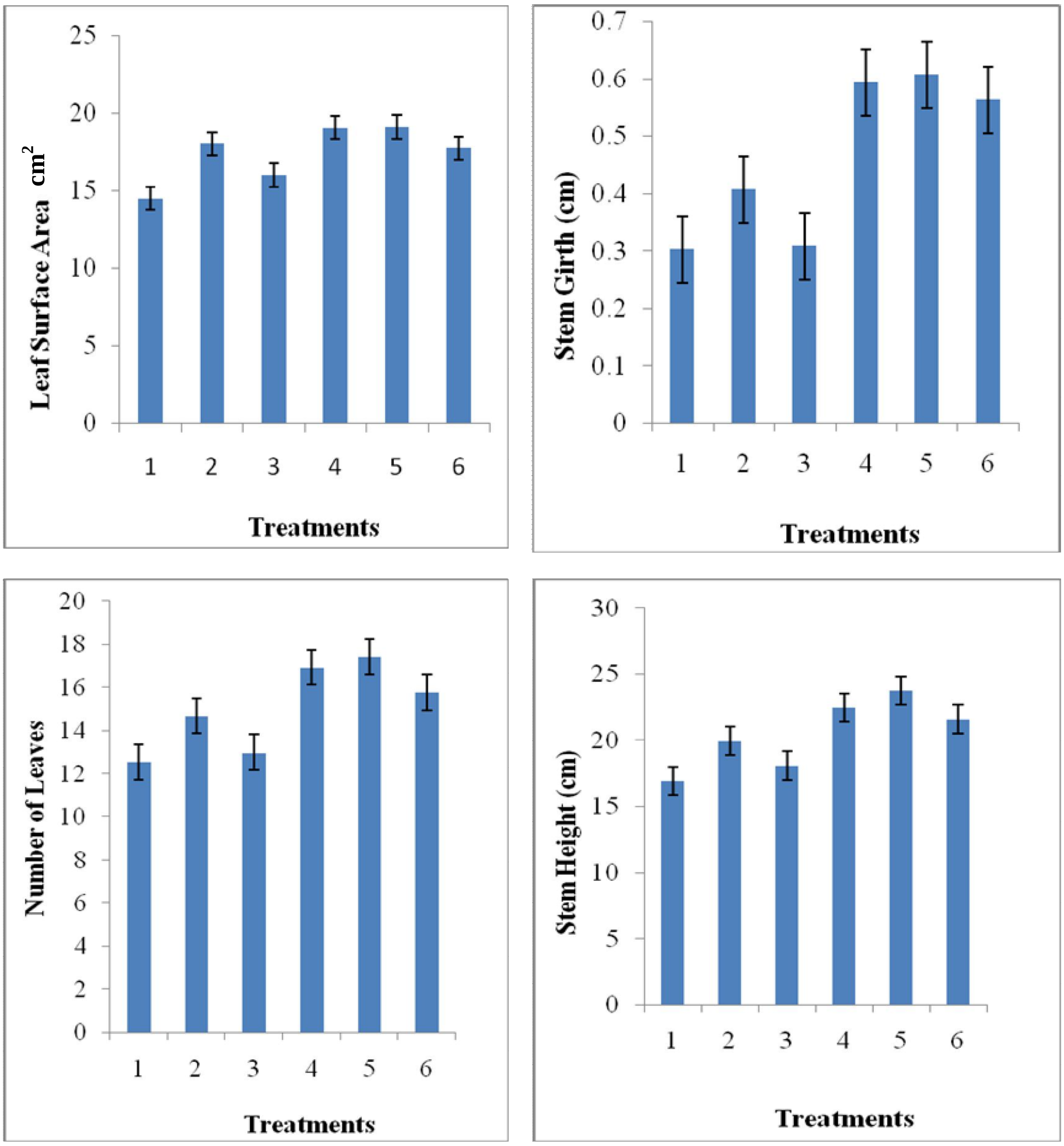

Fig.5: Effect of treatments on the growth parameters of eight weeks old Jute mallow seedlings

TREATMENT:

1……………… pathogen alone
2……………… pathogen and mycorrhizal
3……………… pathogen 2 weeks before mycorrhizal
4……………… mycorrhizal 2weeks before pathogen
5……………… mycorrhizal alone
6……………… control

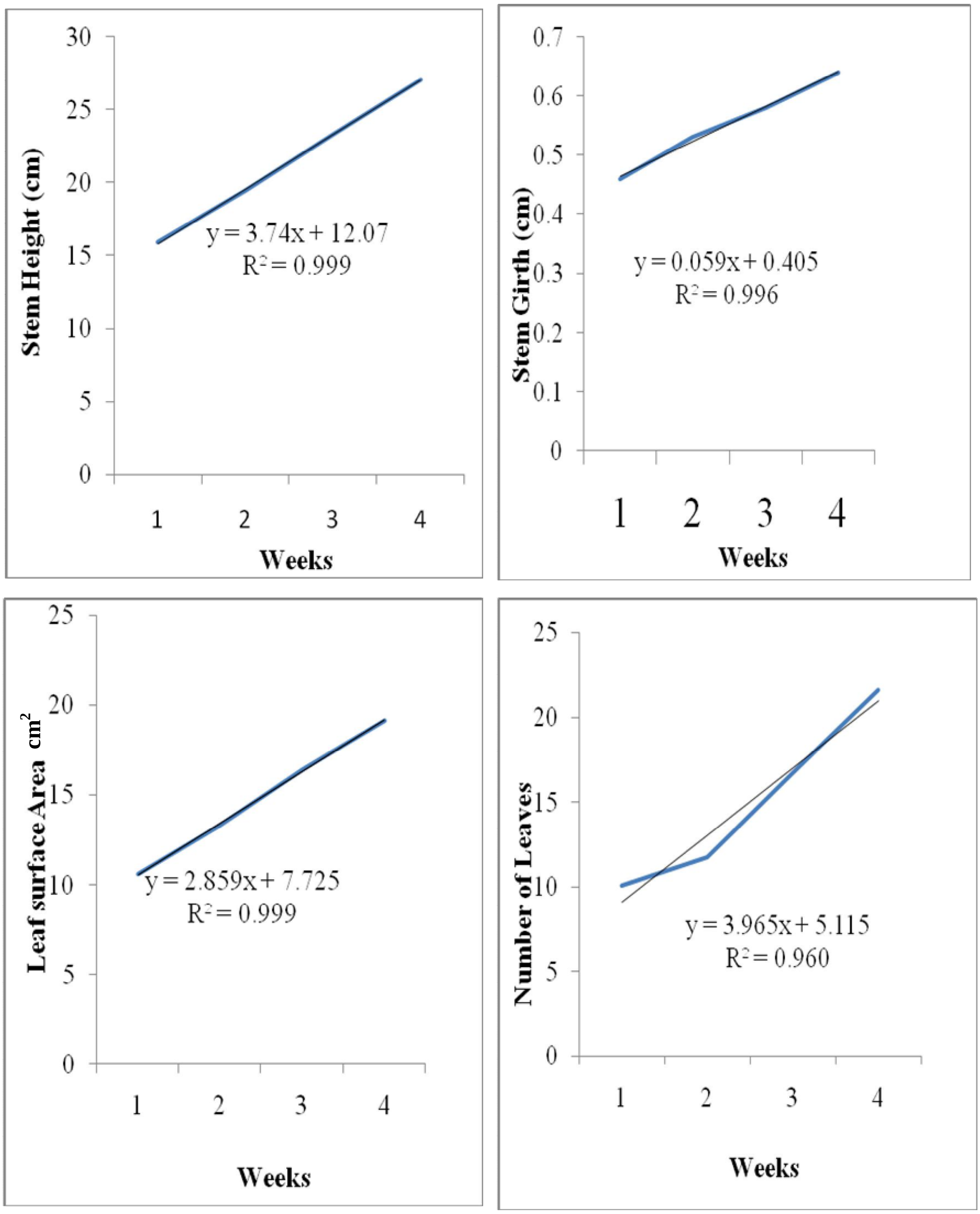

Fig. 6: Regression analysis, showing the effect of 8 weeks of age on the response of Jute mallow seedlings to the ditrophic interaction

DISCUSSION

Ditrophic interaction in food chain reveals the flow of energy in two trophic levels leading to predator-prey interactions (Thilo *et al*., 2004). However, these levels could lead to changes in parameters, sudden and discontinuous transitions to other types of dynamical behavior (Gragnani *et al*., 1998 & Boer *et al*., 1998). The results from this study show that there was a ditrophic interaction between *Glomus mosseae*, an arbuscular mycorrhiza (AM) fungus and *Phytophthora infestans* and that it has a biological control effect on the growth of jute mallow. This observation has been established and it is in line with the findings of McCauley and Murdoch, (1990); Yolande and Marcia, (2004); & Tyler *et al*. (2008). Further to this, is the establishment of the fact that the growth response and biomass of jute mallow seedlings inoculated with *G. mosseae* was significantly higher than that of non-mycorrhizal plants, both in the presence and absence of the pathogen. This shows that the flow of energy in this trophic level has led to the changes in the observed growth parameters (Boer *et al*., 1998). Mycorrhiza has also proven as observed in the levels of interactions in this study, to be a very useful agent of discontinuous transitions to other types of dynamical behavior in agricultural programme such as in nutrient uptake, growth parameters, inhibition of pathogens and improvement of yield. This was also opined by Gragnani *et al*. (1998) & Salami (2002). Plants inoculated with *G. mosseae* (mycorrhiza) had a lower incidence of necrosis; defoliation; and wilting than the non-mycorrhizal ones. This has also been observed by Schelkle and Peterson (1996); Heino Lepp (2002). In this study, mycorrhiza has been found to serve as a bio-control agent and an antagonist that have great potentials for the protection of plants against soil-borne pathogens which also stimulate the growth of plants. This was established in line with the observations of Arnaud *et al*. (1995); Shippers (1999); Quarles (1999); Salami (2008); and Mukerji, *et al*. (2006).

It was discovered from this study that plants inoculated with mycorrhiza two weeks before the pathogens had a lower incidence of the effects of the pathogen and the growth parameters were able to compare well with that of the control. This was also discovered by Dixon (2002) in his work where he found that mycorrhiza if formerly established in plants, acts as bio-stimulants, which are the natural products of organic composition that by their mode of actions, they positively contribute to crop nutrition and the development of healthy plants. It was also observed that AM fungi are recognized as high potential agent in plant protection and pest management especially in a 'predator-prey interactions (Quarles, 1999 & McCauley *et al*., 1999). Various mycorrhizal fungi also help protect the associated plants against pathogenic fungi, a number of soil microbes, heavy metals and toxic compounds. This could be found possible in a ditrophic interaction where the energy flow leads to a dynamic behavior of the organism with the upper hand in a 'predator-prey' interaction (Luckinbill, 1974; Heino Lepp, 2002, & Salami, 2005). Ditrophic interaction was established in this study at the different weeks of data collection after the inoculation exercise was carried out. Inoculation with *P. infestans* alone or before inoculating with mycorrhiza significantly reduced the growth parameters of the plant compared to simultaneous and dual inoculation with mycorrhiza before pathogen (dual inoculations). This greatly reduced the healthy growth of jute mallow seedlings as well as their biomass.

This was also found by Agbenin *et al*. (2000); Beuhamou *et al*. (1994); Dugassa *et al*. (1996); and Donnell *et al*. (2004) in their experiments. The stem height of jute mallow plants treated with *P. infestans* alone had the lowest mean value compared to other treatments. This trend also cuts across the other growth parameters (i.e. the mean values of leaf surface area, number of leaves and stem girth which also followed the same trend). This has been observed by Matsufuji *et al*. (2001); & Mukerji *et al*. (2006).

Jute mallow seedlings treated at age 8week old, showed a relative level of resistance against the pathogenic effect compared to that of 4 weeks. This however might be due to some antibacterial and antifungal compounds (methyl acetate) present in mature jute mallow plants at older ages (Gray and Smith (2005). It is known that extracts from the leaves of *Corchorus olitorius* show level of antibacterial and antifungal activities which in-turn inhibit fungal strains, like yeasts and molds especially in *Botrytis cinera, Aspergillus flavus, Aspergillus parasiticus, Aspergillus fumigatus,* and *Fusarium oxysporum.* (Semra *et al*., 2007). This antimicrobial activity was, however, not so significant at age 4 weeks of jute mallow seedlings but becomes more significant as the age of the seedlings increased. This shows that the antimicrobial activity of the plants responds to the age of the plants with the greatest effect at higher weeks of age. The could mean that the resistance by the older plants is age mediated and makes the levels of energy flow in the food chain to be more of 'predator-prey' interaction in an enrichment and food chain stability (Azuma, 1999; Abou Zeid, 2002; Thilo *et al*., 2004). Regression analysis of this result shows that there is significant difference in the number of weeks and treatments among the growth parameters (i.e. stem height, number of leaves, leave surface area and stem girth). This reveals a coefficient of determination (R^2) that is near perfect fit, which means treatments and age that are on the X axis are the major cause of variation establishing the predator-prey relationship in this ditrophic interaction observed (McCauley *et al*., 1999). It is also an indication that the response of jute mallow seedlings to this ditrophic interaction can be age mediated and could be seen as been incited by the infection of these micro-organisms when the treatments were inoculated into them. Thus, age and treatments used in this study are the major sources of variations that led to significance differences observed in the results. This has been opined by Kirk (1998); Dixon (2002) & Salami *et al.* (2005) in their study of tritrophic interactions between the mycorrhizal fungus, fungal pathogen and soil-borne fungal antagonist. It was reported that the tritrophic interactions was generally beneficial to the plant in moderating the severity of disease incited on it by the fungal pathogen.

Conclusively, this study has shown the beneficial effect of *Glomus mosseae* (mycorrhiza) association against infection by *Phytophthora infestans* (pathogen) in their ditrophic interaction which is a second level in food chain where predator-prey interaction exists. *Glomus mosseae* fungus acted as a bio-protective agent been able to suppress the incidence and severity of *Phytophthora infestans* in the ditrophic interaction. It also enhanced the growth parameters of jute mallow seedlings with respect to the time and mode of inoculations. However, caution should be taken in the time as well as mode of inoculation of these micro-organisms.

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