Assessment of micro-morphology of the leaves of *Solanum nigrum* (L.), *Amaranthus hybridus* (L.) and *Celosia argentea* (L.) cultivated using kola pod extract-mediated AgNPs as biofertilizer

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In this study, assessment was made on the growth response and micro-morphology of the leaves of *Amaranthus hybridus*, *Celosia argentea* and *Solanum nigrum* cultivated using super grow (Golden Neo-Life Diamite-(GNLD) bio-fertilizer, kola pod extract-mediated AgNPs with super grow. AgNPs was synthesized biologically making use of pod extract of *Cola nitida*. A pot experiment was conducted to cultivate the three vegetables on soil from botanical gardens, LAUTECH in 5 kg soil capacity pots and super grow (biofertilizer) and mixture of AgNPs and super grow were applied at the second week after planting. Data were collected from the plant growth parameters. Random collection of the leaves was done at the onset of flowering and prepared for viewing with a binocular light microscope (Olympus) at 100X and 400X magnifications. Photographs were obtained digitally with Android phone using Microsoft image programme for windows. Statistical analysis of variance was done used MINITAB release 17, and means were separated with Fisher’s Least significant different at p<0.05. Leaves of plant treated with AgNPs have their micro organs (stomata and subsidiary/epidermal cells) bigger in sizes and increased in densities. This situation was observed in the leaves of the three plant species used in this study. Conclusively, Kola pod extract-mediated AgNPs strengthened the super grow and influenced the growth response as there was significant difference among the values recorded from the treatments. Also, micro-morphology of the AgNPs treated plant samples recorded significant values.

**Keywords:** Micro-morphology, vegetables, Kola pod extract-AgNPs, light microscopy
1 Introduction

Plants being the most dependable group of living organisms in space; they have vital roles they play in the existence of other living organisms that make them indispensable. Vegetable among plants is recognized as a cheap source of micronutrients needed by man [1]. Among the leafy vegetables that are common on consumers’ table are *Amaranthus hybridus* and *Celosia argentea*, while *Solanum nigrum* rarely appear in the markets. However, their spread nearly round the world bring about various forms and structures as a result of living conditions, genetic structure and phylogenetic characteristics [2]. Micro-morphological parameters of different plants have been used as aids in the taxonomical recognition of plant species [3]. Therefore, the possibility of alteration in micro-morphology of the plant (especially the leaf organs) when cultivated on different media is necessary for investigation. Cultivation of edible leafy vegetables like *Solanum nigrum* (L.), *Amaranthus hybridus* (L.), and *Celosia argentea* (L.), concentrates on leaves as the essential part for consumption which is believed to alleviate malnutrition problems, simply because of their nutrient composition, immune-boosting activities and so on [4-6]. Highest priority is therefore given to leaf production of the vegetables in this study; this is due to the fact that leaf reflects the consequence of nutrient deficiency in vegetables [7].

Generally the use of organic fertilizer is believed to be safe, cost effective and thus available within the reach of every one [8]. Application of green synthesized nanoparticles in agriculture with suitable results has been to the knowledge of many researchers. Nanotechnology is believed to be one of the key events in biotechnologies of the 21st century. The promise of nanotechnology to improve current agricultural practices through the enhancement of management and conservation of inputs in crops has permitted its expansion to different areas of specialization [9]. The use of nanofertilizers has shown many advantages over others as mentioned: (i) increased bioavailability (Easy dissolution; improved penetration/permeation through membranes); (ii) lower doses; (iii) lower dose-dependent toxicity; (iv) controlled release; (v) targeted biodistribution; (vi) reduction of the influence of environment on bioavailability variability [9-12]. For these reasons, acceptability is rapid even though the hazards assessment can never be overemphasised. The application of nanofertilizers as reported by DeRosa [13] has been found to have reduced nitrogen loss due to leaching, emission and long-term assimilation by soil microorganisms [13]. Other credibility to the use of nanofertilizers has been its accessibility to seed and root tissue and modulatory effects of phytochemicals [14-15]. This has established the possibility of developing new nutrient delivery systems that exploit the nanoscale porous domains on plant
surfaces and show sustained release of nutrients on demand, while preventing them from premature conversion into chemical/gaseous forms that cannot be absorbed by plants [16]. The new trend brought by the application of green synthesized nanoparticles in agriculture has permitted the use of biosynthesized AgNPs mixed with super grow (biofertilizer) to cultivate three vegetables in this study. Therefore, this study aim at assessing the growth response and micromorphology (stomata) of the three vegetables in this study to foliar application of super grow (GNLD organic fertiliser) when mixed with Kola pod extract-mediated AgNPs and when applied alone.

2 Materials and Methods

2.1 Study area and plant preparation

The study was carried out in the Research farm of Botanical gardens, LAUTECH, Ogbomoso on Lat. 8.15977° N Long. 4.27426° E. Soil was collected from the same place to cultivate the three (3) vegetables: Amaranthus hybridus, Celosia argentea and Solanum nigrum. Super grow (Organic liquid fertilizer made from poultry droppings and sea bird guano) was bought from Golden Neo-Life Diamite (GNLD) International and the stock concentration was diluted at 1ml/litre of water. Kola pod extract-mediated AgNPs was prepared and characterized as explained by Lateef et al. [17]. Exactly 12.5 µl/ml was added to the diluted super grow. Foliar application of the treatments started from the second week after planting till the onset of the flower. Application was done twice in a week as biofertilizer for the 3 vegetables. The control plant samples of each of the three species were treated with water alone and each of the treatments was replicated 5 times.

2.2 Data collection and microscopic study

Data were taken from growth parameters: plant height, stem girth, number of leaves, and leaf area. At flowering stage of each of the vegetables, 3 freshly harvested leaves from 3 pots of each plant species were randomly plucked; cut into 10 mm-sizes and prepared for observation under light microscope. A strip of lower and upper epidermis from the middle portion of the leaves was carefully peeled off to show the transparent part [18]. Images showing the number, arrangement of stomata with guard cells and subsidiary cells were captured with the Android phone (Infinix Hot 6) fitted in the light microscope as described by Otang et al. [19]. The images were captured digitally using Microsoft image programme for windows. Leaf stomatal density was determined and expressed as the number of stomata per unit area [11, 19]. This was achieved by counting the number of full or half shown stomata within 50 µm unit area at 400X magnification on the lower and upper (adaxial and abaxial) surfaces of the
specimens. Density of stomata per mm$^2$ was calculated for each plant species and recorded for each treatment. With the assistance of the concept of Silva et al. [20], assessment was made based on the number, type of arrangement and position of accessory cells surrounding the guard cells of the stomata.

2.3 Statistical analysis
All data on growth parameters and stomata density were subjected to statistical analysis using MINITAB Release 17. A one-way analysis of variance (ANOVA) was used to compare the mean values among the plant treatments. Means were segregated using Fisher’s Least Significant Difference (LSD) paired wise comparison. The means were treated as significantly different at p < 0.05.

3 Results and Discussion
From the growth parameters measured from the 3 types of vegetables in this study, exponential increases was recorded in the growth values (number of leaves, length of median mid-rib and leaf area) of the treated plant samples cultivated with mixture of AgNPs and super grow, and super grow alone. The increase was observed immediately after the application of the growth promoters in the second week of plant growth as presented on Tables 1a-3d. Plant samples cultivated with mixture of AgNPs and super grow recorded significant increase at P< 0.05 compared to samples treated with super grow alone and the control samples (Figures 1a–3d). These results corroborate those of Ghahremani et al. [21] on the exponential increase in their results from foliar application of nano-calcium chelated fertilizers on *Ocimum basilicum*. However, the control plant samples recorded significantly low values in growth characteristics of the 3 plant types.
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Fig. 1a: Effect of growth promoters on number of leaves of *A. hybridus*

Fig. 1b: Effect of growth promoters on length of median rib of *A. hybridus*

Fig. 1c: Effect of growth promoters on leaf area of *A. hybridus*

Fig. 1d: Effect of growth promoters on stem width of *A. hybridus*
The distribution of the stomata in all the 3 vegetables is hypo-amphistomatic as stomata were present in both adaxial and abaxial surfaces of the leaves but with higher densities in the adaxial surface (Figures 4-6). This result is in line with earlier studies [22-23]. It was observed in the 3 vegetables that stomata densities on both adaxial and abaxial surfaces of the leaf samples treated with mixture of AgNPs and super grow were significantly higher at p <0.05 compared to those of super grow alone and the control samples (Table 1). Paracytic stomatal arrangement which is a simple type of arrangement is of highest frequency in plant samples with mixture of AgNPs and super grow. Control plant leaves recorded high frequency of anisocytic and tetracytic stomatal arrangement (Table 1). The paracytic stomatal arrangement in those leaves treated with AgNPs may be the potential mechanism of the AgNPs to increase the stomatal densities on the leaf surfaces. It accommodates more stomatal complex which might have given the chances of the AgNPs treated leaves to increase the photosynthetic activities and produce more carbohydrates. Furthermore, the possibilities of
the plants’ growth increase at faster rate, as there are evidences of improvement of plant growth under the influence of application of AgNPs [9, 15, 24].

Table 1. Comparative assessment of stomatal arrangement in three studied vegetables

| Vegetables                        | Amaranthus spinosus | Celosia argentea | Solanum nigrum |
|-----------------------------------|---------------------|------------------|-----------------|
| Control abaxial stomata density   | 16<sup>b</sup>      | 16<sup>b</sup>   | 15<sup>b</sup>  |
| AgNPs + Super grow abaxial       | 24<sup>a</sup>      | 26<sup>a</sup>   | 22<sup>a</sup>  |
| Control adaxial stomata density  | 28<sup>b</sup>      | 18<sup>b</sup>   | 18<sup>b</sup>  |
| AgNPs + Super grow adaxial       | 37<sup>a</sup>      | 32<sup>a</sup>   | 29<sup>a</sup>  |
| Types of stomata arrangement abaxial | Control: 16 anisocytic; 12 tetracytic; 5 paracytic; 7 anisocytic | Control: 16 paracytic; 26 paracytic | Control: 13 anisocytic; 2 paracytic; 22 paracytic |
| Types of stomata arrangement adaxial | Control: 18 paracytic; 6 tetracytic; 24 paracytic; 13 anisocytic | Control: 18 paracytic; 32 paracytic | Control: 12 paracytic; 6 anisocytic; 29 paracytic |
Figure 4. Stomata arrangement in *Amaranthus hybridus*

Figure 5. Stomata arrangement in *Celosia argentea*
Figure 6. Stomata arrangement in *Solanum nigrum*

4 Conclusion
In all the 3 vegetables in this study, significant improvements of production characteristics was recorded with Kola Pod extract-mediated AgNPs and super grow mixture. The mixture also increased the stomata densities, and influenced the stomata arrangement in the leaves. The paracytic type of stomata arrangement is to the maximum. This type of arrangement accommodates more stomata on the leaf surface which increases photosynthetic potentials of the stomata thereby ensuring the production of more carbohydrates which may likely lead to accumulation of more plant biomass, and encourages the plant fast growth.

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