ISSN 0331-7277

NIGERIAN JOURNAL OF PLANT PROTECTION

Published by:
THE NIGERIAN SOCIETY FOR PLANT PROTECTION

Volume 29 (2015)

NIGER. J. PLT. PROT. 29:56- 65 (2015)

EFFECTS OF FERTILIZER RATES ON GROWTH AND YIELD CHARACTERS OF OKRA (*ABELMOSCHUS ESCULENTUS* [L.] MOENCH) INOCULATED WITH *CUCUMBER MOSAIC VIRUS*

*Salaudeen, M. T., Abubakar, S. K., Bello, L. Y. and Oyewale, R. O.

Department of Crop Production, Federal University of Technology, P. M. B. 65, Minna, Niger State, Nigeria *mtsalaudeen.fut@gmail.com

SUMMARY

The impacts of NPK 15:15:15 fertilizer rates on growth and yield characters of okra (*Abelmoschus esculentus* [L.] Moench) plants inoculated with *Cucumber mosaic virus* (CMV) were investigated under field conditions in Minna, Nigeria during the 2014 cropping season. Four treatments (CMV only; CMV + 100 kg/ha NPK; CMV + 200 kg/ha NPK; and 300 kg/ha NPK) were arranged in randomised complete block design with four replications. Seedlings were mechanically inoculated with CMV at 10 days after sowing. Virus detection and quantification was accomplished by Enzyme-Linked Immunosorbent Assay (ELISA). Data were collected on the growth and yield characters. The data were subjected to analysis of variance and significance was determined at *p*=0.05. Significance means were separated using the least Significance Difference (LSD). All the plants elicited the typical symptoms of CMV infection, regardless of the treatment. Plants without fertilizer suffered the highest disease severity (4.1) and virus concentration (1.2), and also recorded the lowest fruit weight (30.5 g) per plant. Application of 100 kg/ha of NPK resulted in the lowest disease severity (2.0) and virus concentration (0.6) as well as the highest fruit weight per plant (58.5 g). Application of 100 kg/ha of NPK is recommended for good growth and appreciable yield of okra under CMV disease pressure.

Keywords: CMV, disease tolerance, fertilizer rates, plant growth, virus concentration

Okra (*Abelmoschus esculentus* [L.] Moench) is one of the major vegetable crops in tropical and subtropical Africa. It is a good source of essential vitamins, calcium, potassium, and other minerals (23). The protein (2 %) and fatty acid (20 %) contents of okra seeds are comparable to those in soybean and cotton seed oil, respectively (25). Its leaves and fruits are consumed by cooking or frying and the leaves are sometimes fed to domestic livestock. Okra is rich in fibers which play a significant role in ameliorating various ill-healths such as ulcer (19), asthma, constipation, colon cancer and obesity. In addition to its tremendous medicinal potentials okra's mucilage also has well-known industrial applications (2). According to FAO (11), about 60.9% of the Okra produced in Africa in 2011 is from Nigeria. Okra productivity is generally low in Africa partly owing to weed infestation, and attack by insect pests and pathogens (3, 13). Cucumber mosaic virus (CMV) disease is one of the major causes of significant yield losses in okra fields.

In Nigeria and many parts of Africa okra production is mainly undertaken by smallholder farmers either as sole or in mixtures with cereal crops (15) such as maize, sorghum and millet. Application of inorganic fertilizers for soil improvement (6) is prevalent due to poor soil fertility and high nutrient demand at various growth stages of the crop. Inorganic fertilizer recommendations for okra vary with the variety and soil conditions. Nitrogen (N), phosphorus (P) and potassium (K) are the primary nutrients required in large quantity by crops (8) including okra. According to FAO (10), nitrogen fertilizer makes up 50 % of all the nutrient inputs, and its availability plays an important role in determining crop yield. In Nigeria, the recommended rate for vegetable crops is 50-100 kg N/ha, 20-60 kg P_2O_5/ha and 40-60 kg K_2O/ha. Application of 60 kg/ha of NPK 20:10:10 has been reported to be adequate in a lowland ecology. While Omotoso and Shittu (21) documented that NPK fertilizer application significantly increased yield and yield components of okra at 150 NPK kg/ha, Iyagba *et al.* (13) suggested application of 200 kg/ha.

Mineral nutrition enables the plant to develop mechanical barriers and also to synthesize natural defense compounds such as antioxidants, flavanoids and phytoalexins against pathogens (8). However, the level of tolerance to insect pests and diseases reduces as nutrient concentration deviates from the optimum (26). Potassium is the most important mineral element promoting tolerance to infections because of its vital role in plants' biochemical and physiological processes (27). In another study Omorusi and Ayanru (20) reported that the incidence of cassava mosaic disease (CMD) was suppressed upon application of 100 kg/ha NPK However, balanced nutrient supply ensures optimal plant growth and is usually considered adequate for disease tolerance (8). The objective of this study was to determine the influence of different rates of NPK 15:15:15 fertilizer on growth and yield characters of okra inoculated with *Cucumber mosaic virus*.

MATERIALS AND METHODS

Virus Source and Propagation

The *Cucumber mosaic virus* isolate used was obtained from the International Institute of Tropical Agriculture (IITA), Ibadan. *Cucumber mosaic virus* inoculum was multiplied by rubbing the Carborundum (600-mesh)-dusted leaves of the cowpea cv. Ife Brown plants with CMV sap at 10 days after sowing. Leaves were ground (1:10; w/v) with inoculation buffer, pH 7.2 (0.1M sodium phosphate dibasic, 0.1M potassium phosphate monobasic, 0.01M ethylene diamine tetra acetic acid and 0.001M L-cysteine per litre of distilled water) using a cold sterilized mortar and pestle and a drop of 2-mercapto ethanol (β-mercapto ethanol) was added just before used. The inoculated plants were monitored for symptom development in a screenhouse; symptomatic leaves were harvested and used for subsequent inoculations.

Sowing, Treatments and Experimental Design

The seeds of a popular okra cultivar "Goro" obtained from a local farmer in Minna were used. Two field experiments were established simultaneously at the Teaching and Research Farm of the Department of Crop Production Federal University of Technology, Minna (06.44^{0}E, 09.51^{0}N; 220 m). The textural class of the soil was sandy loam, consisting of 71 % sand, 15 % silt and 14 % clay. Treatments were arranged as randomised complete block design with four replications. Each treatment consisted of five ridges; the two outer ones served as guard rows. Seeds were sown on 24th June, 2014. The seeds were sown at 1 m × 0.30 m inter and intra-row spacing. NPK 15:15:15 fertilizer was applied (0, 100, 200, and 300 kg/ha) at the time of planting. The seeds were treated with Cibaplus at the rate of 10 g per 2 kg of seeds. Five seeds were sown per hole and seedlings were thinned to three plants per stand. Plots were manually weeded when necessary and sprayed fortnightly with an insecticide (Cypermethrin 10% E.C.), according to the manufacturer's instructions.

Sap Inoculations, Data collection and Serological test

Seedlings were inoculated with the virus at 10 days after sowing, using the procedure described above. Disease incidence, disease severity, growth and yield data were collected. Plants were scored for CMV disease severity using a 1 to 5 scale developed by Arif and Hassan (4) based on the magnitude of symptoms on the leaves and general growth conditions of the inoculated plants. In the scale:

1 = no symptoms (apparently healthy plant);

2 = slightly mosaic leaves (10-30 %);

3 = mosaic (31-50 %) and leaf distortion;

4 = severe mosaic (51-70 %), leaf distortion and stunting;

5 = severe mosaic (>70 %), stunting and death of plants.

Leaves from each treatment were sampled on the day the final disease severity data were taken and these were subjected to Enzyme-Linked Immunosorbent Assay (ELISA) according to Koenig (14). Samples for test were prepared by extracting in cold carbonate buffer, pH 9.6 (0.015 M sodium carbonate plus 0.0349 M sodium bicarbonate per litre of distilled water), at a ratio of 1:10 (w/v). Negative control sample was prepared from the leaves of a healthy okra plant maintained in an insect-proof screenhouse. The leaf of a confirmed CMV-infected okra plant was used as a positive control. Each sample was tested in duplicated wells of the polystyrene microtitre ELISA plate (Thermo Scientific "Nunc", Milford, MA). The plate was incubated at 37 °C for 1 h, washed thrice with phosphate buffered saline-Tween (8 g NaCl, 1.1 g Na 2 HPO 4, 0.2 g KH 2 PO_4, 0.2 g KCl, 0.5 mL Tween-20, 1 L distilled water, pH 7.4) (PBS-T) and tap-dried. Two hundred microlitres of a blocking solution [3 % (w/v) of dried nonfat skimmed milk in PBS-T] was dispensed into each well, the plate was incubated at 37 °C for 30 minutes, emptied of its contents and dried. Next was addition of 100 µL of the polyclonal antibody diluted (1:10, 000; v/v) in conjugate buffer [half strength PBS-T containing 0.05 % (v/v) Tween-20, 0.02 % (w/v) egg albumin, 0.2 % (w/v) polyvinylpyrolidone].

The polyclonal antibody raised against a Nigerian CMV isolate at the Virology and Molecular Diagnostics Unit, IITA, Ibadan was kindly provided by Dr. P. Lava Kumar (IITA, Ibadan). One hundred microlitres of a mixture of healthy okra leaf sap and conjugate buffer (1:20; v/v) was added to each well and the plate was incubated at 37 °C for 30 minutes. After washing the plate three times 100 µL of the goat anti-rabbit diluted with conjugate buffer (1:15,000) was added to each well and the plate was incubated at 37 °C for 1 h. The plate was washed and tap-dried and *p*-nitrophenyl phosphate dissolved in substrate buffer (97 mL diethanolamine, 1000 mL H_2O, pH 9.8) at the rate of 1mg/mL and 100 µL of this was added to each well. The plate was incubated in dark at room temperature (37 °C) for 1 h. Plate was assessed by recording the absorbance at 405 nm using a microplate reader (MRX, Dynex Technologies, Inc., USA). Values were considered to be positive when the absorbance values were at least twice that of the average for the negative controls.

Statistical analysis: Data were subjected to analysis of variance and where the *F*-test was significant ($p \leq 0.05$) treatments' means were separated using the Least Significant Difference (LSD). Data were analyzed using statistical analysis system (24).

RESULTS AND DISCUSSION

Symptoms of CMV disease were observed at 10 days after inoculation. Leaf mottling with pockets of light green areas, chlorosis and curling were seen, regardless of the treatment. None of the treatments proved superior by way of limiting symptoms development at the early stage of infection (2 wk after inoculation) (Fig. 1). The impact of NPK fertilizer rates on the severity of infection began to manifest afterwards. From 3 – 5 weeks after inoculation (WAI), disease severity spread rapidly in the plants without fertilizer. Application of 100 kg/ha NPK consistently reduced CMV symptoms to the lowest level, followed by the application of 200 kg/ha NPK. Moreover, in the plants treated with either 100 or 200 kg/ha NPK the progress of infection was slow and the weekly increase was quite negligible. Application of 300 kg/ha NPK promoted disease severity but the intensity of infection was not as dramatic as in the plants without fertilizer. The appearance of CMV symptoms at 10 days after inoculation is similar to the result published by Agrios and Walker (1) when pepper plants were inoculated with CMV. Moreover, the observed symptoms are in agreement with those reported by Balogun *et al*. (7) when some okra lines were challenged with CMV.

The severity of CMV disease was uniform in all the plants at the early period of infection partly because the plants were still young and the applied fertilizer had not been fully utilized. Although the N component of the NPK fertilizer could be released much earlier than P and K the influence of P and K seems to be more important for building plants' resistance to infection (9, 12). Because symptoms expression was lowest in the plants treated with 100 kg/ha of NPK it could be said that excessive fertilizer application suppresses plant's tolerance to infection. This is in line with the finding of Bhaduri *et al.* (8) who reported that symptoms of virus infections sometimes disappear when N supplies are large but the entire plant is infected. Bhaduri *et al.* (8) elucidated that visible symptoms are dependent upon the competition for N between the virus and the host cells. This implies that the extra nutrient supplied is not completely utilized for plant's overall growth and development.

Different crops have different optimum nutrient requirements and a certain amount is required for plants to cope with the stresses resulting from viral infection. Arraudeau (5) recommended that lower dose of N and higher rate of potassium would reduce cassava mosaic disease severity. Ogbe *et al*. (18) suggested application of a balance NPK fertiliser to cassava varieties susceptible to CMD, to control the incidence and severity of infection. Similarly, Omorusi and Ayanru (20) reported that CMD infection was depressed by application of 100 kg/ha of NPK compared to the use of 10 kg/ ha. However, Muengula-Manyi *et al*. (16) found that CMD incidence and severity were increased significantly when NPK dosage of 300 Kg ha was applied, similar to the result obtained in this study. ELISA result showed that virus concentration was highest in the leaves of plants without fertilizer, whereas the lowest titre was found in those treated with 100 kg/ha NPK (Fig. 2). However, the average virus concentration in the leaves of plants treated with 200 was not significantly different from those receiving 300 kg/ha of NPK. These results indicate a positive correlation between visual disease assessment and serological test.

When the first assessment was made at 2

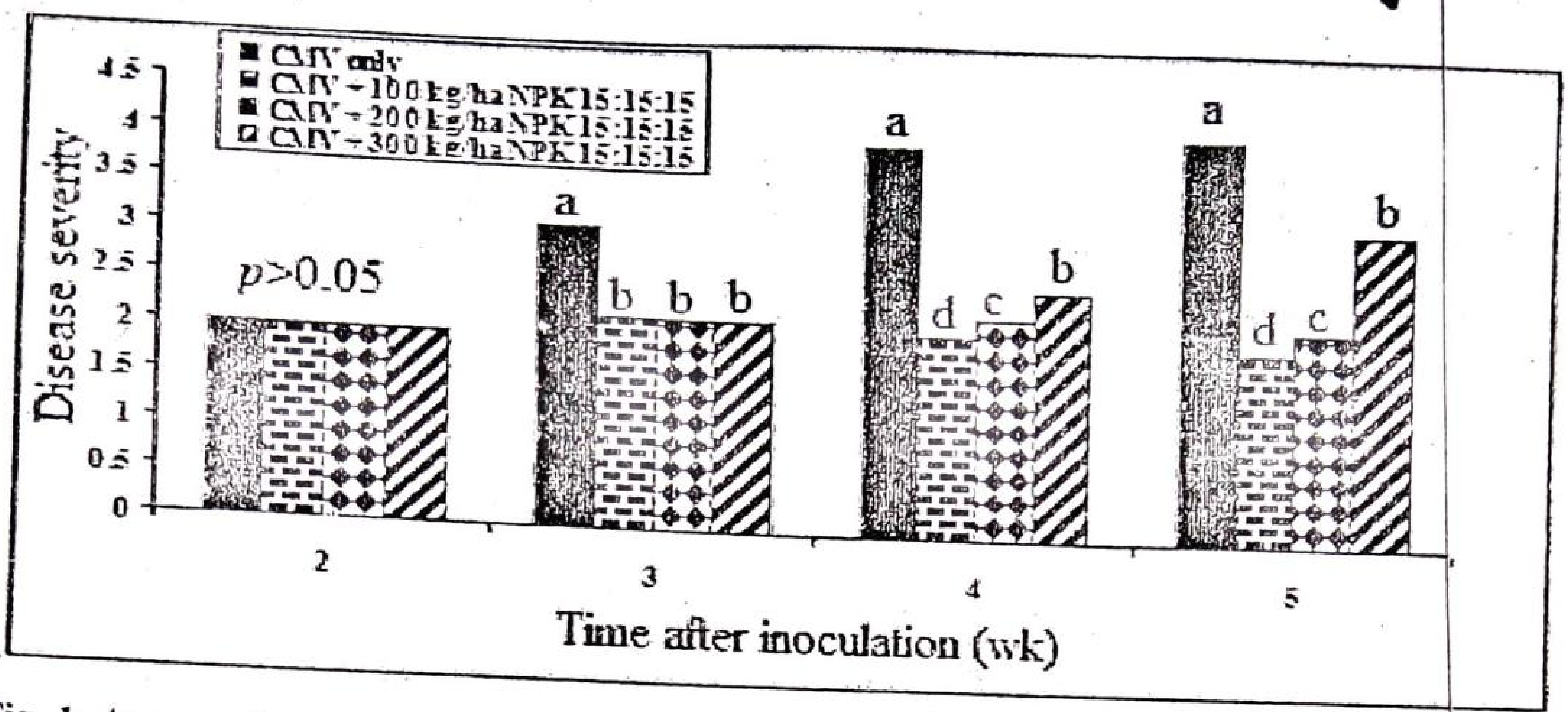

Fig. 1: Average disease severity on okra plants at different rates of NPK 15:15:15 fertilizer and of those without fertilizer after inoculation with *Cucumber mosaic virus*

Bars labelled with dissimilar letter within the same week differ significantly according to Least Significant Difference (LSD) at p=0.05

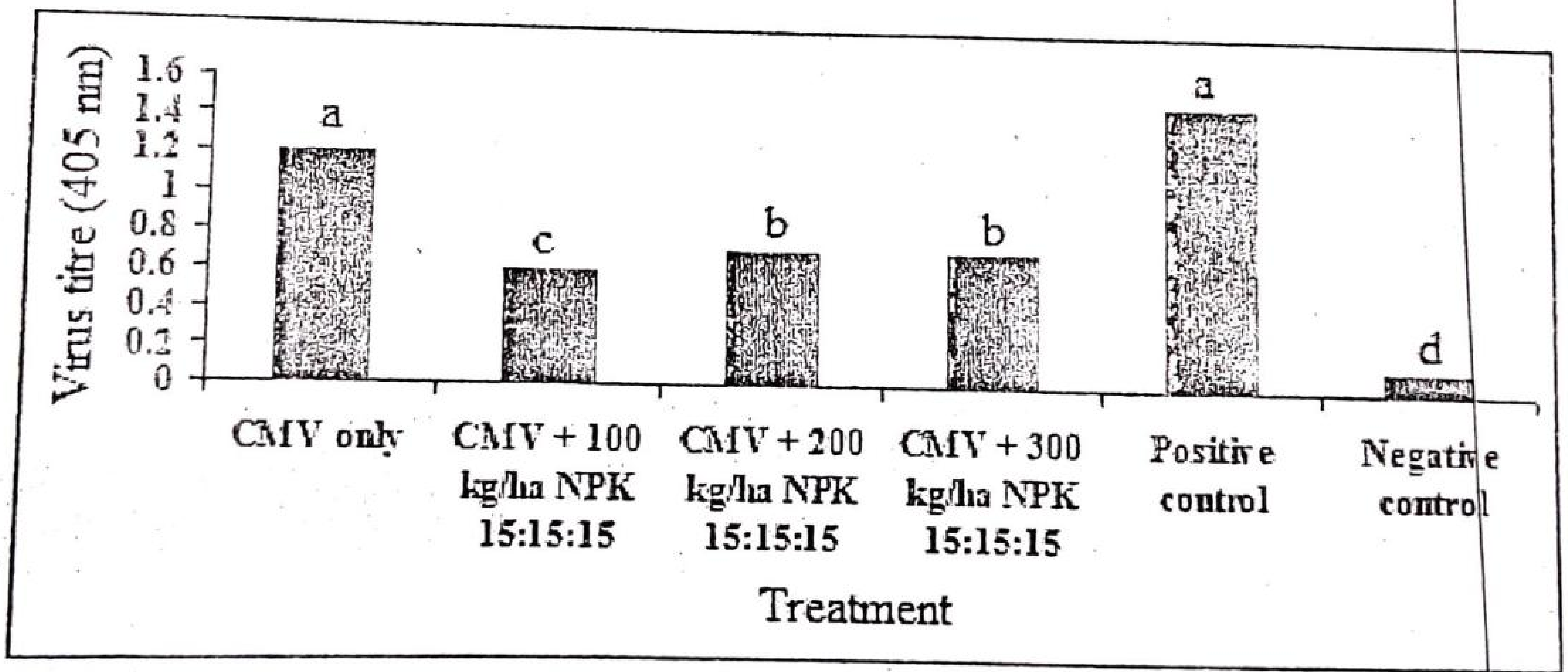

Fig. 2: Relative titre of *Cucumber mosaic virus* (CMV) in okra plants at different rates of NKP 15:15:15 fertilizer and of those without fertilizer after inoculation with CMV

Bars labelled with dissimilar letter differ significantly according to Least Significant Difference (LSD) at p=0.05

WAI, different treatments resulted in the same number of leaves per plant (Fig. 3 A). Subsequently, striking variation was noted between the plants without fertilizer and those treated with NPK fertilizer. This underscores the role of nutrition in host – pathogen interaction. In the plants without fertilizer, average leaf number slightly increased from 6 at 2 WAI to 8 at 3 WAI; in others the value increased from 6 to 10. At 4 and 5 WAI, the number of leaves per plant marginally increased by 1 and 2, respectively in the plants without fertilizer but in others it increased by 2 and 4,

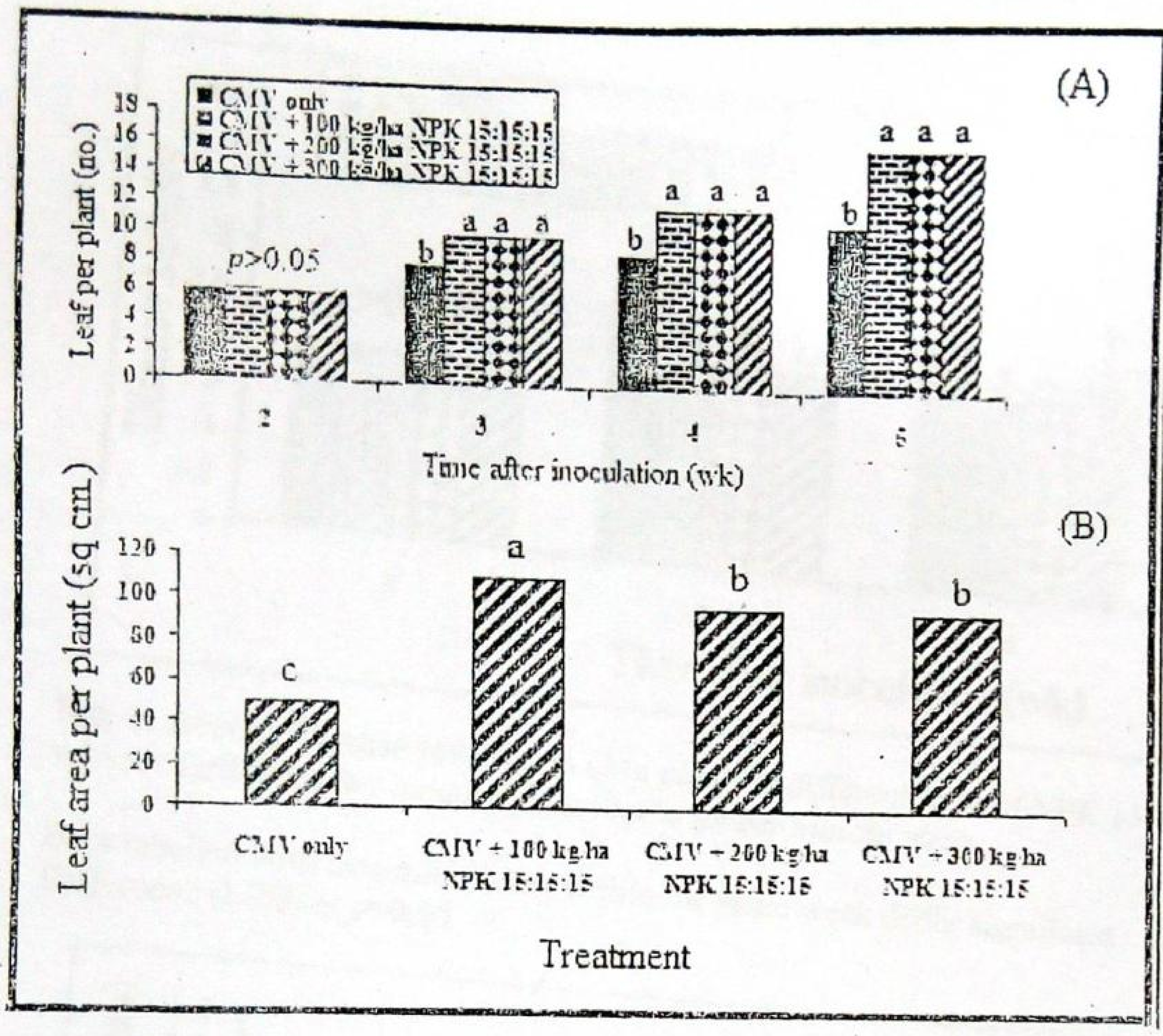

Fig. 3: Average (A) number of leaf per plant and (B) leaf area per plant of okra plants at different rates of NPK 15:15:15 fertilizer and of those without fertilizer after inoculation with *Cucumber mosaic virus*

Bars labelled with dissimilar letter differ significantly according to Least Significant Difference (LSD) at p=0.05

respectively. Application of 100 kg/ha NPK resulted in the broadest leaf area of about 56 % over the plants without fertilizer (Fig. 3 B); plants without fertilizer treatment produced conspicuously narrow leaves. Use of either 200 or 300 kg/ha NPK fertilizer resulted in similar leaf area but the former gave higher figure. The data in Fig 3 B also reveal that increasing NPK fertilizer rate from 100 to 200 or 300 kg/ha resulted in about 13 % leaf area reduction. The higher number of leaves produced by the plants treated with fertilizer portends better level of photosynthesis. The narrow leaf area observed in plants without fertilizer was due to the deleterious impact of the virus. Reduction in leaf area has a direct and positive relationship with the level of photosynthesis (22), which ultimately influences plant's growth and yield.

The differences in the heights of plants treated with 100, 200 and 300 kg/ha of NPK fertilizer were not significant but those treated with 300 kg/ha were the tallest (Fig. 4). The plants in the control treatment (without fertilizer) were severely affected by CMV with characteristic stunting. In addition, plant height was less than in those receiving other treatments. Height increase from 2 – 3 WAI was approximately 42 % in the control plants (without fertilizer). On the other hand, an increase of about 58.1, 58.6 and 59.4 was observed in the plants where 100, 200 and 300 kg/ha of NPK, respectively was applied. Generally, the

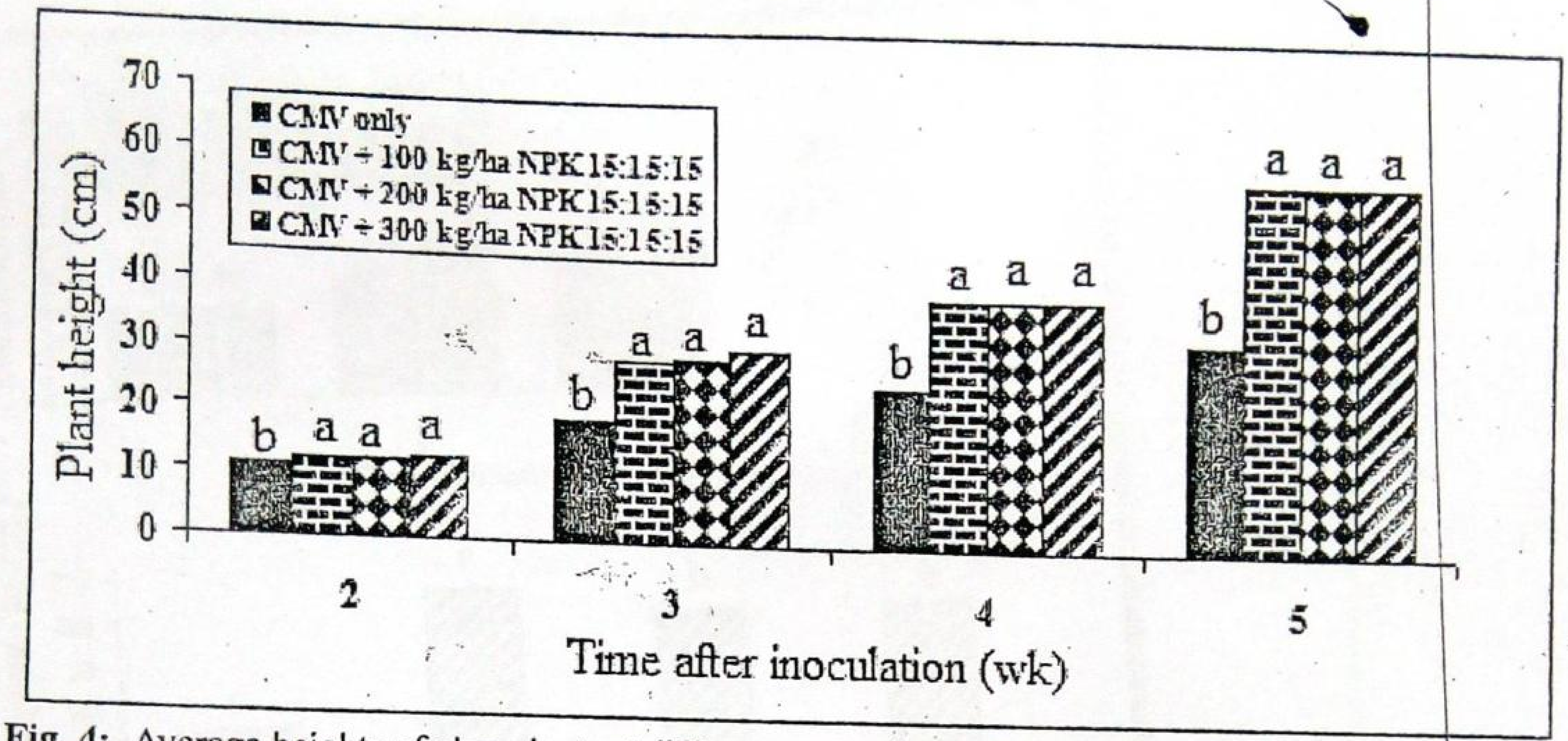

Fig. 4: Average heights of okra plants at different rates of NPK 15:15:15 fertilizer and of those without fertilizer after inoculation with *Cucumber mosaic virus*

Bars labelled with dissimilar letter differ significantly according to Least Significant Difference (LSD) at *p*=0.05

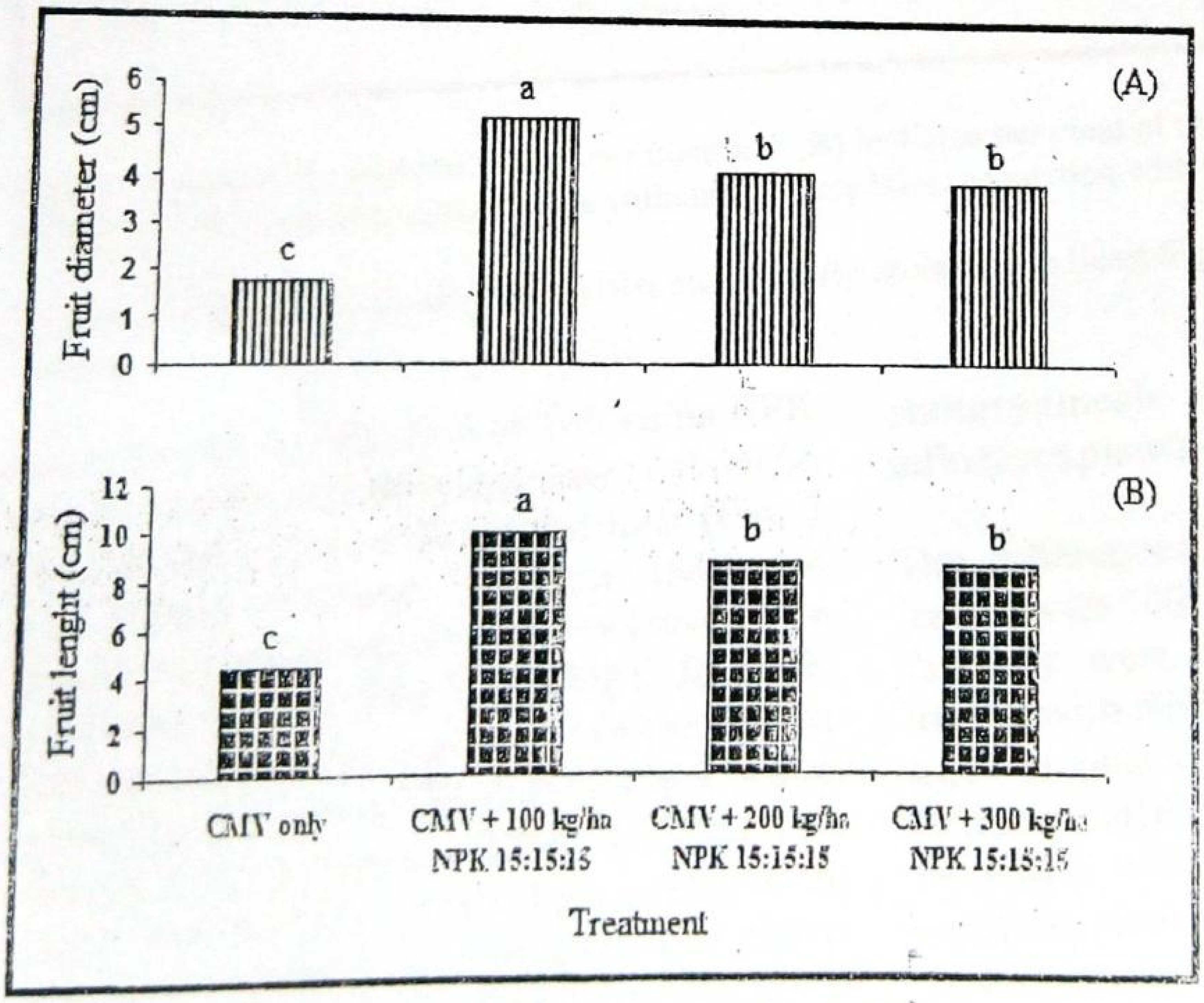

Fig. 5: Average (A) diameter and (B) lengths of fruits from okra plants at different rates of NPK 15:15:15 fertilizer and of those without fertilizer after inoculation with *Cucumber mosaic virus*

Bars labelled with dissimilar letter differ significantly according to Least Significant Difference (LSD) at *p*=0.05

magnitude of height increase declined during the subsequent growth stages (3 – 5 WAI) but in most cases the performance of 100 kg/ha fertilizer was superior. The negative effect of CMV on plant height agrees with Balogun *et al*. (7) who reported that CMV caused substantial reductions in the heights of okra plants. Fruit diameter (Fig 5A) and length (Fig. 5B) assumed a common trend such that application of NPK at 100 kg/ha resulted in the longest and widest fruit, contrary to the lowest values observed in the plants without fertilizer; the difference between the effect of 200 and 300 kg/ha of NPK fertilizer was not significant. Application of NPK at 100 kg/ha resulted in the heaviest fruit, followed by the use of 200 kg/ha. Fruit weight from the plants without fertilizer was the lowest (Fig. 6). The lowest values of the growth and yield characters in plants without fertilizer could be attributed to combined negative effects of CMV on leaf number and area, as well as fruit length and diameter. The observed contribution of NPK to plant's growth and yield agrees with Nam *et al*. (17). It could be concluded that application of higher doses of NPK fertilizer may not translate to significant positive effect in okra plants infected with CMV. Okra production in sub-Saharan Africa (SSA) is on subsistence level and mainly by resource-poor farmers. Therefore, excessive amount of fertilizer is not only wasteful,

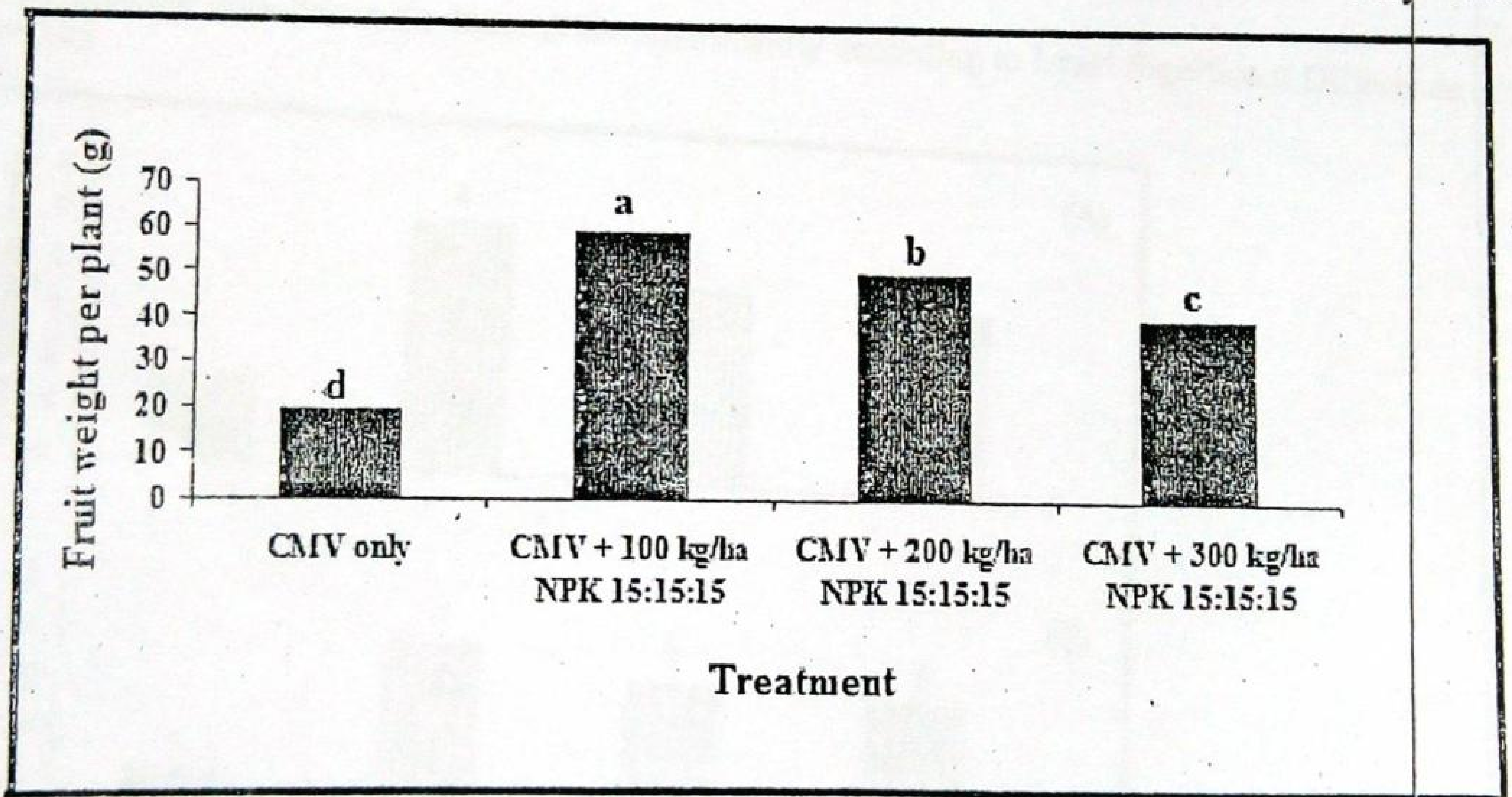

Fig. 6: Average weights of fruits from okra plants at different rates of NPK 15:15:15 fertilizer and of those without fertilizer after inoculation with *Cucumber mosaic virus*

Bars labelled with dissim ilar letter differ significantly according to Least Significant Difference (LSD) at p=0.05

which inflates productions costs but may also aggravate disease severity. Application of 100 kg/ha of NPK 15:15:15 is recommended for good growth and appreciable yield of okra under CMV disease pressure.

LITERATURE CITED

1. **Agrios, G. N., Walker, M. E. and Ferro, D. N. 1985.** Effect of *Cucumber mosaic virus* inoculation at successive weekly intervals on growth and yield of pepper (*Capsicum annuum*) plants. *Plant Dis.*, **69**: 52 – 55.

2. **Akinyele, B. O. and Temikotan, T. 2007.** Effect of variation inn soil texture on the vegetative and pod characteristic of okra [*Abelmoschus esculentus* (L.) Moench]. *Int. J. Agric. Res.*, **2**: 165-169.

3. **Alegbejo, M, Ogunlana, M. and Banwo, O. 2008.** Survey for incidence of Okra mosaic virus in northern Nigeria and evidence for its transmission by beetles. *Spanish J. Agric. Res.*, **6**: 408-411.

4. **Arif, M. and Hassan, S. 2002.** Evaluation of resistance in soybean germplasm to *Soybean mosaic Potyvirus* under field conditions. *Online J. Biol. Sci.*, **2**: 601–604.

5. **Arraudeau, M.** 1987. African cassava mosaic disease and its control. In: Proceedings of the International Seminar: African cassava mosaic disease and its Control, CTA, FAO, ORSTOM, IITA, IAPC. Yamoussoukro, 4-8, May 198, Ivory-Coast, Pp. 177-182.

6. **Babatola, L. A. 2006.** Effect of NPK 15:15:15 on the performance and storage life of okra (*Abelmoschus esculentus)*. Proceeding of Horticultural Society of Nigeria, Pp: 125-128.

7. **Balogun, O. S., Bakare, R. A. and Babatola, J. O. 2007.** Evaluation of the pathogenic responses of some okra lines to *Cucumber mosaic virus*. *J. Agric. Res. Dev.*, **6**: 63-73.

8. **Bhaduri, D., Rakshit, R. and Chakraborty, K. 2014.** Primary and secondary nutrients-a boon to defense system against plant diseases. *Int. J. Bio-resource Stress Manage.*, **5**: 461- 466.

9. **Dordas, C. 2009.** Role of Nutrients in Controlling Plant Diseases in Sustainable Agriculture: A Review. In: Lichtfouse, E., (Ed.), Sustainable Agriculture, DOI 10.1007/978-90-481-2666-8_28, Springer.

10. **FAO (Food and Agriculture Organization). 1998.** FAOSTAT agriculture data, land use domain.http://faostat.fao.org.defult.htm>.

11. **FAO (Food and Agriculture Organization). 2011.** http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor.

12. **Huber, D. M. and Graham, R. D. 1999.** The role of nutrition in crop resistance and tolerance to disease. In: Rengel, Z., (Ed.), Mineral Nutrition of Crops Fundamental Mechanisms and Implications. Food Product Press, New York, 205-226.

13. **Iyagba, A. G., Onuegbu, B.A. and Ibe, A. E. 2012.** Growth and yield response of okra (*Abelmoschus esculentus* (L.) Moench) varieties to weed Interference in South-Eastern Nigeria. *Global J. Sci. Frontier Res. Agric. Vet. Sci.*, 12: 23 – 31.

14. **Koenig R.** 1981. Indirect ELISA methods for the broad specificity detection of plant viruses. *J. Gen. Virol.*, 55: 53–62.

15. **Lombin, G., Owonubi, J. J. and Yayock, J. Y. 1988.** Crop science and production in warm climate. Macmillan Intermediate Agric Science pp 214-216.

16. **Muengula-Manyi, M., Nkongolo, K. K., Bragard, C., Tshilenge-Djim, P. Winter, S. and Kalonji-Mbuyi, A. 2012.** Effect of NPK fertilization on cassava mosaic disease (CMD)

expression in a sub-Saharan African region. *American J. Exp. Agric.*, 2: 336–350.

17. **Nam, M. H., Jeong, S. K., Lee, Y. S. Choi, J. M. and Kim, H. G. 2006.** Effects of nitrogen, phosphorus, potassium and calcium nutrition on strawberry anthracnose. *Plant Pathol.*, 55: 246–249.

18. **Ogbe, F. O., Ohiri, A. C. and Nnodu, E. C. 1993.** Effect of NPK fertilization on symptom severity of *African cassava mosaic virus*. *Int. J. Pest Manage.*, 39: 80-83.

19. **Okonmah, L.U. 2011.** The effects of pig manure rates on some agronomic parameters of three okra (*Abelmoschus esculentus* (L.) Moench) cultivars in the Asaba agro-ecological Zone. *Int. J. AgriSci., 1: 427-43.*

20. **Omorusi, V. I. and Ayanru, D. K. G. 2011.** Effect of NPK fertilizer on diseases, pests, and mycorrhizal symbiosis in cassava. *Int. J. Agric. Biol., 13: 391-395.*

21. **Omotosho, S. O. and Shittu, O. S. 2007.** Effect of NPK fertilizer rates and method of application on growth and yield of okra (*Abelmoschus esculentus* (L.) Moench). *Res. J. Agron.*, **1**: 84-87.

22. **Pazarlar, S., Gümüş, M. and Öztekin, G. B. 2013.** The effects of *Tobacco mosaic virus* infection on growth and physiological parameters in some pepper varieties (*Capsicum annuum* L.). *Not Bot Horti Agrobo.*, **41**: 427-433.

23. **Santamaria, P. 2006.** Nitrate in vegetables: Toxicity, content, intake and EC regulation. *J. Sci., Food Agric.*, **86**: 10-17.

24. **SAS (Statistical Analysis System) 2008.** Statistical Analysis System SAS/STAT User's guide, ver. 9.2. SAS Institute Inc., Cary, N.C.

25. **Siemonsma, J.S. and Hamon, C. S. 2002.** *Abelmoschus caillei* (A.Chev.) stevels Record from Protabase.Oyen, L.P.A. and Lemmens, R.H.M.J (Eds.) PROTA (Plant Resources of Tropical Africa/Resources Vegetables de l'Afrique tropicale), Wageningen, The Netherlands.

26. **Spann, T. M. and Schumann, A. W. 2010.** Mineral nutrition contributes to plant disease and pest resistance. Series No. HS1181, Horticultural Sciences Department, UF/IFAS Extension, University of Florida, Gainesville, FL.

27. **Wang, M., Zheng, Q., Shen, Q. and Guo, S. 2013.** The critical role of potassium in plant stress response. *Int. J. Mol. Sci.*, **14**: 7370–7390.