Vol. 1 No. 2, December 2021

FUAM Journal of Pure and Applied Science

Available online at
www.fuamjpas.org.ng

An official Publication of
College of Science
Joseph Sarwuan Tarka University,
Makurdi.

FUAM Journal of Pure and Applied Science
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https://fuamjpas.org.ng/
Vol.1. No. 2. December, 2021

Effect of Plant Growth Regulators and Coconut Milk on Vegetative Growth of *Amaranthus hybridus* and *Telfairia occidentalis*

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Received: 18/10/2021 **Accepted:** 26/10/2021 **Published online:** 30/10/2021

Abstract
The study assessed the single and combined effects of plant growth regulators (indole-3-acetic acid, IAA; 50 ppm), Gibberellic acid (GA_3; 100 ppm) and coconut milk (CM; 15%) on vegetative growth – plant height, leaf length, leaf breadth, leaf area, number of leaves and number of branches – of *Telfairia occidentalis* and *Amaranthus hybridus*. The experiment consisted of two plants, five (5) replicates, and eight (8) treatments – GA_3;IAA; CM; GA_3 + IAA; IAA + CM; GA_3 + CM; and GA_3 + IAA + CM; including control – in a completely randomized design. Plant height in both *A. hybridus* and *T. occidentalis* were highest in treatment GA_3, and were significantly different ($P<0.05$) from all other treatments. Leaf length of *A. hybridus* was highest in treatment GA_3; however, in *T occidentalis*, it was highest in treatments GA_3+ IAA and CM, respectively. Moreover, treatments GA_3, IAA + CM, and GA_3 + IAA + CM had the highest leaf breadth in both plants. Furthermore, leaf area was highest in treatments IAA+CM, and CM in *A. hybridus* and *T. occidentalis*, respectively. Treatments GA_3, had the highest number of leaves in both plants. The number of branches varied significantly ($P<0.05$) between treatments, especially in *T. occidentalis*, but was not significantly different ($P>0.05$) between treatments in *A. hybridus*. In conclusion, the study suggests that single or combined treatments of GA_3 had the most impact on overall growth of these vegetables.

Keywords: Auxin, Gibberellic Acid, Coconut milk, Cytokinin, Vegetables

Introduction

Vegetables, being rich sources of many essential micronutrients – vitamins and minerals – including vitamins C and K, folate, thiamine, carotenes, and dietary fibres. Vegetables are important components of diets in Nigeria. Enhancement of their production is important in meeting the challenges of feeding the ever-increasing population in Nigeria and in overcoming the challenges of food security in the country [1].

Amaranthus is a genus of the family Amaranthaceae. *Amaranthus hybridus* L. is popularly called amaranth, green, or smooth pigweed. It is an annual herbaceous plant of 1-6feet in height. The leaves are alternate petioles, 3-6 inches long, dull green and rough, hairy, ovate or rhombic with wavy margins [2] It is considered a weed in North America [3], but forms part of the staple vegetable in most parts of Nigeria [2].

A. hybridus was reported to have a thirty higher protein value than cereals such as rice, wheat flour, oats and rye [4]. Cultivation of the various *Amaranthus* species is acquiring increasing importance in Nigeria and other parts of the African continent where the available species are grown for their leaves, with research on the effect of fertilizer on their growth [5]. As a traditional food plant in Africa, *Amaranthus* has the potential to improve nutrition, boost food security, foster rural development and support sustainable land care [6, 2].

Telfairia occidentalis (Hook F.),otherwise called fluted pumpkin, is an important staple vegetable in Nigeria. It is a perennial vegetable commonly called 'ugu' by the Igbos of the South Eastern Nigeria where the crop is most popularly grown [7]. The plant produces luxuriant edible green leaves which are cut at intervals or wheneaten as potherbs,rich in iron and vitamins [7, 8].

Plant hormones are signal molecules produced at specific locations that regulate physiological processes in target cells at other locations in very low concentrations [9]. Plant growth regulators (PGRs) have gained much importance due to their consistent effects on germination and growth of various plant species [10, 11, 12]. Gibberellic acid (GA_3), is a member of a type of plant hormone called gibberellins, which regulates the growth and development of plants [11, 13]. GA_3 is a well-known stimulator of cell expansion, cell elongation and elongation of internodes [14, 15]. Studies have shown that high IAA concentrations have an inhibitory effect on root elongation [16, 17]. Indole-3-acetic acid and gibberellic acid promote seedling growth in various concentrations [17, 18]. The spray of IAA and GA_3 on wheat plant showed significant yield of plant to the extent of reducing the effect of salinity on the vegetative measurement and some physiological components of the plant [19]. Coconut water as an organic supplement in culture media had significant effect on growth and shoot production [20]. Also, *Hibiscus sabdariffa* when treated with 15% coconut milk showed higher levels of phosphorus and potassium [21].

The study was aimed at investigating the single and combined effects of indole-3-acetic acid, gibberellic acid and coconut milk on the vegetative growth of *Telfairia occidentalis* and *Amaranthus hybridus*. This should help in elucidating potential uses of these PGRs in the cultivation of these vegetables.

Materials and Methods

Plant materials

Seeds of *Amaranthus hybridus* and *Telfairia occidentalis* were sourced from the research farm of Joseph Sarwuan Tarka University Makurdi, Nigeria. The experiment was conducted at the Botanical Garden, Department of Botany, Joseph Sarwuan Tarka University Makurdi, Nigeria. Seeds of *Amaranthus hybridus* and *Telfairia occidentalis*were sown on prepared nursery beds (2 x 2 m spacing), and subsequently transplanted to ridges after three weeks.

Experimental design and treatments

A 2*5*8 factorial experiment was carried out using a completely randomized design. Plant growth regulators used in the experiment were auxin (indole-3-acetic acid, IAA; 50 ppm), Gibberellic acid (GA_3; 100 ppm) and coconut milk (CM; 15%). The same concentrations of PGRs were used for the single and combined treatments, respectively. The experiment consisted of two plants (*A. hybridus* and *T. occidentalis*), five (5) replicates, and eight (8) treatments (GA_3; IAA; CM; GA_3 + IAA; IAA + CM; GA_3 + CM; and GA_3 + IAA + CM), including control. Foliar applications of 30 mL of various combinations of plant growth regulators were sprayed on the vegetables, at 4 and 6 weeks after planting, using a pressurized hand sprayer. Furthermore, the control plants were also foliar sprayed twice with 30 mL of distilled water only [21].

Growth analysis

Growth parameters including plant height, leaf length, and leaf breadth were measured using a meter rule. The leaf number and number of branches were also counted and recorded. The leaf area was calculated by multiplying the leaf length by the leaf breadth [21].

Statistical analysis

One way analysis of variance (ANOVA) was carried out using IBM Statistical Package of Social Science (SPSS) 25 software. Means (vertical bars) and standard error (error bars ±) were presented in graphs. Mean separation (post hoc) was done using Tukey Honest Significant Difference (HSD). A p value of <0.05 was considered statistically significant.

Results and Discussion

Growth analysis of Amaranthus hybridus and Telfairia occidentalis

Plant height

There were significant differences (P<0.05) in plant height between GA_3(31.35 cm) and all other treatments (Figure 1A). Furthermore, significant differences (P<0.05)were recorded in plant height between treatments IAA (25.13 cm);GA_3 + IAA (24.95 cm), and other treatments. Also, there were significant differences(P<0.05) between GA_3 + CM (23.13 cm);GA_3 + IAA + CM (22.59 cm); IAA+CM (18.74 cm); CM (20.52 cm); and Control(18.16 cm), respectively (Figure 1A).Plant height in *T. occidentalis*varied significantly (P<0.05) between treatments, with treatment GA_3(145.3 cm) having the tallest plants, which was significantly different(P<0.05) from all other treatments (Figure 2A). Plant height in other treatments were significantly different (P<0.05) from each other (Figure 2A). Increases in plant height observed in GA_3 treatments could be because gibberellic acid promotes cell division and elongation, resulting in taller plants [13]. Similarly, these studies [22, 23] elucidated rapid cell division and elongation upon application of GA_3 to strawberry plants. Likewise, [24] increases were observed in plant height of carrot upon application of 100 ppm of GA_3.

Leaf length

Leaf length in *A. hybridus* was highest in treatment GA_3 (8.22 cm) and was significantly different (P<0.05) from all other treatments (Figure 1B). All other treatments, with the exception of treatments IAA + CM (5.44 cm) and GA_3 + CM (5.41 cm), were significantly different (P<0.05) from each other (Figure 1B). Leaf length in *T. occidentalis* was highest in treatment GA_3 (12.13 cm) and CM (11.45 cm). However, GA_3 was significantly different (P<0.05) from all other treatments with the exception of CM. CM was significantly different (P<0.05) from IAA (10.36 cm) and Control (10 cm)only. Furthermore, leaf length was not significantly different (P>0.05) in all other treatments (Figure 2B).GA_3 promotes cell elongation and cell division [13, 22, 23, 24], leading to the longest leaf lengths observed in both *A. hybridus* and *T. occidentalis*. Furthermore, [26] also reported that GA_3 application increased the growth of bean and lettuce leaf length. Similarly, [27] highlighted increased petiole length in GA_3treated plants.

Leaf breadth

Leaf breadth in *A. hybridus* was highest in treatments GA_3 + IAA + CM (3.05 cm) and IAA + CM (3.066 cm) and were significantly different (P<0.05) from all other treatments. Additionally, all other treatments were also significantly different (P<0.05) from each other (Figure 1C). Additionally, leaf breadth in *T. Occidentalis* varied significantly (P<0.05) between treatments. Treatments GA_3 + IAA + CM (5.64 cm); IAA + CM (5.61 cm); and CM (5.59 cm) had the highest values that were significantly different (P<0.05) from all other treatments.

Additionally, treatments GA_3 + CM (5.40 cm), and IAA (5.29) were also significantly different ($P<0.05$) from treatments GA_3+ IAA (4.70 cm), Control (4.74 cm) and GA_3(4.10), correspondingly. Similarly, treatments GA_3 + IAA, and control were also significantly different ($P<0.05$) from GA_3 (Figure 2C). Gibberellic acid has been shown to alter leaf shape and reduce leaf breadth in various vegetables, including tomato [31] by promoting longitudinal elongation of leaves. This supports the reduction observed in GA_3 treatments in both *A hybridus* and *T. occidentalis*.

Leaf area

Leaf area varied significantly ($P<0.05$) between all treatments in *A. hybridus*. Treatment IAA + CM (16.68 cm^2) produced the largest leaf area, which was significantly different ($P<0.05$) from all other treatments. All other treatments were also significantly different ($P<0.05$) from each other, with treatment GA_3 + IAA (9.28 cm^2) having the least leaf area (Figure 1D). Leaf area in *T. occidentalis* was highest in treatments CM (64.11 cm^2); IAA + CM (61.03 cm^2); and GA_3 + IAA + CM (60.89cm^2), respectively (Figure 2D). Leaf area of treatment CM was significantly different ($P<0.05$) from all other treatments except treatments GA_3 + IAA + CM; and IAA + CM, respectively. Furthermore, treatments GA_3 + IAA + CM; IAA + CM; and GA_3 + CM were not significantly different ($P>0.05$) from each other. Similarly, treatments GA_3 + IAA + CM; and IAA + CM were significantly different ($P<0.05$) from all other treatments. Also, GA_3 + CM (57.29 cm^2) was significantly different ($P<0.05$) from GA_3 + IAA (51.00 cm^2); GA_3, (49.78cm^2) and Control (47.41 cm^2), respectively, but not significantly different ($P>0.05$) from treatment IAA (54.89cm^2; Figure 2D).Coconut milk, containing cytokinin, stimulates cell division and differentiation [28, 29], leading to larger leaf area. In combination with coconut milk, IAA promotes cell enlargement, tissue differentiation and response to light and gravity [11, 12]. GA_3 promotes longer and narrower leaves, leading to overall reduction in leaf area [32]. Similar observation was also established in a previous study [25], which postulated that narrow leaves in GA_3 treated plants could be as a result of anisotropy and higher lateral and dorso-ventral growth compared to width growth of leaves.

Number of leaves

Treatment GA_3 (24) produced the highest number of leaves in *A. hybridus*, which was significantly different ($P<0.05$) from treatment IAA (17.33) only. All other treatments were not significantly different ($P>0.05$) from each other (Figure 1E).Number of leaves in *T. occidentalis* was highest in treatment GA_3 (120.33) which was significantly different ($P<0.05$) from all other treatments. Additionally, all other treatments were also significantly different ($P<0.05$) from each other (Figure 2E). Studies [30, 31] have elucidated the effect of GA_3 in improving leaf number in different species of plants due to its effect on cell elongation and proliferation.

Number of branches

There was no significant difference ($P>0.05$) in the number of branches in *A. Hybridus* between all treatments (Figure 1F). Furthermore, number of branches in *T. occidentalis* was highest in treatment GA_3 + CM (9.33), which was also significantly different ($P<0.05$) from treatmentsGA_3 + IAA + CM (7), CM (6), and IAA (6), respectively. However, all other treatments were not significantly different($P>0.05$) from each other (Figure 2F).The significant increase observed in gibberellic acid and cytokinin (coconut milk) combination in *T. occidentalis* is indicative of the aforementioned roles of these PGRs in cell elongation and cell division respectively [11, 28]. This could be quite useful in the cultivation of *T. occidentalis* as more branches would produce more leaves and improve the overall plant productivity, consequently enhancing the nutritional and economic gain of consumers and farmers, respectively.

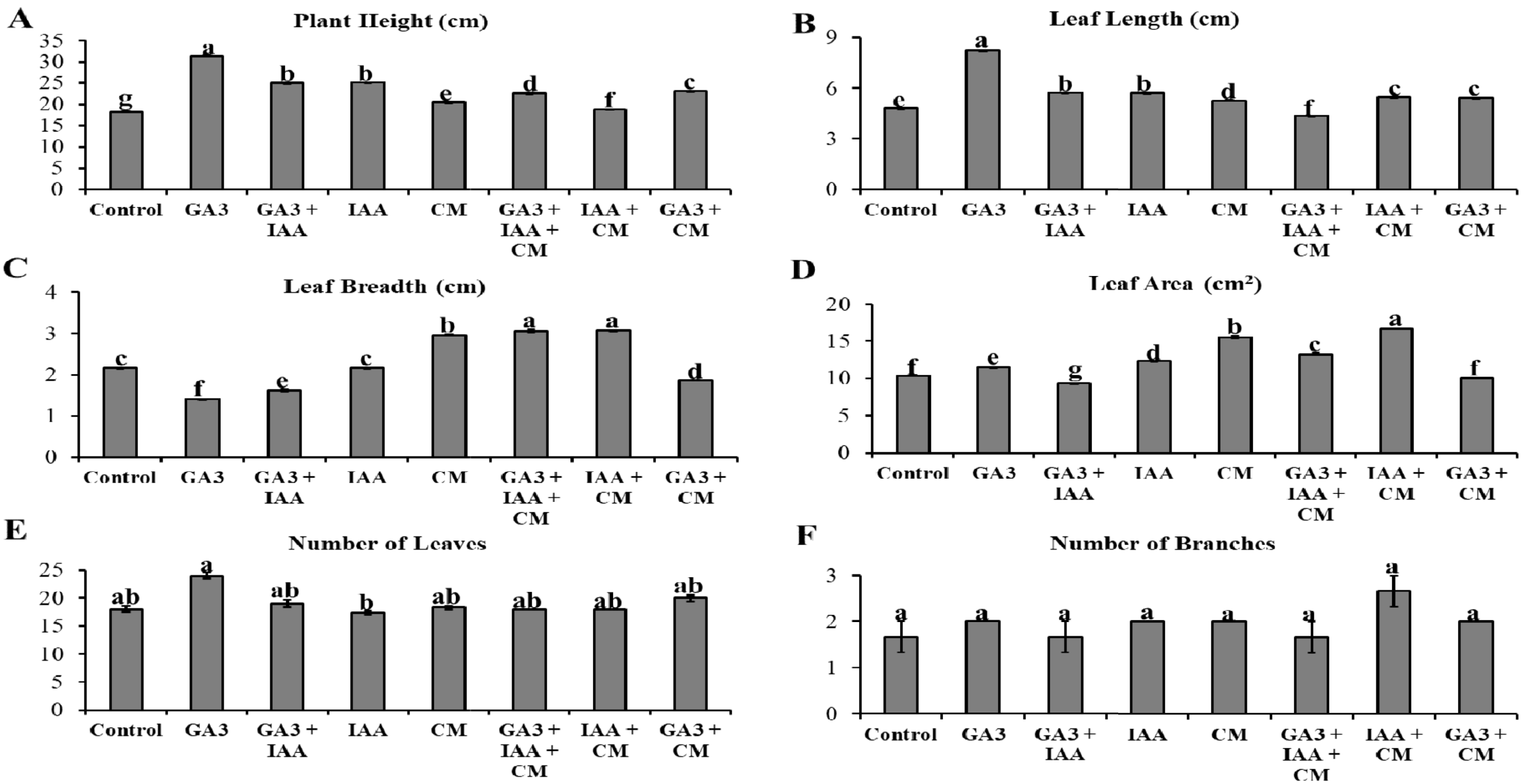

Figure 1.Comparative growth analysis of effects of plant hormones and coconut milk on *Amaranthus hybridus*

(A) Plant height (B) Leaf length (cm) (C) Leaf breadth (cm) (D) Leaf area (cm^2) (E) Number of leaves (F) Number of Branches. Vertical bars represent means; error bars represent ± standard error; n = 5; bars with different alphabets are statistically significant at 95% confidence interval ($p < 0.05$; Tukey HSD). GA_3: Gibberellic acid; IAA: Indole-3-acetic acid; CM: Coconut milk.

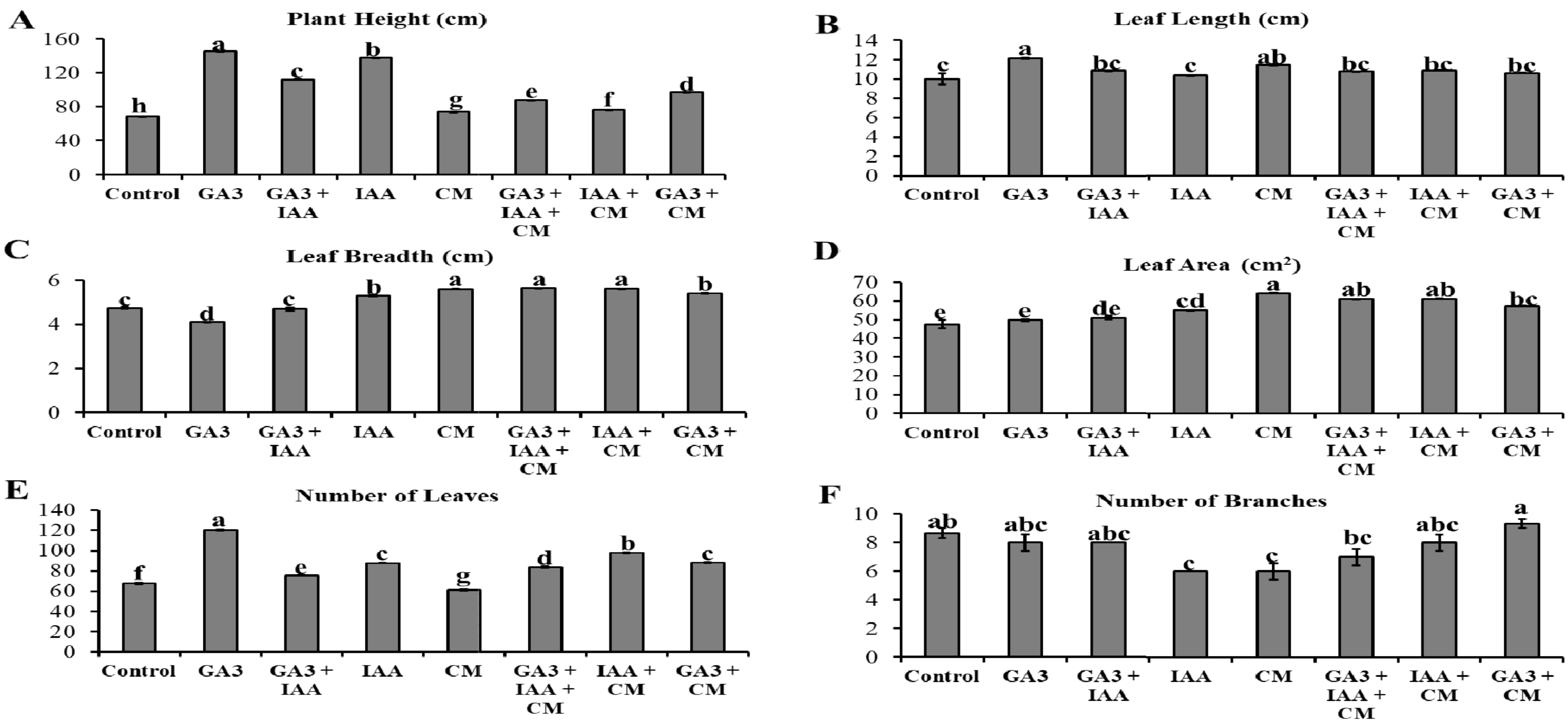

Figure 2. Comparative growth analysis of effects of plant hormones and coconut milk on *Telfairia occidentalis*

(A) Plant height (B) Leaf length (cm) (C) Leaf breadth (cm) (D) Leaf area (cm^2) (E) Number of leaves (F) Number of Branches. Vertical bars represent means; error bars represent ± standard error; n = 5; bars with different alphabets are statistically significant at 95% confidence interval ($p < 0.05$; Tukey HSD).

GA_3: Gibberellic acid; IAA: Indole-3-acetic acid; CM: Coconut milk.

Conclusion

Plants treated with GA_3 resulted in longer plant height and leaf length, while plants sprayed with 15% coconut milk significantly stimulated increase in leaf breadth and leaf area. The study suggests that single or combined treatments (with coconut milk) of GA_3 had the most impact on overall growth of *A. hybridus* and *T. occidentalis*.

Declaration of conflicting interests

The authors declared no potential conflicts of interest.

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Cite this article

Okoh T., Okekporo E.S. and Oche L.A. (2021). Effect of Plant Growth Regulators and Coconut Milk on Vegetative Growth of *Amaranthus hybridus* and *Telfairia occidentalis.* FUAM Journal of Pure and Applied Science,**1**(**2**):68-74