ORIGINAL RESEARCH ARTICLE

Effects of indigenous arbuscular mycorrhizal fungi on growth of selected Carica papaya L. hybrids in Kenya

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ABSTRACT

Arbuscular Mycorrhizal Fungi (AMF) are naturally occurring root symbionts known to improve the uptake of essential nutrients by host plants due to their extra-radical hyphae. However, the effect of indigenous AMF inoculation on the growth of papaya hybrids has not been investigated. This study evaluated the effect of AMF inoculation on the growth characteristics of papaya hybrids (JKUAT and Malkia) at the vegetative stage. A greenhouse experiment consisting of three treatments—AMF inoculum, compost manure, and a combination of inoculum and compost manure—was set up in a completely random design and replicated six times. Non-treated seedlings were included as controls. Spores were isolated from rhizospheric soil samples and bulked in a potted sorghum crop for four months to obtain AMF inoculum. The treatments were applied to papaya seedlings between the second and third leaf stages. Growth parameters including plant height, leaf length, stem girth, and number of leaves were recorded every 4 weeks for a period of 20 weeks after planting (WAP). All data were subjected to one-way ANOVA at the $p \leq 0.05$ level, with significant and nonsignificant means separated using Tukey's HSD test in Genstat's 15th edition. Results showed that plants treated with a combination of compost manure and AMF inoculum were significantly different ($p \leq 0.05$) for all growth parameters tested, compared to compost manure alone and the control treatments. Malkia hybrids treated with a combination of compost manure and AMF inoculum (MIC) had the highest plant height of 53.2 cm at 20 WAP. JKUAT hybrids treated with AMF inoculum and composted manure and the control treatment had a root biomass of 49 and 11.80 grammes at 20 WAP, respectively. In conclusion, soil media amendment with compost manure and AMF inoculum enhanced overall papaya seedling growth, and the growth response was dependent on papaya hybrids.

Key words: Compost manure, indigenous AMF inoculums, papaya hybrid response, seedling growth effects

1.0 Introduction

Arbuscular mycorrhizal fungi (AMF) are soil microorganisms that form associations with the roots of the majority of plant species (Abbott and Lumley, 2014). AMF plays a major role in processes associated with soil aggregation, the acquisition of nutrients by plants, and

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ecosystem function (Abbott and Lumley, 2014). Arbuscular mycorrhizal fungi form symbiotic relationships with over 80 percent of terrestrial plant species (Wang and Qiu, 2006). This symbiotic relationship is characterised by its association with phosphorus, nitrogen, sulfur, and micronutrient uptake by host plants and the enhancement of water uptake through the extra-radical fungal hyphal networks (Brundrett, 2002).

AMF symbiosis can also prompt physiological and molecular signals at subcellular levels, modifying the structure of the plant community and thus escalating plant tolerance to several abiotic and biotic stresses. Ectomycorrhizal (ECM) fungi form associations with only 3 percent of terrestrial plant families (Smith and Read 2008). When a symbiosis occurs, both ECM and AMF can demand 20–40% of photosynthetically fixed carbon supplied by their host plants to complete their life cycle (McNear, 2013). In return, AMF supplies nutrients, including phosphorous, nitrogen, and zinc, to the plant through the arbuscules, the nutrient exchange sites (Solaiman and Saito, 1997; Harrison, 1999; Balestrini and Bonfante, 2005). Mycorrhizal plants usually display better performance compared to non-mycorrhizal plants in high-input agricultural systems where nutrients are limited (Janos, 2007). Inoculation with AMF becomes effective in plants when it is introduced during the early plant development stages (Guillemin *et al*., 1993), and thereafter, colonisation by AMF follows root establishment of the already inoculated seedlings and the plant will be extensively mycorrhizal (Lovato *et al*., 1995). Plants' survival rate during the acclimatisation phase is highly enhanced, plant growth and development are stimulated, and overall high production of the plants is achieved at the vegetative and flowering stages (Lovato *et al*., 1996; Estaún *et al*., 1999). Plants Inoculation with indigenous AMF similarly aided the general growth of the plants, nitrogen fixation, and phosphorous acquisition by plants (Jeffries *et al*., 2003).

Papaya (*Carica papaya*) is a tropical and sub-ttropical fruit (Yadava *et al*., 1990), partaking in commercial importance due to its high nutritive and medicinal value (Pinnamaneni, 2017). The papaya crop is primarily propagated by seeds and grows well in warm places. It requires full sunlight and well-drained soils (Heena Dave and Sunil Trivedi, 2019). However, the cost of certified papaya seeds is prohibitively expensive, making increasing percentage germination for healthier seedlings a challenge for papaya farmers (Bhardwaj, 2013).Growing media is an essential part of the majority of horticultural crops as it directly affects germination, enlargement, and the proper function of the rooting system (Abad *et al*., 2002). An appropriate propagation medium serves as a nutrient and water reservoir for the plants, provides adequate anchorage, and allows sufficient gaseous exchange between the roots and their substrates (Abad *et al*., 2002). According to Wilson *et al*. (2001), the superiority of seedlings is mostly dependent on the constituents of the growing medium. Agbo and Omaliko (2006) stated that the potting medium used in the nursery influences the quality of the seedlings formed, which in turn increases the yield (Baiyeri, 2006).

Various biofertilizers have been used for inoculation, including rhizobium, which enhanced root nodulation in cowpea, and this reduced the need to incorporate nitrogen fertiliser in the soil medium (Anitha *et al*., 2004). Further experiments with vesicular arbuscular mycorrhizal

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(VAM) fungus inoculation facilitated more efficient uptake of phosphorous from the soil and boosted absorption of water, potassium, nitrogen, and micronutrients in the inoculated cowpea plants (Anitha *et al*., 2004). A fungus such as *Trichoderma harzianum* has been found to be beneficial in plant root development, enhancing effective mineral absorption from the soil and resistance to stresses due to abiotic elements, resulting in improved crop productivity (Harman 2000). Mycorrhizal fungi act as a solid sink for photosynthates and also condense the nutrient imbalances in cowpea plants (Muthukumar and Udaiyan, 2000). Herrera *et al*. (1993) stated that inoculation of plants with mycorrhizal fungi in re-vegetation systems not only helps plant establishment but also improves soil physical, chemical, and biological properties, thus contributing to improved soil quality (Carrillo-Garca *et al*., 1999). However, varied progress has been attained in exploring the use of microorganisms for enhancement of soil fertility and ultimately increased crop productivity. Growth of the root system is suppressed in soils with inadequate drainage, and the plants become more susceptible to soil-borne diseases (Beattie and White 1992). Organic matter is incorporated into the soil to enrich it with enough nutrients for the plants, improve the rooting system, and increase resistance against pest and disease attack (Akanbi *et al.* 2002).

Efforts are therefore being made towards the exploitation of indigenous soil microbes such as AMF, which contribute to enhanced soil fertility and increased plant growth and protection, especially in papaya (Abbott and Lumley, 2014). This study sought to explore the growth benefits of inoculating *Carica papaya* hybrids with indigenous AMF at the seedling stage.

2.0 Materials and methods

2.1 Sampling procedure and soil collection

Soil samples were collected from the roots' rhizosphere of papaya, banana, and grass plants in Mwea, Mitunguu, and Juja, at a depth of 0–20 cm, following a zigzag pattern across a paddock. The soil was packed in sterilised 500-gram polyethene bags. The selection of the sampling areas was based on the existing, established papaya orchards, which were the focus of this study. Banana and grass plants were chosen for their mycorrhizal nature as well as their proximity to papaya orchards in this region.

2.2 Isolation of arbuscular mycorrhizal fungi (AMF)

Spore isolation was carried out as described by Schenk and Perez (1990). Grid-calibrated petri dishes were used for examining the extracted spores.

2.3 Bulking of arbuscular mycorrhizal fungi (AMF) inoculum

As a trap culture crop, Sorghum bicolor (L.) Moench, a fast-growing annual grain plant, was used. Disposable glasses, 300 ml, were perforated and half filled with sterile coarse sand mixed with the isolated AMF spores. Sorghum seeds were planted in a greenhouse with temperatures ranging from 36°C to 41°C and relative humidity ranging from 60-90%. The plants were watered as necessary and grown for 4 months before a sample of roots and medium from the sorghum rhizosphere was obtained for AMF inoculum preparation.

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2.4 Papaya treatments

Papaya seeds of JKUAT and Malkia F1 hybrids at the 2 and 3 leaf stages were transplanted in a mixture of sterilised soil and sand media at a ratio of 1:1. AMF inoculum, compost manure, and a combination of the AMF inoculum and the compost manure (1:1) were used as treatments. The treatments were combined with the sterilised soil at a ratio of 1:9 (one part of the treatment to nine parts of the soil). As a control, only sterilised soil media were used. Eighteen plants from each hybrid were grown in each treatment, and watering of the plants was done when necessary.

2.5 Assessment of seedling growth

Every 2 weeks, seedlings were assessed for height, leaf length, number of leaves, and stem girth. Roots, shoots, and leaf biomass data were recorded every 4 weeks for a period of 20 weeks. Three plants were randomly uprooted from every treatment and from the two hybrids (JKUAT and Malkia). The roots were carefully and thoroughly cleansed to remove any debris and soil particles. They were then air dried, carefully cut from the stem, weighed, and recorded as root biomass. The upper portion of the plant was weighed and recorded as shoot biomass. All leaves were then plucked from the shoots and weighed separately to obtain leaf biomass.

2.6 Arbuscular mycorrhizal fungi (AMF) root colonization

Papaya seedlings in all the treatments were assessed for root colonisation according to the procedures of Koske and Gemma (1989), while the frequency and intensity of mycorrhizal colonisation were done using the subjective visual technique developed by Kormanik and McGraw (1982). Rhizospheric roots were obtained through destructive sampling at 20 weeks after transplanting. The roots and soil samples were put in an aqueous solution of 2.5% potassium hydroxide before autoclaving at 120 ^{o}C for 3 to 5 minutes. The samples were then baked at 70 degrees Celsius for one hour before being rinsed in several changes of water to remove the KOH. Alkaline hydrogen peroxide was added to the samples and placed in an oven at 70 ^{o}C for 20 minutes so as to remove phenolic substances. The samples were then rinsed with tap water and acidified with 1% HCL for 30 minutes. Without rinsing, the samples were stained in an acidic glycerol solution containing 0.05% trypan blue. They were then baked for one hour at 70 degrees Celsius. The stain was decanted, and a destaining solution comprising acid glycerol was added. The samples were randomly picked and mounted on slides under a compound microscope to assess the frequency and intensity of mycorrhizal colonization.

2.7 Statistical analysis

All study data were subjected to two- way analysis of variance (ANOVA) using Genstat statistical package, 15th edition and means found to be significantly different at $p \leq 0.05$ were separated using Tukey's HSD test.

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3.0 Results

3.1 Effect of AMF inoculation, compost manure and a combination of AMF inoculation and compost manure on papaya hybrid growth and AMF colonization

The media amendment in the nursery had variably significant ($p \leq 0.05$) effects on the growth of JKUAT and Malkia F1 papaya hybrids and also among the hybrids. AMF inoculum and compost manure (MIC) significantly ($p \leq 0.05$) increased the height of Malkia F1 hybrid (53.2 cm) at 20 weeks after transplanting compared to all other treatments (Table 1). Similar effects were observed in leaf length, stem girth, and the number of leaves for Malkia hybrids inoculated with inoculum and composted manure (MIC). When compared to the other treatments, the JKUAT control had the fewest leaves (14.3) and the fewest branches (10), followed by the JKUAT hybrid with compost manure treatment (JKC), which had 26 and 16.3, respectively. Plants inoculated with MIC had the highest percent AMF colonisation (81.3%), followed by JKUAT hybrid plants inoculated with AMF and compost manure (JKIC) (78%), while the non-inoculated JKUAT and Malkia control plants had a significantly ($p \leq 0.05$) lower percent AMF colonisation of 32.3% (Table 1).

3.2 Leaf biomass

A gradual increase in leaf weight over time was observed in all the treatments. Malkia F1 hybrid seedlings treated with a combination of AMF inoculum and compost manure treatment showed significant differences ($p \leq 0.05$) in growth from JKUAT hybrid seedlings with a similar treatment during the entire growth period (Table 2). Leaf biomass of the JKUAT hybrid with compost manure treatment weighed 21.9 g, while the JKUAT control weighed 14.8 g at 20 weeks after transplanting.

3.2 Shoot biomass

JKUAT hybrid with the combination of AMF inoculum and compost manure treatment was significantly different ($p \leq 0.05$) from JKUAT hybrid with compost manure treatment during the seedling growth period. JKUAT hybrid with compost manure treatment recorded a shoot biomass of 35.8 g, while JKUAT hybrid treated with a combination of AMF inoculum and compost manure had a shoot biomass of 56.5 g at 20 weeks after transplanting. Non-treated control plants (JK Control and M Control) had the least shoot biomass throughout the data collection period (Table 3).

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Table 1: Effect of soil amendment on growth of JKUAT and Malkia F1 papaya hybrid seedlings 20 weeks after inoculation

Treatment	JKUAT Hybrid					Malkia Hybrid				
	Plant Height (cm)	Stem girth (cm)	No. of leaves	Length (cm) of longest leaf	Root colonization (%)	Plant height (cm)	Stem girth (cm)	No. of Leaves	Length (cm) of longest leaf	Root colonization (%)
AMF Inoculum	42.9^{b}	5.8^{b}	27.3^{b}	15.7^{b}	70.7^{b}	47.4^{b}	6.5^{a}	28.7^{b}	16.3^{b}	73.3^{b}
AMF Inoculum + compost	49.4^{a}	6.1^{a}	32.0^{a}	18.5^{a}	78.0^{a}	53.2^{a}	7.0^{a}	36.3^{a}	19.4^{a}	81.3^{a}
Compost	40.3^{c}	5.9^{b}	26.0^{b}	16.2^{b}	41.0^{c}	43.7^{c}	6.5^{a}	28.3^{b}	19.3^{a}	53.7^{c}
Control	25.7^{d}	3.7^{c}	14.3^{c}	11.7^{c}	32.3^{d}	25.7^{d}	3.7b	14.3^{c}	11.7^{c}	32.3^{d}
LSD	0.69	0.13	1.76	0.43	3.03	0.66	0.48	1.15	0.33	3.60
CV%	0.9	1.2	3.5	1.4	2.7	0.8	4.1	2.1	1.0	3.0
SE	0.34	0.06	0.88	0.22	1.52	0.34	0.24	0.58	0.16	1.80

Means within each column followed by a different letter differ significantly at $p \leq 0.05$

Table 2: Leaf biomass (g) of JKUAT and Malkia F1 papaya hybrid seedlings treated with AMF inoculum, compost manure and a combination of AMF inoculum and compost manure, 4-20 weeks after treatment

Time in Weeks	JKUAT Hybrid					Malkia Hybrid				
Treatment	4	8	12	16	20	4	8	12	16	20
AMF Inoculum	7.7^{b}	11.3^{b}	16.8^{b}	18.5^{b}	23.2^{b}	11.5^{b}	15.0^{b}	20.3^{b}	27.5^{b}	28.2^{b}
AMF Inoculum + compost	15.2^{a}	18.3^{a}	22.0^{a}	27.3 ^{a}	30.5 ^{a}	19.0^{a}	22.7^{a}	25.0^{a}	30.3^{a}	32.5^{a}
Compost	6.1^{c}	10.2^{b}	14.3^{c}	17.9^{b}	21.5^{b}	8.5^{c}	12.3^{c}	16.3^{c}	20.4^{c}	22.7^{c}
Control	4.7^{d}	7.1^{c}	11.6^{d}	12.8^{c}	14.8^{c}	5.1^{d}	7.6^{d}	12.6^{d}	13.5^{d}	15.2^{d}
LSD	0.76	0.93	0.81	0.53	1.47	1.31	0.86	0.74	0.78	1.86
CV%	4.5	3.9	2.5	1.4	3.3	6.0	3.0	2.0	1.7	3.8
SE	0.38	0.46	0.41	0.27	0.74	0.66	0.43	0.37	0.39	0.93

Means within each column followed by a different letter differ significantly at $p \leq 0.05$

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Table 3: Shoot biomass (g) of JKUAT and Malkia F1 papaya hybrid seedlings treated with AMF inoculum, compost manure and a combination AMF inoculum and compost manure, 4-20 weeks after treatment

	JKUAT Hybrid					Malkia Hybrid				
Time in Weeks / **Treatment**	**4**	**8**	**12**	**16**	**20**	**4**	**8**	**12**	**16**	**20**
AMF Inoculum	25.8[b]	38.8[a]	45.1[a]	49.3[a]	53.3[b]	27.4[b]	41.2[a]	44.8[a]	50.3[b]	54.4[b]
AMF Inoculum + compost	28.2[a]	39.2[a]	44.5[a]	51.0[a]	56.4[a]	33.5[a]	41.8[a]	46.2[a]	52.4[a]	65.1[a]
Compost	18.9[c]	24.5[b]	28.5[b]	33.0[b]	35.8[c]	20.2[c]	24.9[b]	32.5[b]	37.5[c]	39.1[c]
Control	14.2[d]	17.6[c]	20.7[c]	24.4[c]	26.2[d]	15.5[d]	18.9[c]	22.5[c]	26.3[d]	28.1[d]
LSD	0.89	0.87	4.20	1.36	1.32	1.37	1.76	1.02	1.14	1.52
CV%	2.0	1.5	6.1	1.7	1.5	2.8	2.8	1.4	1.4	1.6
SE	0.45	0.44	2.1	0.68	0.66	0.68	0.88	0.51	0.57	0.76

Means within each column followed by a different letter differ significantly at $p \leq 0.05$

3.3 Root Biomass

JKUAT and Malkia hybrids inoculated with AMF had significantly ($p \leq 0.05$) higher root biomass throughout the growth assessment period compared to the compost manure treatment and the control (Table 4). JKUAT hybrid with AMF inoculum treatment (JKI) was significantly different ($p \leq 0.05$) from MALKIA F1 hybrid with similar treatment (MI) at 12 weeks after inoculation, with JKI having a lower root biomass of 16.4 g compared to the MI root biomass of 18.8 g (Table 4).

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Table 4: Root biomass (g) of JKUAT and Malkia F1 papaya hybrid seedlings treated with AMF inoculum, compost manure, and a combination of AMF inoculum and compost manure, 4–20 weeks after treatment.

	JKUAT Hybrid					Malkia Hybrid				
Time in Weeks	**4**	**8**	**12**	**16**	**20**	**4**	**8**	**12**	**16**	**20**
Treatment										
AMF Inoculum	8.3^{b}	11.6^{b}	16.4^{b}	21.4^{b}	23.5^{b}	9.5^{b}	14.5^{b}	18.8^{b}	26.3^{b}	32.6^{b}
AMF Inoculum + compost	18.5^{a}	29.7^{a}	38.0^{a}	41.6^{a}	49.0^{a}	24.7^{a}	36.7^{a}	41.4^{a}	48.3^{a}	58.5^{a}
Compost	3.7^{c}	4.9^{c}	6.5^{c}	11.1^{c}	16.1^{c}	4.7^{c}	5.7^{c}	8.7^{c}	14.8^{c}	18.2^{c}
Control	3.3^{d}	4.1^{c}	4.7^{c}	8.1^{d}	11.8^{d}	4.1^{c}	4.4^{d}	5.3^{d}	8.3^{d}	12.5^{d}
LSD	0.23	0.97	1.55	1.3	0.94	0.67	0.78	0.94	1.18	1.55
CV%	1.4	3.9	4.7	3.2	1.9	3.1	2.5	2.5	2.4	2.5
SE	0.12	0.49	0.78	0.65	0.47	0.34	0.39	0.47	0.59	0.78

Means within each column followed by a different letter differ significantly at $p \leq 0.05$

Figure 1: Root and shoot biomass of papaya seedlings 20 weeks after transplanting and soil media amendment AMF and compost manure treatment B = AMF treatment, and C = compost manure treatment.

4.0 Discussion

Mycorrhizas are known to increase the absorption of elements such as phosphorous and other major elements found in the soil, as well as zinc and copper absorption, especially in nutrient-scarce conditions. These elements provide the roots, leaves, and stems of the plants with the required nutrients for their effective development, thereby increasing the yield through the intensified dry matter (Ortaş, 1996). On the other hand, compost manure is a beneficial source of organic matter for crop production. However, low application of the compost manure may lead to low crop yields due to a deficiency of essential nutrients, whereas excess application results in the leaching of nitrates and phosphorous and their unavailability to the plants (Oades, 1993).

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Our study evaluated the performance of JKUAT and Malkia F1 papaya hybrids established on soil media with arbuscular mycorrhizal fungi (AMF) and compost manure treatments. The observed increase in growth rate in plants treated with a combination of AMF and composted manure compared to either treatment alone could be attributed to increased AMF colonisation (reported in this study).Previous studies on the combination of compost manure and mycorrhiza showed that the hyphae of the mycorrhiza and the roots of the plants contribute independently to the stability of soil aggregates, and their overall effects on the plants are enhanced by their combination (Andrade *et al*., 1998).

The rate of emergence of new leaves and branches as well as the average length of the leaves were higher on the plants treated with both AMF and compost manure, suggesting a synergistic effect between the two. Disturbed and eroded soil has been found to contain extremely low amounts of nitrogen, phosphorous, pH, and organic matter content (Shrestha Vaidya *et al.,* 2008). According to Geetha and Fulekar (2008), when organic matter was added to eroded soil, the AM spore count increased, which led to soil stabilisation as well as effective plant establishment.

The increased stem girth in plants inoculated with both AMF and compost manure may also suggest a compost-induced AMF colonization, providing avenues for greater nutrient exploration and uptake by the plant. The combined effect of mycorrhiza provides a great mass of external mycelium that can extend even beyond the rhizospheric area of the plant, searching for more water and nutrients from the soil. The absorbed minerals are then directed to the intraradical mycelium and transported to the host plant (Ramos *et al.,* 2009). This may also explain why the roots, shoots, and leaves of the plants treated with both AMF and composted manure performed better than plants treated with either composted manure or AMF alone.

Useful effects of incorporating AM fungi on soil media have been stated for various plants, such as apple rootstocks (Schubert and Lubraco, 2000) and the rootstocks of pistachios (Kafkas and Ortas, 2009). Mycorrhiza also enables plants to cope with saline and dry conditions, along with other biotic and abiotic stress factors (Pozo *et al*., 2010). According to Trotta *et al*. (1996), AMF prompts the plants to produce growth promoters that support them in the management of plant pathogens. Other studies have shown that grapevines grown on sterilised media that were inoculated with AMF developed faster than those in non-inoculated media (Ozdemir *et al*., 2010). Shrestha Vaidya *et al*. (2008) concluded that organic amendments boost AMF spore production. Previous experiments have also indicated that the combination of soil as the basic medium, sand for porosity, organic matter to enrich the soil, and trichoderma to reduce the incidence of soil-borne diseases produced superior results on the performance of papaya seedlings (Rakibuzzaman *et al*., 2019). In the current study, the effect of amending the soil medium with AMF inoculum was clearly indicated on the roots and significantly differed on the percentage levels of root colonisation depending on the treatment and papaya hybrid. The overall performance indicated that the Malkia F1 papaya hybrid portrayed better results in the

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vegetative phase of growth compared to the JKUAT papaya hybrids. According to Tim Jumah (2022), the Malkia-Fi papaya hybrid is currently the best-performing in Kenya.

5.0 Conclusion

Current research has shown that supplementing organic manure with AMF improves the performance of papaya seedlings regardless of hybrid. This study also reports for the first time the isolation, bulking, inoculation, and growth response of papaya hybrid seedlings with indigenous AMF inoculum. This study therefore offers a more environmentally friendly soil amendment for papaya seedling growth and establishment, providing an alternative to the continuous use of inorganic soil amendments conventionally used by farmers for improvement.

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6.2 General acknowledgement

None

6.3 Declaration of interest

None

6.4 Conflict of interest

None.

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