



## Effects of Single and Mixed Virus Infections on the Germination and Longevity of Some Cultivars of Cowpea

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### Abstract

A field trial was carried out to assess the response of twenty five cultivars of cowpea to single and mixed infections with Black eye cowpea mosaic virus (BICMV) and Cowpea mottle virus (CPMoV) on seed quality. The field trial was conducted at the Teaching and Research Farm of the Faculty of Agriculture, Ahmadu Bello University (ABU), Zaria, Mokwa Station (09°21'N and 5°13' E, 201 m above sea level) situated in the Southern Guinea Savannah agro - ecological zone of Nigeria. The seed viability test was determined at the Crop Production Laboratory, Department of Crop Production, Federal University of Technology, Minna, Nigeria. Four independent trials were conducted simultaneously, for single and mixed infections. The field was cleared, ploughed, harrowed and ridged at 0.75 m apart then marked out into plots and replications. The trial was a randomized complete block design (RCBD) replicated three times giving a total land area of 900 m<sup>2</sup>. Three cowpea seeds of each cultivar were sown after dressing with Apron – star (methylthiuram + metalaxyl + carboxin) at the of rate 3.0 kg seed per 10 g of the chemical. Seeds were sown at an intra and inter–row spacing of 0.30 × 0.75 m along the ridges and later thinned to two per stand at 2 weeks after sowing (WAS). For the single virus infection, seedlings of the twenty five cultivars were inoculated at 10 days after sowing(DAS) while for the mixed virus infections, seedlings were inoculated at 10 and 17 DAS. The results of the experiment revealed that all cultivars were susceptible to single and mixed infections of the two viruses but to seemingly different extents. The viability of seeds from single infection with CPMoV was slightly reduced in some instances, but, even when seeds viability was not much affected, test of accelerated ageing for four weeks indicated that seed vigour was seriously impaired as compared to the other three virus treatments.

**Keywords:** Blackeye cowpea mosaic virus, Cowpea mottle virus, cowpea seeds, Seed quality, Germination

### INTRODUCTION

Cowpea (*Vigna unguiculata* [L.] Walp) is one of the ancient crops known to man. Its origin and domestication occurred in Africa near Ethiopia and subsequently was developed mainly in the farms of the African Savannah (Gómez, 2012). Today, it is widely adapted and grown throughout the world but Africa predominates in production. It is a major staple food crop in sub-Saharan Africa, especially in the dry savanna regions of West Africa (Dugjie *et al.*, 2009). The seeds are a major source of plant protein and vitamins for man, feed for animals, and also a source of cash income. The young leaves and immature pods are eaten as vegetables (Dugjie *et al.*, 2009). It

has been estimated that the annual world cowpea crop is grown on 12.5 million hectares, and the total grain production is 3.9 million tonnes (FAO, 2016). More than 8 million hectares of cowpea are grown in West and Central Africa. Also, it is known that Nigeria is the largest producer with 4 million hectares accounts for 45 % of the total on 1.15 million hectares annually (Dugjie *et al.*, 2009). Other producers are Niger, Mali, Burkina Faso and Senegal (Gómez, 2012). The major cowpea producing areas in Nigeria include Niger, Kwara, Kaduna, Borno, Taraba and Yobe States in the northern part while Oyo, Ogun and Ondo also produce appreciable quantities in the southern part of the country (IITA, 2013).



Virus diseases are considered to be a major limiting factor for the production and productivity of legumes in the tropical and sub-tropical countries (Bashir *et al.*, 2000). Out of more than 20 viruses reported on legumes from different parts of the world, (Kareem and Taiwo, 2007) nine are known to infect cowpea naturally in Nigeria. *Blackeye cowpea mosaic virus* was first reported on cowpea in the U.S. in 1955 (Alegbejo, 2015). It is distributed in all ecological zones and cowpea- growing areas of Nigeria. Local symptoms appear as large reddish lesions that spread along the veins, while systemic symptoms appear as severe mottle, mosaic, vein-banding, veinal chlorosis, distortion and stunting of the plant. Disease symptoms vary with virus strain and host cultivar. Incidence varies from 1-40 % on farmers' fields. Yield losses due to the virus vary from 10-85% on individually infected plants and vary with time of sowing. *Cowpea mottle virus* is a positive sense single-stranded RNA, unipartite, isometric virus, 30 nm in diameter (Alegbejo, 2015). The pathogen is distributed in all ecological zones of Nigeria, particularly in the riverine areas of the middle belt which has a Southern Guinea Savanna climate and where a lot of bambara groundnut is grown (Reddy and Devi, 2010). Infected plants display severe mosaic, mottling or bright yellow mosaic. Leaf distortion and reduction in leaf size sometimes leading to a witches' broom appearance in cowpea occurs (Bhat *et al.*, 2011).

Seed-borne viruses are important for source of diseases at the beginning of production even at low rates of seed transmission (Kareem and Taiwo, 2007). In addition, seed-borne viruses can aggravate other transmission methods and cause disease to spread rapidly. Seed-borne and seed

transmitted viruses are also damaging to cowpea productivity owing to inherent primary inoculum and potential for their wide dispersal. Information on the possibility of seed transmission in virus infected cowpeas will be valuable to numerous cowpea farmers. Information on germination of infected seeds and survival of resulting plants, virus disease progress during the growing season, magnitude of yield loss and amount of infection in harvested seeds in replicated field experiments is required to establish acceptable threshold levels of seed-borne infections. The study is essential to develop preventive and management measures for cowpea virus diseases in Niger State. Therefore, this research aimed at examining the effects of virus infections on seed quality.

## MATERIALS AND METHODS

### Field trial

This was conducted during the 2017 wet session at the Teaching and Research farm of the Faculty of Agriculture, Ahmadu Bello University (ABU), Mokwa Station (09°21'N and 5°13'E, 201 m above sea level) situated in the Southern Guinea Savannah agro-ecological zone of Nigeria. The site used was under continuous cropping between 2012 till the commencement of the study.

### Screening site, treatments and experimental design

Four independent trials were conducted simultaneously, for single and mixed infections of the two most common viruses in the study area. In each trial, 25 cowpea cultivars namely Ife Brown, IT90K – 277 – 2, IT96D – 610, IT97K – 499 – 35, IT97K – 568 – 18, IT97K – 573 – 2 – 1, IT98K – 205 – M8, IT98KD – 288, IT99K – 316 – 2, IT99K – 377 – 1, IT00K – 901 – 5, IT03K – 337 – 6, IT04K – 267 – 8, IT04K – 291 – 2, IT04K – 321 – 2, IT04K – 332 – 1, IT06K –



124, IT06K – 137 – 1, IT07K – 211 – 1 – 8, IT07K – 222 – 2, IT07K – 243 – 1 – 10, IT07K – 251 – 3 – 3, IT07K – 292 – 1 – 10, IT07K – 299 – 6 and IT07K – 318 – 33) constituted the treatments. The cultivars were photosensitive and high yielding under virus free conditions. The field was cleared, ploughed, harrowed and ridged with tractor at 0.75 m apart then marked out into plots and replications. Each cultivar was evaluated in 0.375 m ridge wide, 3 m long and 0.75 m apart giving a total plot size of 18.75 m per replicate. The trial was arranged as randomized complete block design (RCBD) replicated three times giving a total land area of 900 m<sup>2</sup>.

#### **Source of inoculum and multiplication**

The *Blackeye cowpea mosaic virus* (BICMV) and *Cowpea mottle virus* (CPMoV) isolates used were obtained from the Department of Crop Production, Federal University of Technology, Minna Niger State. The virus isolates were extracted by grinding 1g/ml of each isolate in extraction buffer containing 0.1M sodium phosphate dibasic, 0.1M potassium phosphate monobasic, 0.01M ethylene diamine tetra acetic acid and 0.001M-cystine per litre of distilled water using a pre-cooled sterilized mortar and pestle as described by Kumar (2009). Two microlitres of  $\beta$ -mercapto-ethanol was added to the extract just before use. Thereafter, cowpea seedlings were infected with BICMV and CPMoV inoculum at 10 days after sowing (DAS) by rubbing the virus extracts on the upper surface of the leaves that was dusted with carborundum powder (600- mesh). The leaves of inoculated plant were rinsed with sterile distilled water. Symptomatic cowpea leaves were collected from the infected plants at 3 weeks after inoculation (WAI) and used for inoculation during the main experiment. The leaves were preserved at

room temperature in airtight via bottle on silica gels covered with a thin layer of non-absorbent cotton wool.

#### **Agronomic practices**

The study site was manually cleared of the previous plant remains and ridged in the second week of August, 2017. Cowpea seeds were sown one week after the land preparation. Three cowpea seeds of each cultivar were sown after dressing with Apron – star (methylthiuram + metalaxyl + carboxin) at the rate of 3.0 kg seed per 10 g sachet of the chemical to protect seed against soil borne pathogens. The sowing was carried out at an intra and inter-row spacing of 0.30 × 0.75 m along the ridges and later thinned to two per stand at 2 weeks after sowing (WAS). The BICMV and CPMoV infected cowpea leaves previously preserved on silica gels were used for inoculation. For the single virus infection, seedlings of the twenty five cultivars were mechanically inoculated singly with BICMV or CPMoV at 10 days after sowing while for the mixed virus infections, seedlings were inoculated at 10 and 17 DAS. Weeds were manually controlled through hand weeding at 4 and 6 weeks after sowing. Insect pests were controlled by spraying D-D force (Cypermethrin plus Dimethoate) and pods were harvested at physiological maturity. The pods were processed and packaged for seed quality assessment in the laboratory.

#### **Assessment of Virus Infection on Seed Quality**

Seed lots from the various virus treatments were subjected to seed quality test as follows;

Germination and longevity of seeds of all the virus treatment combinations were determined by germination test after harvest and at four weeks of storage respectively at the Crop Production Laboratory,



Department of Crop Production, Federal University of Technology, Minna. There were 25 seeds placed in distilled-water moistened filter paper lined in Petri-dish in three replicates. The filter paper in the petri-dishes were kept moist as found necessary. The petri-dishes were arranged inside the seed germination chamber. Germination counts were taken at 1, 2, 3, 4 and 5 days after sowing. Seeds were considered germinated when the tip of the radicle had grown free from the seed coat (El Balla *et al.*, 2011). Germination percentage (GPCT) was calculated as follows:

$$\text{GPCT} = \frac{\text{Total number of seedlings that emerged on the final day}}{\text{Total number of seeds planted}} \times 100$$

planted

Cowpea seeds were also subjected to accelerated ageing tests at two and four weeks as described by El Balla *et al.* (2011) for vigour determination. The seeds of all the treatments were stored in open plastic plates and arranged inside an incubator at 35 °C and 86 % relative humidity. This was aimed at accelerating the ageing of the seeds so that the relative longevity of the seed samples could be determined. Twenty five seeds from each treatment that were artificially aged in three replications were counted and placed on layer of distilled water moistened-filter paper placed in Petri-dishes over a wire mesh screen inside a growth chamber at 30 °C. Germination count was taken as described above.

#### Data analysis

Data were subjected to analysis of variance (ANOVA) using Statistical Analysis System (SAS, 2008) to verify if there were significant differences among the cultivars. Significance was determined at 5 % level of probability. Where the *F*-test ratio was significant, means were separated using Student-Newman-Keuls (SNK) test.

## RESULTS

### Effects of single and mixed virus infections on seed quality

The study revealed significant impairments in germination before and after four weeks of storage of the 25 cultivars of cowpea both in single and mixed infections of the viruses used. The variation in seed germination of cowpea cultivars with respect to virus infections is presented in Table 1. Prior to storage of seeds, the difference between the lowest and highest mean value for seed germination was wide and significant ( $p < 0.05$ ). Seed germination percentage varied from 77.4 to 99.7 % for the BICMV infected cultivars, 77.4 to 98.7 % for CPMoV infected cultivars, 74.8 to 98.5 % for BICMV + CPMoV infected cultivars and 78.6 to 98.5 % for CPMoV + BICMV inoculated cultivars (Table 1). Seeds obtained from IT97K-568-18, IT04K-332-1 and IT07K-292-1-10 cowpea cultivars infected with BICMV had significantly ( $p < 0.05$ ) higher germination percentage of 99.7 which was statistically similar to 97.6 and 97.3 % germination obtained from seeds of cultivar IT07K-243-1-10 and IT03K-337-6 respectively. Seeds from cultivars IT90K-277-2, IT07K-211-1-8 and IT06K-124 had germination values of 94.7, 94.3 and 93.7 % respectively which were not significantly different among each other. Seeds of cultivars IT07K-251-3-3 and IT07K-222-2 had 92.3 and 92.5 % germination values respectively which were statistically similar while seeds from the remaining cowpea cultivars had germination percentages ranging between 77.4 and 91.3.

Furthermore, seed germ in ability of 98.7 % was highest in IT90K-277-2 with CPMoV infected cowpea seeds which was not significantly ( $p > 0.05$ ) different from seeds obtained from cultivars IT04K-332-1 (98.5





%), IT07K-243-1-10 (98.4 %), IT04K-267-8 (98.2 %) and IT96D-610 (97.7 %), while significantly lowest seed germination percentage of 77.4 was recorded in seeds of cowpea cultivar IT07K-292-1-10 (Table 1). On the other hand, co-infections of cowpea seeds significantly ( $p < 0.05$ ) affected seed germ in ability across the cowpea cultivars investigated. BICMV + CPMoV infected IT04K-332-1 exhibited the highest germination percentage of 98.5 % than all other cultivars, whereas IT96D-610 and IT97K-499-35 gave 97.6 % each. Seeds of cultivars IT07K-292-1-10 and IT97K-573-2-1 had 96.0 and 94.8 % germination respectively, while seeds of cultivar IT07K-222-2 gave in the lowest germination percentage of 74.8. Seeds obtained from cultivar IT97K-568-18 infected with CPMoV + BICMV exhibited the highest germination percentage of 98.5 before storage which was not significantly ( $p > 0.05$ ) different from 97.3 % obtained from seeds of IT99K-316-2. Next to these with high germination percentage of 96 were seeds obtained from IT90K-277-2, IT96D-610, IT98K-205-M8, IT98KD-288, IT04K-332-1 and IT07K-222-2 whereas the significantly lowest germination percentage of 78.6 was recorded in seeds of cowpea cultivars IT04K-321-2 and IT07K-211-1-8. (Table 1).

Similarly, the difference between the lowest and highest percentage mean values for the longevity test was also wide and significant ( $p < 0.05$ ) when seeds were stored for four weeks. Significantly highest germination percentage of 77.9 was recorded in seeds of BICMV infected Ife Brown followed by IT90K-277-2, IT00K-901-5 and IT96D-610 with 76.6, 70.6 and 70.3 germination percentage, respectively. Seeds of cultivar IT97K-568-18, IT07K-292-1-10 and IT07K-

299-6 exhibited germination values of 69.5, 64.4 and 62.1 %, respectively whereas the least germination values of 46.6 % was obtained from seeds of IT06K-124. Mean value for accelerated ageing germination (AAG) on CPMoV infected cowpea cultivars showed that seeds of IT98K-205-M8 had 70.6 % germination. This was closely followed by seeds of Ife Brown with 69 % while 68, 66.8 and 66.5 % were obtained from cultivars IT90K-277-2, IT03K-337-6 and IT96D-610, respectively. The germination capacity of 64 % was recorded from seeds of cultivars IT99K-316-2 while IT07K-299-6 and the remaining cultivars had AAG percentages ranging from 53.4 to 62.7 % (Table 1).

For the mixed infection treatments, germination value of 58.6 % was obtained from IT90K-277-2, IT06k-124 and IT07K-292-1-10 BICMV + CPMoV infected cowpea cultivars. This value (58.6 %) was significantly ( $p < 0.05$ ) higher than the values obtained from seeds of other cultivars. Seeds from cultivars IT98K-205-M8, IT97K-499-35, IT06K-137-1 and IT07K-211-1-8 gave germination values of 56.5, 55, 54.5 and 53.4 % respectively. Seeds of cultivars IT96D-610 and IT00K-901-5 exhibited similar germination percentage of 52 while the remaining cowpea cultivars had germination percentages of between 44.0 and 50.6. Also, seed germ in ability of 57.3 % was highest in IT07K-292-1-10 with CPMoV + BICMV infected cowpea seeds which was statistically ( $p > 0.05$ ) similar to the performance of seeds of IT97K-499-35 with 56 %. Seeds of cultivar IT04K-267-8 and IT07K-222-2 exhibited 54.6 and 53.7 % respectively, while IT96D-610 and IT04K-291-2 had germination values of 52 % which did not differ from one another. The lowest AAG percentage of 31.6 was



recorded in seeds of cowpea cultivar IT99K-377-1 (Table 1).

## DISCUSSION

Germination and longevity are two major indices used for determining the performance capability of seed lot. Seed quality is influenced by the environment where it is produced. Pathogens namely virus, nematode, fungi, bacteria among others are integral components of the environment of any seed crop; failure to effectively manage their competition could mean zero harvest (Adesina *et al.*, 2012) as found in this study. However the imperative of understanding the impact of virus management strategies and management for quality seed production arises from the paucity of information on the agronomy of seed production (Adesina *et al.*, 2012), more so that seed production efforts are judged on the basis of quality of the produce rather than quantity. The result of this study has established a clear negative influence of virus infection on cowpea seed quality and that the differential ranking of the virus infection treatments in different seed quality test is an indication of the response of the developing seeds on the mother plant to competing virus infection situations. Differences in time of flower initiation, pod setting, seed formation and maturity to virus infections are critical factor to tropical farming. The results obtained from this study revealed that there was a variation in germination percentage before and after four weeks of storage which is a measure of seed viability and longevity. When seed that has this trait is sown on the field for production, it exhibits a wide variation in performance after sowing due to the differences in quality (Adesina *et al.*, 2012). Since seeds did not ripen at the same time amongst virus treatments across the test cowpea cultivars, variation in seed germination and longevity

due to age at harvest is inevitable (Singh, 2014).

It is known that cowpea seedlings are susceptible to virus infection at different stages of development (Agrios, 2005). This is supported by the differential responses of cowpea seeds harvested from the different virus treatment seed lots in the present study. The initial general high germination percentage recorded in seeds of all treatment combinations in this study is an indication that the seeds did not exhibit dormancy contrary to what is known with most vegetable seeds when freshly harvested. This rapid germination also showed that the activities of the pathogens (viruses) on the seeds were not severe enough to impaired germination (Anjorin and Mohammed, 2014). Mandhare and Gawade (2010) reported that thoughseeds obtained from mosaic infected bean at harvest exhibited high seed germination, a significant sharp decline in germination percentage of the seeds was recorded following four weeks of storage at 32 °C and 50 % relative humidity. Following storage of seeds for four weeks in this study, a sharp decline in the germination capability of seeds of all the treatment combinations was recorded. This sharp decline in the quality of seeds is abnormal according to the normal/natural seed ageing process (Hamim *et al.*, 2014). The reason may be that the pathogen activities must have been activated which resulted in the sudden and heavy decline in the germination percentages (Ahmad *et al.*, 2006). Furthermore, the variation in germination percentages amongst the cultivars and treatments as shown in this study suggest genetic superiority (Anjorin and Mohammed, 2014) and tolerance level of the cultivars over one another.

## CONCLUSION AND RECOMMENDATIONS



The results of the experiment revealed that all cultivars were susceptible to single and mixed infections of the two viruses but to seemingly different extent. The germination of seeds as seen from this study was generally high before storage; the high initial germination percentage was not sustained (short lived); an indication that conservation of infected seeds of all cultivars was impaired. More so, all the cowpea cultivars did not exhibit dormancy which is a problem with most freshly harvested vegetable seeds. The benefits of increased cowpea production include improved nutrition for humans and livestock, improved soil properties and substantial opportunities for greater income. The monitoring and management of these viruses therefore is crucial to sustainable cowpea production most especially in sub-Saharan Africa. There is the need, therefore, for constant monitoring of legume fields through regular field sanitation, disease surveys to identify new and emerging viruses because these facts present a good starting point for legume virus diseases diagnosis in the study area. Finally, there is also need to ensure availability of acceptable horticultural desirable cowpea cultivars with a high level of resistance to cowpea viruses for the nation to sustain its high level of production.

## REFERENCES

- Adesina, G. O., Ajayi, S. A and Olabode, O. S. (2012). Influence of weed control methods on viability and vigour of maize (*Zea mays* L.) seeds. *Nigerian Journal of Weed Science*, 25: 117-124.
- Ahmad, Z., Ghafoor, A. and Bashir, M. (2006). Effect of seed-borne pathogens on seed longevity in chickpea and cowpea under storage at 25 °C to 18 °C. *Seed Science Technology*, 34: 69-75.
- Agrios, G. N. (2005). *Plant Pathology*, Fifth Edition. Elsevier Academic Publishers. Amsterdam.
- Alegbejo, M. D (2015). Virus and virus-like diseases of crops in Nigeria. Zaria, Nigeria. Ahmadu Bello University Press. 273p.
- Anjorin, S. T and Mohammed, M. (2014). Effects of seed-borne fungi on germination and seedling vigour of watermelon (*Citrullus lanata* Thumb). *African Journal of Plant Science*, 8(5): 232-236.
- Bashir, M., Ahmad, Z and Murata, N. (2000). *Seed-borne Viruses: Detection, Identification and Control*. Islamabad, Pakistan, Pakistan Agricultural Research Council, National Agricultural Research Center, 156p.
- Batiano A. (2011). Fighting poverty in sub-Saharan Africa: *The multiple roles of legumes in integrated soil fertility management*. New York, Dordrecht.
- Dugje, I. Y., Omoigui, L. O., Ekeleme, F., Kamara, A. Y and Ajeigbe, H. (2009). *Farmers' Guide to Cowpea Production in West Africa*. Ibadan, Nigeria, IITA, pp. 5-12.
- El Balla, M. M. A., Saidahmed, A. I and Makkawi, M. (2011). Effects of moisture contents and maturity on hardseededness and germination in okra (*Albemoschus esculentus* [L] Moench.) seeds. *Weed science* 35, 45-51
- FAO (Food and Agriculture Organization) (2012). Available from <http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#anchor>



- Gómez, C. (2012). *Cowpea: Post-harvest management*; Rome, Italy. Food and Agriculture Organization (FAO), 71p
- Hamim, I., Mohanto, D. C., Sarker, M. A and Ali, M. A. (2014). Effect of seed-borne pathogen on germination of some vegetable seeds. *Journal of Phytopathology and Pest Management*, 1(1): 34-51.
- IITA (International Institute for Tropical Agriculture). (2013) *Research Highlights*, Ibadan, Nigeria IITA. pp 143-146
- Kareem, K. T and Taiwo, M. A (2007). Interactions of viruses in cowpea: Effects on growth and yield parameters. *Virology Journal*, 4(1): 234-240.
- Kumar, L. (2009). *Methods for the diagnosis of Plants Virus diseases. Laboratory Manual*, Ibadan IITA, 94p.
- Mandhare, V. KandGawade, S. B (2010). Effect of seed-borne *Soybean mosaic virus* infection on quality and yield parameters in soybean. *Legume Research*, 33,(1),43-49.
- SAS [Statistical Analysis System](2008). *Statistical Analysis System SAS/STAT User's guide*, version. 9.2. Cary, N.C SAS Institute Inc.
- Singh, G. (2014). Response of soybean (*Glycine max*) genotypes to plant population and planting geometry in Northern India. *International Journal of Agricultural Resources*, 6: 653-659.





**Table 1: Cowpea seed quality as affected by single and mixed infections of Blackeye cowpea mosaic virus (BICMV) and Cowpea mottle virus (CPMoV) at Mokwa in 2017**

Cultivar	Germination Test (%)				Accelerated Ageing Germination (%) 4 Weeks of Storage			
	BICMV	CPMoV	BI + CP	CP + BI	BICMV	CPMoV	BI + CP	CP + BI
Ife Brown	93.5 <sup>bcd</sup>	90.5 <sup>c-f</sup>	86.7 <sup>f</sup>	86.5 <sup>gh</sup>	77.9 <sup>a</sup>	69.0 <sup>b</sup>	56.6 <sup>b</sup>	46.2 <sup>l</sup>
IT90K – 277 – 2	94.7 <sup>bc</sup>	98.7 <sup>a</sup>	78.5 <sup>c</sup>	96.0 <sup>bc</sup>	76.6 <sup>a</sup>	68.0 <sup>bc</sup>	58.4 <sup>a</sup>	51.6 <sup>e</sup>
IT96D – 610	87.3 <sup>g</sup>	97.7 <sup>a</sup>	97.6 <sup>b</sup>	96.0 <sup>bc</sup>	70.3 <sup>b</sup>	66.5 <sup>d</sup>	52.0 <sup>e</sup>	52.0 <sup>de</sup>
IT97K – 499 – 35	88.0 <sup>fg</sup>	86.9 <sup>i</sup>	97.6 <sup>b</sup>	92.0 <sup>e</sup>	61.5 <sup>de</sup>	60.0 <sup>g</sup>	55.0 <sup>c</sup>	56.0 <sup>ab</sup>
IT97K – 568 – 18	99.7 <sup>a</sup>	91.2 <sup>c</sup>	81.3 <sup>j</sup>	98.5 <sup>a</sup>	69.5 <sup>b</sup>	57.2 <sup>h</sup>	48.0 <sup>h</sup>	41.2 <sup>l</sup>
IT97K – 573 – 2 – 1	87.8 <sup>g</sup>	93.4 <sup>b</sup>	94.8 <sup>d</sup>	94.5 <sup>d</sup>	50.6 <sup>l</sup>	57.1 <sup>hi</sup>	45.5 <sup>i</sup>	35.6 <sup>m</sup>
IT98K – 205 – M8	87.6 <sup>g</sup>	89.2 <sup>efg</sup>	77.5 <sup>m</sup>	96.0 <sup>bc</sup>	57.5 <sup>gh</sup>	70.6 <sup>a</sup>	56.3 <sup>b</sup>	41.5 <sup>l</sup>
IT98KD – 288	91.3 <sup>c-g</sup>	90.7 <sup>cde</sup>	82.6 <sup>i</sup>	96.0 <sup>bc</sup>	48.0 <sup>m</sup>	62.7 <sup>f</sup>	48.3 <sup>gh</sup>	51.3 <sup>ef</sup>
IT99K – 316 – 2	92.1 <sup>c-f</sup>	93.4 <sup>b</sup>	85.0 <sup>gh</sup>	97.3 <sup>ab</sup>	53.3 <sup>jk</sup>	64.0 <sup>ef</sup>	57.3 <sup>ab</sup>	46.0 <sup>j</sup>
IT99K – 377 – 1	88.9 <sup>efg</sup>	90.8 <sup>cd</sup>	85.4 <sup>gh</sup>	92.0 <sup>e</sup>	60.0 <sup>ef</sup>	60.0 <sup>g</sup>	50.6 <sup>f</sup>	31.6 <sup>n</sup>
IT00K – 901 – 5	88.8 <sup>efg</sup>	86.1 <sup>i</sup>	81.3 <sup>j</sup>	89.3 <sup>f</sup>	70.6 <sup>b</sup>	65.0 <sup>e</sup>	52.0 <sup>e</sup>	47.0 <sup>ij</sup>
IT03K – 337 – 6	97.3 <sup>ab</sup>	89.4 <sup>d-g</sup>	84.6 <sup>h</sup>	89.3 <sup>f</sup>	50.5 <sup>l</sup>	66.8 <sup>cd</sup>	46.4 <sup>i</sup>	41.4 <sup>l</sup>
IT04K – 267 – 8	92.2 <sup>c-f</sup>	98.2 <sup>a</sup>	81.3 <sup>j</sup>	86.5 <sup>gh</sup>	56.0 <sup>hi</sup>	62.6 <sup>f</sup>	49.5 <sup>fg</sup>	54.6 <sup>bc</sup>
IT04K – 291 – 2	87.8 <sup>g</sup>	86.9 <sup>i</sup>	89.3 <sup>e</sup>	87.7 <sup>g</sup>	54.6 <sup>ij</sup>	58.7 <sup>g</sup>	57.4 <sup>ab</sup>	52.0 <sup>de</sup>
IT04K – 321 – 2	90.5 <sup>c-g</sup>	93.8 <sup>b</sup>	85.3 <sup>gh</sup>	78.6 <sup>k</sup>	58.6 <sup>fg</sup>	56.3 <sup>hi</sup>	48.0 <sup>h</sup>	50.6 <sup>efg</sup>
IT04K – 332 – 1	99.7 <sup>a</sup>	98.5 <sup>a</sup>	98.5 <sup>a</sup>	96.0 <sup>bc</sup>	60.0 <sup>ef</sup>	53.4 <sup>k</sup>	49.3 <sup>fgh</sup>	48.1 <sup>hi</sup>
IT06K – 124	93.7 <sup>bc</sup>	90.1 <sup>c-g</sup>	80.0 <sup>k</sup>	81.2 <sup>j</sup>	46.6 <sup>m</sup>	56.8 <sup>hi</sup>	58.6 <sup>a</sup>	49.6 <sup>fgh</sup>
IT06K – 137 – 1	77.4 <sup>h</sup>	87.2 <sup>hi</sup>	78.8 <sup>l</sup>	80.0 <sup>jk</sup>	52.0 <sup>kl</sup>	56.0 <sup>hij</sup>	54.5 <sup>cde</sup>	44.0 <sup>k</sup>
IT07K – 211 – 1 – 8	94.5 <sup>bc</sup>	88.5 <sup>gh</sup>	89.3 <sup>e</sup>	78.6 <sup>k</sup>	53.3 <sup>jk</sup>	56.0 <sup>hij</sup>	53.4 <sup>d</sup>	49.3 <sup>gh</sup>
IT07K – 222 – 2	92.5 <sup>cde</sup>	93.0 <sup>b</sup>	74.8 <sup>n</sup>	96.0 <sup>bc</sup>	54.2 <sup>j</sup>	56.4 <sup>ik</sup>	45.3 <sup>ij</sup>	53.7 <sup>bc</sup>
IT07K – 243 – 1 – 10	97.6 <sup>ab</sup>	98.4 <sup>a</sup>	89.3 <sup>e</sup>	94.8 <sup>cd</sup>	57.5 <sup>gh</sup>	54.7 <sup>jk</sup>	50.5 <sup>f</sup>	50.8 <sup>efg</sup>
IT07K – 251 – 3 – 3	92.3 <sup>cde</sup>	88.5 <sup>gh</sup>	82.8 <sup>i</sup>	85.3 <sup>h</sup>	57.3 <sup>gh</sup>	56.6 <sup>ij</sup>	44.0 <sup>k</sup>	47.0 <sup>ij</sup>
IT07K – 292 – 1 – 10	99.7 <sup>a</sup>	77.4 <sup>j</sup>	96.0 <sup>c</sup>	81.3 <sup>j</sup>	62.1 <sup>d</sup>	57.3 <sup>h</sup>	58.3 <sup>a</sup>	57.3 <sup>a</sup>
IT07K – 299 – 6	80.3 <sup>h</sup>	89.0 <sup>gh</sup>	86.8 <sup>f</sup>	82.6 <sup>i</sup>	64.4 <sup>c</sup>	64.0 <sup>ef</sup>	49.7 <sup>f</sup>	44.0 <sup>k</sup>
IT07K – 318 – 33	89.3 <sup>d-g</sup>	77.6 <sup>j</sup>	85.6 <sup>g</sup>	80.0 <sup>jk</sup>	59.8 <sup>f</sup>	58.7 <sup>g</sup>	44.9 <sup>jk</sup>	46.5 <sup>ij</sup>
SE ±	1.27	0.5	0.26	0.43	0.54	0.46	0.42	0.61

Means with the letter (s) within the same column are not significantly ( $p \leq 0.05$ ) different by Student-Newman-Keuls (SNK) test