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Improvement of Okra (*Abelmoschus esculentus* L.) Hardseedness by Using Micro Elements Fertilizer

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ABSTRACT

The occurrence of hardseedness and the low percentage of seed germination are major challenges when growing okra. For this reason, this study was conducted to determine the effects of micro elements fertilizer in relation with time of harvest and plant parts on germination behavior of cultivars of okra (Abelmoschus esculent L.). The study was carried out at Agricultural farm of Thessaly University in 2011. The design was a randomized complete block with a factorial arrangement with three replications. Four different cultivars of okra contain Boiatloy, Beloudo, Clemsson and Pleas and three treatments of micro fertilizer were studied. Results showed micro fertilizer treatment had positive effect on germination. Both level of micro fertilizer (F_2 and F_3) raised noticeable seed germination than control (F_1). Increasing level of fertilizer from first level of fertilizer (F_1) to third level of fertilizer (F_3) was more effective in varieties with more seedhardness; In this case application of level three of fertilizer (F_3) in Beloudo variety was more positive than other varieties. By increasing time of harvest percentage of seed germination was increased until third harvesting time (T_3) and after that reduced. Level two (F_2) and three (F_3) of micro fertilizer had a same effect on seeds that harvested in second harvesting time (T_2). Application of level three of fertilizer (F_3) was more effective on seeds that harvested in three harvesting time (T_3). Application level three of fertilizer (F_3) on seeds in middle part of the plant produced more seed germination.

Keywords: Okra, Germination, Seedhardness, Fertilizer, Dormancy.

INTRODUCTION

Okra (*Abelmoschus esculentus* (L.) Moench) crop exhibits seed hardness that complicates its management. This seed hardness interferes with seed germination, weed control, harvesting and other management factors (Mohammadi et al., 2011). As a starting generation, seed is structurally and physiologically equipped as a dispersal unit and providing food for growing seedlings until it establishes itself as an autotrophic organism (Bewley, 1997). To do this the mature seed should germinate first. This physiological reaction begins with water uptake by the dry seeds and terminates with the initial elongation of the embryonic axis. Germination tests give some information about seed constituents (Malo, 2000). Nevertheless, in many cases desired germination rates could not be attained due to the dormancy requirement that is one of the major obstacles especially for most legume forage species (Ersin CAN et al., 2009). The dormancy of dormant seed must be broken to induce germination. Various methods are used for this, depending on the plant species and type of dormancy (Koyuncu, 2005). It could be that the seeds exhibit some form of dormancy, possibly associated with the seed coat. This is the first factor to be checked if seeds do not germinate after a reasonable after-ripening period (Budy et al., 1986) because the capacity of many seeds to germinate is determined by the seed coat. Tough seed coats may regulate germination by establishing a permeability barrier that can interfere with the water uptake required for imbibition and subsequent radicle emergence; for gaseous exchange, particularly oxygen uptake required for respiration; and/or for the outward diffusion of endogenous germination inhibitors (Mmolawa, 1987). Typical characteristics of hard seeds are seed coats that are permeable to water but not to gases or vice versa (Budy et al., 1986; Mmolawa, 1987).

The percentage of seed germination of okra is relatively low, due to occurrence of hardseedness in this plant (Luis Felipe et al., 2010). The percent of hardseedness in okra cultivar is different, in some cultivars they don't have hardseedness or they have a little hardseedness that doesn't create any impedance for their germination, but in other cultivars their hardseedness doesn't let them to germinate, or low percent of them germinate. Ellis et al., (1985) and Standifer et al., (1989) emphasized that hardseededness in okra vary among cultivars. Also El Balla et al. (2011) reported that percentage of hardseedness highly affected by the cultivars.

Time of harvest of pods, seed moisture content, fertilizer, growth regulators, priming, scarification, growth regulators are some of the methods that proposed that have effect on okra seedhardness. It was reported, the percentage of hardseedness increased significantly in all cultivars with the increase in the maturity of seed. This may be due to the deposition or development of hard cuticle or impermeable cell layer of the seed coat during the later stages of seed development (El Balla et al., 2011). Similarly, Egley (1987) noted that impermeability in showy crotalaria (*Crotalaria spectabilis*) seed was acquired during the later stages of maturation on the parent plants. In okra plant, Chauhan and Bhandri (1971) reported that seeds that harvested at 30 days from anthesis had the maximum percentage of germination. Castro et al. (2008) concluded that okra seeds that harvested in 34 and 41 DAA had a highest germination. While, Demir (1997) reported that okra seeds failed to germinate until 32 DAA, as soon as seeds began to germinate at 36 DAA, the percentage of hard seeds increased.

Seed moisture content is another factor that proposed has effect on seedhardness. As the seed matured and by decreasing its moisture content the percentage of hardseedness increased. This affair was confirmed by another scientist (El Balla et al., 2011; Quinlivan (1971); Ellis et al., (1985); Standifer et al., (1989) and Demir (2001). El Balla (2011) in another experiment showed that when the seed moisture content of *Trifolium patense*, *Trifolium repens* and *Lupinus arborcus* reach to 14% their seedhardness increased.

Pod position in the plant also proposed that can effect on hardseedness. In this case seeds that harvested in pods in middle and lower part of the plant than upper part of plant produced more seed germination and less seed hardness. For example Felipe V Purquerio, (2010), Malik et al., (2000) has reported that seeds from pods located in the middle of the plant had higher germination, but Yadav and Dhankhar (2001); Naik et al., (2004); Prabhakar et al., (1985) and Malik et al., (2000) reported that harvested seed from the lower pods had more seed germination.

Fertilizer is another factor that can effect on hardseedness. It was revelled that okra gives good response to different plant nutrients (Mishra and Singh, 2005). But in the case of the effect of fertilizer on hardseedness, it was reported that seed germination doesn't affect by the fertilizer application (Anjum and Amjad, (1999), Amjad et al., (2001). Several attempts have been made to study the response of okra to various levels of nitrogen (Majanbu et al., 1982; Shrestha, 1983; Kabura et al., 2002). Amjad et al., (2001) in their experiment on the cultivar of okra cv. Pusa Sawaniit reported that germination percentage, green pod length and yield were not influenced by different levels of fertilizers used.

The objective of this study was to determine the effect of micro fertilizer in relation of time of harvest of pods and plant parts on seed germination and investigate effective level of micro fertilizer for overcoming okra seedhardness.

MATERIALS AND METHODS

The experiment was conducted in Agricultural Farm of Thessaly University in Greece in 2011. Climate characteristics of experimental place were shown in Table (1). Each experiment was laid out in Randomized Complete Block Design with factorial arrangement with three replications. 4 different cultivars of okra contain Clemson, Boiatloy, Beloudo and Pleas and three micro fertilizer treatments were studied. Two different micro fertilizer (called Proten and Basfolir 36 extra from Compo and IKO-Hydro Company) treatment as fallow gave to the plants:

F_1= control (0 cc of micro fertilizer).
F_2= 20 cc micro fertilizer (20 cc Proten and 20 cc Basfolir 36 extra) solved in 10 lit water.
F_3= 40 cc micro fertilizer (40 cc Proten and 40 cc Basfolir 36 extra) solved in 10 lit water.

Land preparation operations plowing, disk and trowel to the desired way, before transplanting was done in the first half of May. After taking track, map test was implemented on the ground. Seeds first were planted inside planting shelf (foam) in the greenhouse and when the height of the plants was 10 cm they transplanted in the farm. Transplanting of the plants was done in the 21 of May by hand. Each replicate contain 12 plots and each plot was 9 m^2 (3×3). Total area of the land for each experiment was 539.5 m. Between the two replicate two meters distance was considered. Crop irrigated regularly one time each week. Broadleaves weeds were control through hoeing manually and narrow leaves by Fusilade herbicide. Plants 33 days after transplanting in the farm started to flowering. Each flower tagged and put pocket. Pods were collected at four harvesting time as fallow:

T_1= Harvesting pods 30 days after flowering.
T_2= Harvesting pods 35 days after flowering.
T_3= Harvesting pods 40 days after flowering.
T_4= Harvesting pods 50 days after flowering.

Each individual crop of okra divided in 3 different parts and pods tagged as a pod of low-middle and upper part of the plant. Initial seed moisture contents were calculated by using low constant temperature oven method (ISTA, 2009). 5 g of seeds from each treatment were taken. After grinding the seeds in grinding mill the weight ground material were poured in a small container with cover and kept in an oven maintained at a temperature of 103°C for a period of 17 hours. The moisture content of seeds was determined by the following formula (ISTA, 2009):

$$\% MC = \frac{(M2 - M3)}{(M2 - M1)} \times 100$$

M1=Wt. in grams of container and its cover.
M2= Wt. in grams of container, its cover + ground material before drying
M3= Wt. in grams of container, its cover + ground material after drying

To study of the seedhardness seed germination was conducted using 400 seeds (four replications of 100 seeds) per each treatment (ISTA, 2009). Seeds sterilized by Mankuzeb fungicide and were placed on double layered Wathman No.1 filter paper moistened with distilled water in sterilized Petri dishes. At final, all Petri dishes were placed in a germinator at 25°C for 21 days and every 2 days the number of germinated seeds was counted and removed; in the case of moisture deficiency distilled water was added. A seed was considered germinated when the tip of the radical had grown free of the seedcoat (Wiese and Binning, 1987; Auld et al., 1988). Seeds that in the last of the test did not absorb water and last as a rigid were considered hardseed. Germination percentage was calculated by following formula (ISTA, 2009):

$$\text{Germination (\%)} \frac{\text{Number of seeds germinated}}{\text{Number of seeds tested}} \times 100$$

For study seed and pod characteristics 5 plants were tagged in each plot randomly to record the data on 100 seed weight, Average seed per pod, pod length, pod diameter and seed yield.

Data were analyzed by using SAS statistical program and Duncan test was employed to classify mean values of different treatments when F-values were significant (P<0.05). Graphs were generated by using EXCEL software.

RESULTS AND DISCUSSION

Micro fertilizer:
Micro fertilizer treatment had positive effect on germination. Both level of micro fertilizer (F_2 and F_3) raised noticeable seed germination than control (F_1) (Fig 1). The level three of micro fertilizer (F_3) with 75.8% produced highest percentage of germination (Fig 1); this level of fertilizer increased 26.8% seed germination than control (F_1). In the experiment to find out the effect of micronutrients on seed production in okra, the maximum seed germination, seed vigor and seed yield was recorded 86.33%, 6.78 and 4.53 q ha^{-1}. respectively, followed by commercial formulation (Multiplex) (Patil et al., 2007).

Variety:
Different varieties showed different seed germination under the effect of micro fertilizer. In Boiatloy and Beloudo varieties in compare to other varieties produced less seed germination under different fertilizer levels (Fig 2). Level three of micro fertilizer (F_3) more raised percentage of seed germination in Beloudo and Pleas varieties than other varieties (Fig 2). Increasing level of fertilizer from first level of fertilizer (F_1) to third level of fertilizer (F_3) was more effective in varieties with more seedhardness. In this case application of level three of fertilizer (F_3) in Beloudo variety was more effective than other varieties; in this variety using third level of fertilizer (F_3) give 63.8% seed germination that in compare to first level of fertilizer (F_1) with 47.8% raised 16% more seed germination (Table 2), whereas increasing fertilizer level was less effective in Clemsson variety; in this variety difference between seed germination of first (F_1) and third level of fertilizer (F_3) with 69.7 and 77.2% was only 7.5% (Table 3).

In okra, seed germination is influenced by the cultivar, Batt and Srinivasa Rao (1998) found considerable reduction in seed germination under field conditions compared with the controlled conditions in different cultivars of okra. In some cultivars, the occurrence of hard seeds becomes more frequent the longer the pods remain in the plant (V Purquerio, 2010). Study of seed moisture content of varieties showed that in all varieties using third level of fertilizer (F_3) that produced higher percentage of germination had more moisture content (Table 4). In this case Boiatloy variety that in compare to other varieties had less seed germination also had less moisture content (Table 4).

El Balla *et al*. (2011) reported that seeds of *Hibiscus esculentus* L. (Moench) at 13% moisture content tend to show little or no hardseededness, but once the seeds have been dried and their moisture content was dropped to 4 to 6%, their seedhardness becomes prevalent.

Harvesting time:
Study of effect of micro fertilizer on percentage of germination of seeds from different harvesting times showed that by increasing time of harvest percentage of seed germination was increased until third harvesting time (T_3) and after that reduced (Fig 3). Level two (F_2) and three (F_3) of micro fertilizer had a same effect on seeds that harvested in second harvesting time (T_2) (Fig 3). Application of level three of fertilizer (F_3) was more effective on seeds that harvested in three harvesting time (T_3) (Fig 3). In Boiatloy variety seeds that harvested in four harvesting time (T_4) showed a less seed germination and more seed hardness than other varieties in this harvesting time (Table 5).

Several studies are available on development of the seed within the capsule and the days to physiological maturity and harvesting. The highest seed germination was recorded when the pods were harvested 35 days (Ewete, 1980; Kanwar and Saimbhi, 1987) and 40 days after anthesis (Neupane et al., 1991). Delay in harvesting time reduced seed weight and germination percentage.

Study of moisture content of seeds from different harvesting times showed that the amount of moisture content of seeds from the first (T_1) to four (T_4) harvesting time hah reduced (Table 4). Seeds that harvested at different harvesting times under the effect of third level of fertilizer (F_3) produced more seed moisture content (Table 4). Probably reduction of moisture content of seeds that harvested in four harvesting time (T_4), in Boiatloy and Beloudo varieties can be associate with creating seeds with hard coat, but this reduction in Pleas and Clemsson varietites is vice versa and reduction of moisture content doesn’t create any seeds with hard coat. It is quite obvious that plants that harvest earlier, did not complete their physiological maturity and have more moisture content, but plants that reach to their physiological maturity their moisture content was decreased.

In the experiment that was done by Demir (1997), when the moisture content of seeds that harvested 50 DAA dropped to 10%, 52% of them represent hardseedness.

Plant part:
Study of effect of micro fertilizer on seed germination from different parts of plant showed that application level three of fertilizer (F_3) on seeds on middle of the plant produced more seed germination (Fig 4).

In upper and middle of part of plant seeds that harvested in Pleas and Boiatloy variety under the effect of first (F_1) and third (F_3) level of fertilizer produced higher and lower of seed germination, respectively (Table 6 and 5). In lower part of plant seeds that harvested in Clemsson and Boiatloy variety under the effect of third (F_3) and second (F_2) level of fertilizer had higher and lower percentage of seed germination, respectively (Table 3 and 5).

Fruit position on the plant or the fruit-bearing node position has influence on seed quality. Yadav & Dhankhar (2001) observed higher values of seed per fruit, test weight, germination, vigor index, and seed yield in fruits harvested from lower positions on the plant at Hisar, India under semiarid conditions. The lowest value for these parameters was from seeds harvested from upper part of the plant. Pod position in the plant also affects seed quality (V Purquerio et al., 2010).

Study of seed moisture content in different parts of plant showed that by increasing level of fertilizer from control (F_1) to third level of fertilizer (F_3) percentage of seed moisture content was increased (Table 4). The highest (31.1%) percentage of seed moisture content was achieved in lower part of plant under the effect of third level of fertilizer (F_3) (Table 4). Mohammadih et al., (2011) in previous experiment showed that seeds from the middle part of the plant had more seed moisture content at the time of harvest.

Table 1 - Climate characteristics of experimental place

Month	Minimum temperature (°C)	Maximum Temperature (°C)		Average of humidity (%)	Precipitation (mm)
April.	18.4	7.5		75.0	0.8
May.	24.5	11.9		69.0	0.9
Jun.	30.3	16.4		58.0	0.9
July.	34.5	18.9		46.4	0.8
Aug.	32.5	18.2		59.5	0.7
Sep.	30.1	15.6		64.0	0.7
Oct.	19.8	9.0		74.8	1.2
Average	27.1	13.9		63.8	0.8
Soil Structure	S	Si	C	pH	Organic Matter
	48%	29%	23%	7,7- 8,1	1,3gr/100gr Soil

Table 2: Effect of micro fertilizer on percentage of seed germination of Beloudo variety in relation to plant part and time of harvest

Variety: **Beloudo**												
Plant part	Upper				Middle				Lower			
Fertilizer	F1	F2	F3		F1	F2	F3		F1	F2	F3	
Time of harvest	G (%)	G (%)	G (%)	Mean	G (%)	G (%)	G (%)	Mean	G (%)	G (%)	G (%)	Mean
T1	39bc	37.3	41c	39.1	38b	40.6c	51.3c	43.3	40.3b	47.3b	46.3c	44.6
T2	49.6ab	49.3	50.6b	49.8	48b	57b	64.3b	56.4	45.3b	49.3b	50.3c	48.3
T3	62a	65.3	74.3a	67.2	65a	71.3a	75.3a	70.5	67.3a	68.6a	74.3a	70.0
T4	40.6c	41.3	49.6b	43.8	43b	41.6c	64.6b	49.7	40.3b	42.3b	65.3b	49.3
Mean	47.8	48.3	53.8		48.2	52.6	63.8		48.3	51.8	59.0	

Table 3: Effect of micro fertilizer on percentage of seed germination of Clemsson variety in relation to plant part and time of harvest

Variety: **Clemson**												
Plant part		Upper				Middle				Lower		
Fertilizer	F1	F2	F3		F1	F2	F3		F1	F2	F3	
Time of harvest	G (%)	G (%)	G (%)	Mean	G (%)	G (%)	G (%)	Mean	G (%)	G (%)	G (%)	Mean
T1	55b	54.6c	56c	55.2	53b	54b	52.3b	53.1	50.6b	51.3b	56.3b	52.7
T2	63.6b	61.3a	64b	62.9	61.6b	62b	60.6b	61.4	57.3b	57.6b	63.6b	59.5
T3	80.3a	83.6ab	87.6a	83.8	82.3a	83.3a	84.3a	83.3	82.6a	86.3a	90.3a	86.4
T4	85.6a	90.3a	92.3a	89.4	90.6a	88.6a	90.6a	89.9	88.3a	90.6a	98.6a	92.5
Mean	71.1	72.4	74.9		71.8	71.9	71.9		69.7	71.4	77.2	

Table 4: Effect of micro fertilizer on seed moisture content of variety, harvesting time and plant part

Fertilizer	Variety	MC (%)	Harvesting time	MC (%)	Plant part	MC (%)
F_1	Boiatloy	28.6	T_1	49.0	Upper	29.4
	Beloudo	29.8	T_2	42.6	Middle	29.6
	Pleas	29.7	T_3	14.9	Lower	29.1
	Clemson	29.6	T_4	11.1		
F_2	Boiatloy	28.5	T_1	49.5	Upper	30.1
	Beloudo	30.9	T_2	43.6	Middle	30.1
	Pleas	30.8	T_3	15.6	Lower	30.0
	Clemson	30.1	T_4	11.7		
F_3	Boiatloy	28.9	T_1	49.5	Upper	30.4
	Beloudo	30.2	T_2	43.7	Middle	30.8
	Pleas	32.0	T_3	17.0	Lower	31.1
	Clemson	31.9	T_4	12.7		
Anova		66.9	Fertilizer (F)	66.9	Fertilizer (F)	66.6
Main effects		101.0	Harvesting time (T)	38980.5	Plant part (P)	1.2
Fertilizer (F)		15.5	Interactions (FxT)	5.8	Interactions (FxP)	3.5
Variety (V)						
Interactions (FxV)						

Table 5: Effect of micro fertilizer on percentage of seed germination of Boiatloy variety in relation to plant part and time of harvest

Variety: **Boiatloy**												
Plant part		Upper				Middle				Lower		
Fertilizer	F1	F2	F3		F1	F2	F3		F1	F2	F3	
Time of harvest	G (%)	G (%)	G (%)	Mean	G (%)	G (%)	G (%)	Mean	G (%)	G (%)	G (%)	Mean
T1	33.6^{c}	38.3b	35.3^{d}	35.7	35b	39b	39.6c	37.8	37	33c	38	36
T2	43.3^{b}	43.3a	45^{b}	43.8	42b	51.6a	53b	48.8	42	45b	48.3	45.1
T3	53^{a}	61a	61^{a}	58.3	56.3a	63a	68a	62.4	57	57a	60.6	58.2
T4	25.6^{d}	37b	41.6^{c}	38.7	32.3b	34.3b	35c	33.8	34	32c	36	34
Mean	38.8	44.9	45.7		41.4	46.9	48.9		42.5	41.7	45.7	

Table 6: Effect of micro fertilizer on percentage of seed germination of Pleas variety in relation to plant part and time of harvest

Variety: **Pleas**												
Plant part		Upper				Middle				Lower		
Fertilizer	F1	F2	F3		F1	F2	F3		F1	F2	F3	
Time of harvest	G (%)	G (%)	G (%)	Mean	G (%)	G (%)	G (%)	Mean	G (%)	G (%)	G (%)	Mean
T1	47b	50.6b	54.3b	50.6	49.3b	50.6c	54.3	51.4	48.6b	50.6b	50.6c	49.9
T2	56.3b	55.3b	60.6b	57.4	57.6b	58.3b	58.6	58.1	53.3b	54.3b	59.3b	55.6
T3	74a	77.3a	94a	81.7	84.3a	91.6a	92.3	89.4	70.3a	75a	95.3a	80.2
T4	75.3a	84.6a	96a	85.3	77a`	85.3a	88.6	83.6	73.6a	80a	90.6a	81.4
Mean	63.1	66.9	76.2		67.0	71.4	73.4		61.4	64.9	73.9	

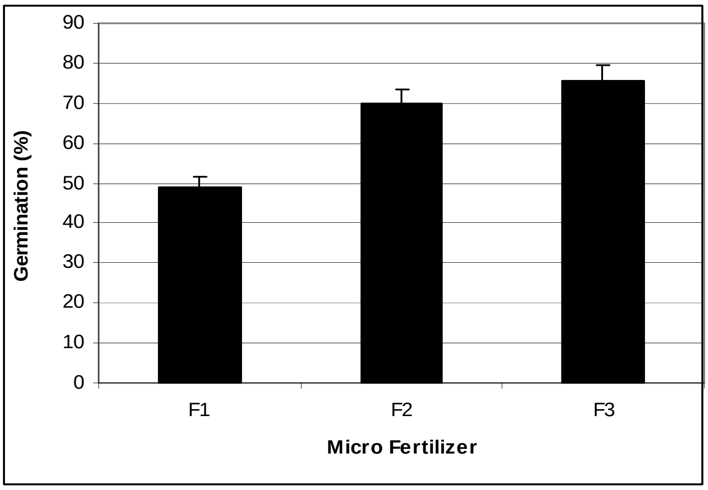

Figure 1: Effect of micro fertilizer on seed germination (%).

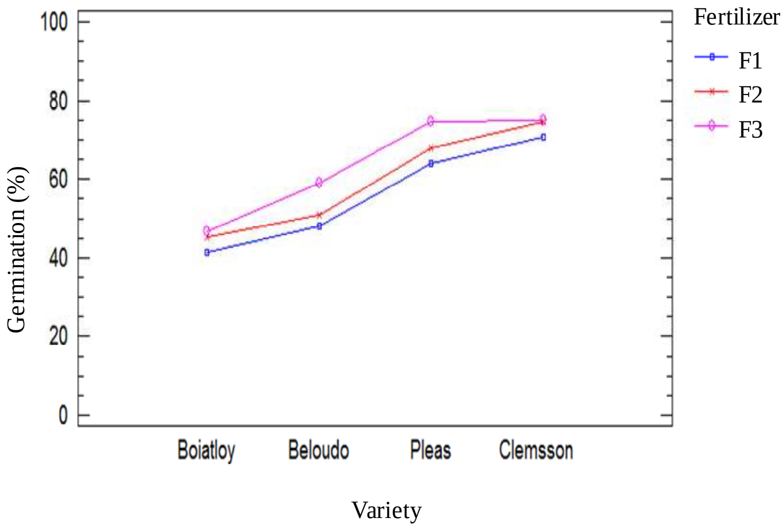

Figure 2: Effect of micro fertilizer on percentage of seed germination of different varieties.

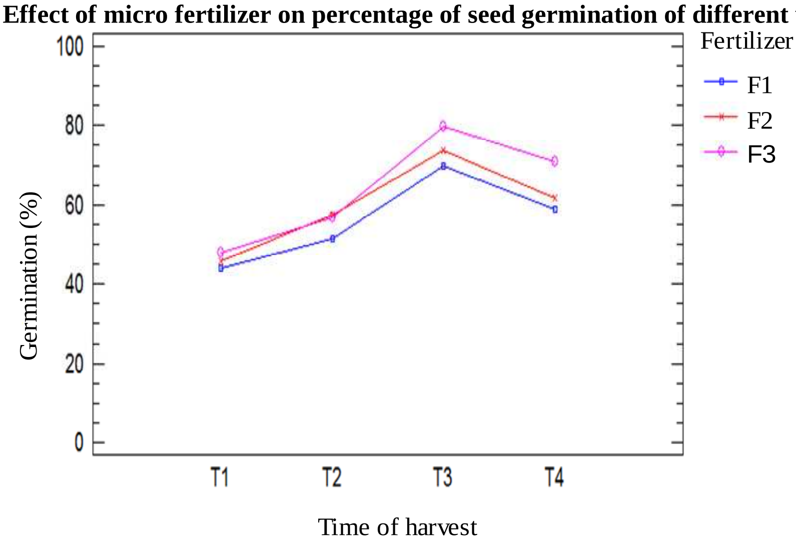

Figure 3: Effect of micro fertilizer on percentage of seed germination of seeds in different harvesting times.

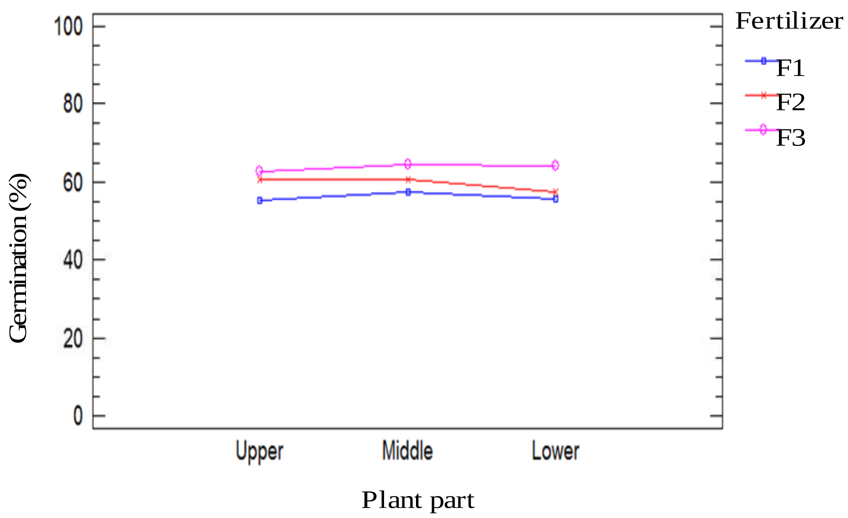

Figure4: Effect of micro fertilizer on percentage of seed germination of different pars of plant.

CONCLUSION

Using level three of micro fertilizer (F_3) has a positive effect on seed germination. Increasing level of fertilizer from first level of fertilizer (F_1) to third level of fertilizer (F_3) was more effective in varieties with more seedhardness. In this case application of level three of fertilizer (F_3) in Beloudo variety was more effective than other varieties. Application of level three of fertilizer (F_3) was more effective on seeds that harvested in three harvesting time (T_3). Application level three of fertilizer (F_3) on seeds on middle of the plant produced more seed germination.

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