GROWTH RESPONSE OF *HELIANTHUS ANNUUS* (SUNFLOWER) INFLUENCED BY FOLIAR SPRAY OF *CYMBOPOGON CITRATUS* (LEMON GRASS) AQUEOUS LEAVES EXTRACT

MUHAMMAD ASAD¹, AFSHEEN KHAN^{2*} AND BUSHREEN JAHAN¹

¹Department of Botany Federal Urdu University Gulshan Iqbal Campus Karachi, 73500, Pakistan ²Dr. Moinuddin Ahmed Research Laboratory of Dendrochronology and Plant Ecology, Department of Botany Federal Urdu University Gulshan Iqbal Campus Karachi, 73500, Pakistan ^{*}Corresponding author's email: khanafsheen913@ymail.com

Abstract

The paper explains possible outcomes in physical parameters of sunflower growth when irrigated by foliar spray of different concentrations of lemongrass aqueous leaf extract. Germination period begins from 48^{th} hour and lasts up to 72^{nd} hour in which vigor seems to be highest in 1% samples. The observed average time period employed by seeds to germinate and the vigor of the treated seeds show significantly (p<0.1 and p<0.05 respectively) different pattern in the early period of germination than the later hours. Seedling growth is determined indicating highest growth in 0.5% and 1% samples in all evaluated parameters. After seedling establishment, plants gained maturity where potential of growth is weakly significant (p<0.1), relatively greater in 0.5% and 1% samples as indicated in their growth parameters *i.e.*, root/shoot length, root/shoot dry weight and shoot length/leaf area. Growth correlation slightly declines in 2% and 3% samples. Lemon grass treatment found effective for germination and growth of sunflower plants in lower concentration hence can be utilized for better yield of sunflower crops and capable to minimize the use of other hazardous chemical fertilizers.

Key words: Agriculture, Biological fertilizers, Cymbopogon citratus, Physical parameters, Sunflower.

Introduction

Today's agriculture goal is to meet food requirements for the ever-increasing population. For this consistently increasing population, it is undoubtedly a challenge to provide healthy crops that can mitigate global food crisis in future. Timsina (2018) stated that it is crucial to maintain crop quality with lesser resources worldwide. Many insecticides, pesticides and chemical fertilizers have been tremendously used in agriculture since ages to increase crop yield. But now it has been discovered that these chemical based products are responsible for deterioration in the nutritional quality of crops at a greater extent (Agarwal et al., 2018). Depletion in soil minerals can also be caused by using chemical fertilizers (Khan et al., 2018 and 2014; Noshad & Khan, 2019). Use of biological products for a better crop yield can become a good replacement and a savor for crop quality (Badgley et al., 2007; Seufert & Foley, 2012). Reason behind using organic products for crop growth enhancement is not only to raise nutritional quality but to avoid the distortion of soil structure and mineral nutrients in soil as well as to maintain environmental and human health (Timsina, 2018). Several workers have advocated for using an alternative way in farming to reduce the negative impacts of agricultural activities on environment like Ullah et al., (2018); Timsina (2018); Pimentel (1996); Tilman et al., (2002); Avery (1996); Kirchmann et al., (2008). Tilman et al., (2002) suggested that there is a need to develop new and healthy methods in agriculture that would not compromise with natural resources nor involve any biodegradable process. The objective of this study is to assess the effectiveness of lemongrass extract in the growth of sunflower. This would suggest agriculturists to replace chemical products by plants extracts/ biological products for crop growth, hence utilizing organic products in farming may bring some productive outcomes in a less expensive and healthy way. Keeping in consideration the above highlights, current study encompasses the impacts and responses of sunflower plants on their growth and yield. This study determines the effects of a commonly found herb, *Cymbopogon citratus* on growth of *Helianthus annuus* (sunflower) which is an important cash crop in Pakistan and covers country's maximum oil requirement (Shah *et al.*, 2013).

Sunflower is one of the crops that are in demand worldwide for their extensive oil contribution in the food industry. In Pakistan, its commercial and economical value has increased every year as it is harvested 407224 tons or more per year, claimed by Shah *et al.*, (2013) and Rashid *et al.*, (2002). They considered sunflower to be a promising crop for Pakistan as its production fits well in the environment in most of the areas of Pakistan.

Due to therapeutic quality of lemongrass as it is used to treat headache, stomach ache, rheumatic pains (Leite *et al.*, 1986; Borrelli & Izzo, 2000), it can be utilized in various agricultural purposes (Cheel *et al.*, 2005; Runnie *et al.*, 2004). However, there is a lack of information of the scientific use of this herb; hence there is still need to explore the effectiveness of leaf extract of the herb on growth of plants (Cheel *et al.*, 2005). This research provides an adequate knowledge to understand the role of lemongrass leaf extract on growth of sunflower plants from early germination period to later developmental stages.

Materials and Methods

Sunflower seeds were brought from local vegetable market in Karachi, Pakistan. Seeds were sterilized and sown in lab and later on transferred to the pots in the field located in Department of Botany, Federal Urdu University Karachi, Pakistan. Lemongrass was also planted in the same place and harvested when fully grown for the preparation of extracts concentration *i.e.*, 0.5%, 1%, 2% and 3% following Sousa *et al.*, (2010). Five replicates of each concentration were prepared. After gaining germination, Germination % and speed of germination were evaluated following Khan and Ungar, (1997) and Khandakar & Bradbeer, (1983); Woodstock (1976) respectively. Following formulae were utilized:

Germination percentage (GP) = $\frac{\text{Total number of germinated seeds}}{\text{Total number of seeds}} \times 100$

Speed of germination = $[N1/2+N2/2+N3/3+Nn/n] \times 100$

For the exploration of relationship among different growth attributes, regression analysis was performed in Excel 2013.

Results

Under different exposures of treatment (0.5%, 1%, 2% and 3% lemongrass extract), sunflower plants produced a variety of responses throughout their consecutive growth stages.

Seed germination and growth: Germination of sunflower seeds did not show activity within 12 hours while cotyledons emerged after 24^{th} hour in all the treatments including control. Germination occurred at the same rate in all treatments while control samples proceeded germination activity at a greater rate right after initiation. All the samples completed germination in 72 hours. Among the treatments, 2% seeds showed highest (53%) rate of germination (Table 1a) whereas 3% samples achieved lowest rate *i.e.*, only 44%. Analysis of variance (ANOVA) showed a weakly significant (p<0.1) relationship among all samples, germination sought to be

increased at every 12th hour interval as represented in Fig. 1a. Therefore, null hypothesis can be rejected. An abrupt increase in seed germination at 60th hour indicated late hours (60 and 72 hours) to be effective for the course of germination in all samples. By skipping earliest 12th hours, germination sequentially increased from 24 to 48 hours showing overlapping in their medians while 60 and 70 hours achieved higher germination and a little overlapped median (Fig. 1a).

Speed of germinating seeds showed a slightly different scenario from that of % germination description, (Table 1b). An average evaluation of seed's vigor per time indicated a higher rate of seeds germinated at 48th hours *i.e.*, 6 ± 2 consecutively up to 72^{nd} hour in Control samples. While in treatments, the average vigor varied by time. The highest speed found in 1% and 2% samples after control plants *i.e.*, 5 ± 2 per at 72^{nd} hour. It was evident that 48th hour was the influential time frame for the speed of germination in which ANOVA evidenced for variability in speed of germination at early period from 12 to 24th hour which abruptly increased at 48th hour and remain constant up till 72^{nd} hour (Fig. 1b). Hence, there was significant difference (p < 0.05) observed in the germination speed at 12 hour difference till the achievement of maximum germination (Fig. 1b).

 Table 1a. Summary of % germination of seeds in respective samples within 72 hours.

T ¹	Samples (% germination)						
Time (hours)	Control	0.5%	1%	2%	3%		
12	0	0	0	0	0		
24	48	31	36	33	23		
36	54	36	40	41	33		
48	60	42	47	46	41		
60	82	48	49	51	44		
72	100	48	51	53	44		

Table 1b. Summary of speed of germination of seeds in respective samples within 72 hours.

Time (hours)	Samples (number of seeds/12 hour)						
	Control	0.5%	1%	2%	3%		
12	0	0	0	0	0		
24	5 ± 2	4 ± 2	3 ± 1	3 ± 1	3 ± 1		
36	5 ± 2	4 ± 1	3 ± 1	3 ± 1	3 ± 1		
48	6 ± 2	4 ± 1	5 ± 2	4 ± 1	4 ± 1		
60	6 ± 2	4 ± 2	5 ± 2	4 ± 1	4 ± 1		
72	6 ± 2	4 ± 2	5 ± 2	5 ± 2	4 ± 1		

Table 2. Average seedling growth with standard errors (root, stem and leaf parameters).

								F		
Samples	RL(cm)	RFW(gm)	RDW(gm)	SL(cm)	SFW(gm)	SDW(gm)	$\mathbf{L}_{\mathbf{n}}$	LA (cm)	LFW(gm)	LDW(gm)
Control	12.64 ± 1	1.4 ± 0.1	0.2 ± 0.03	62.5 ± 0.7	6.2 ± 0.1	1.4 ± 0.02	16 ± 1	25.8 ± 3	2.2 ± 0.1	0.72 ± 0.1
0.5%	11.03 ± 1	0.8 ± 0.1	0.2 ± 0.01	54 ± 4.5	4.1 ± 0.1	1.1 ± 0.02	14 ± 1	28 ± 8	1.5 ± 0.2	0.43 ± 0.1
1%	10 ± 1.7	1.4 ± 0.1	0.3 ± 0.01	56 ± 3.7	7.6 ± 0.2	2 ± 0.1	17 ± 1	21.14 ± 3	3.1 ± 0.1	0.71 ± 0.03
2%	5.6 ± 1.2	0.7 ± 0.02	0.2 ± 0.01	43 ± 4	4.5 ± 0.1	1.3 ± 0.01	9 ± 2	10 ± 3	1.4 ± 0.1	0.5 ± 0.01
3%	7 ± 3	0.3 ± 0.02	0.11 ± 0.003	35 ± 5.2	2.2 ± 0.1	1 ± 0.04	6 ± 1	6.4 ± 1.2	0.72 ± 0.02	0.24 ± 0.03

Note: RL = Root length, RFW = Root fresh weight, RDW = Root dry weight, SL = Shoot length, SFW = Shoot fresh weight, SDW = Shoot dry weight, $L_n = number of leaves$, LA = Leaf area, LFW = Leaf fresh weight, LDW = Leaf dry weight

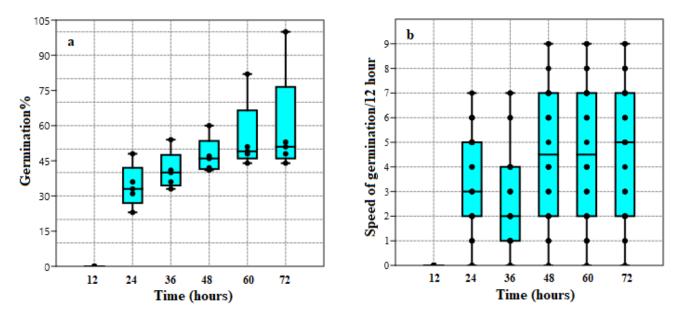


Fig. 1a and b. Germination% and speed of germination /12 hour respectively.

Plant growth: After seed germination, plant growth was monitored. The physical growth parameters were recorded and averaged with respect to their concentrations (Table 2). There was a remarkable gain in average root and shoot length (12.64±1cm and 62.5±0.7cm) attained by control seedlings followed by 0.5% samples and 1% samples. Among the treatments, 1% seedlings attained highest growth rate in most of the parameters like root dry weight, shoot fresh and dry, number of leaves and leaf fresh weight (0.3±0.01gm, 7.6±0.2gm, 2±0.1gm, 17±1, 3.1±0.1gm respectively). The second highest position in growth attainment was recorded from 0.5% samples in all parameters while highest value recorded only in leaf area *i.e.*, 28 ± 8 cm². 2% samples steadily attained growth in all the featured variables while 3% appeared to be the slowest growing sample likewise in case of its germination % and vigor.

To get deeper insight of growth in seedlings in all treatments, regression analysis was performed to elaborate the relationship between root and shoot length, root and shoot fresh weight and dry weight, shoot length and leaf area, shoot length and leaf fresh weight. The obtained statistics showed only few cases that were significant, presented in Fig. 2. Summary statistics of regression analysis is shown in Table 3.

R squared deviation claimed the existence of weak correlation (p<0.1) between root and shoot fresh weight of control samples (Fig. 2a). Significant correlation

(p<0.1 and p<0.05) also found in root/shoot length and shoot length/leaf area in 0.5% samples (Fig. 2b and c) respectively. Shoot length and leaf area correlation was weakly significant (p<0.1) in 1% samples (Fig. 2d) as well as in 3% samples (Fig. 2e) while in 2% samples the same parameter remained non-significant likewise other parameters. The rest of all parameters in all samples remained non-significant (Table 3). Root-shoot fresh weight highlighted its significance for the first time in control samples inspite of being in a weak relationship this showed a gradual effectiveness on biomass acquisition. A similar case occurred in root/shoot length of 0.5% samples while sharing a weak but significant correlation (p<0.1) between shoot length and leaf area (Fig. 2b, Table 3).

Discussion

Synthetic fertilizers have adverse effects on crops, this has encouraged biologists to investigate and formalize a well-managed application mechanism using natural products. Induction of bio-extracts from different plants can replace the use of chemical fertilizers hence can act as a principle bio-stimulant at initial growth stages or sometimes at different growth levels of a plant life.

Seed germination and establishment of seedlings were studied by many workers like Beckett & Staden, (1989); Hankins & Hockey, (1990); Norrie & Keathley, (2006). They determined that early germination of seeds and seedling establishment took place under the influence of other plant extracts. In the current study, control samples germinated at a greater rate than treated seeds. Among treated seeds, there was a greater germination rate and vigor seen at lower concentration whereas upon induction of higher concentrations, the growth and vigor declined. Azza et al., (2010) used tea compost to improve soil fertility. Nardi et al., (2009) claimed that plant growth and their macroflora can be greatly effected by bio-stimulants. Jang & Kuk, (2019) studied the effects of Chinese chive and soybean leaves and shoot extracts on growth of lettuce. They found significant increase in shoot fresh weight while plant height did not produce any visible differentiation in their treatments (1%, 3% and 5%). However, current study also provided a similar response from sunflower shoots in their fresh weight and height (length). Our treated roots failed to respond dynamically against the

provided extract. The response of leaves in their fresh weight was remarkable than other parameters. Noshad & Khan, (2019) concluded positive effects of food industrial residues on *Solanum melongena* growth as their shoot growth and fruit production enhanced at a greater rate. Khan *et al.*, (2018) provided several examples of positive allelopathic effects on crop production and growth yield such as tomato extracts on wheat and rice crops.

Our study revealed a gradually changing behavior in the response of roots shoots and leaves growth after 10, 20, 30 and 40 (times) exposures of lemongrass leaf extract. It was clearly visible that as the exposure number increased, fresh and dry weight of roots decreased consistently but in case of shoots and leaves, 3% treatments dramatically gave highest biomass as well as physical growth. In the light of above explained responses of sunflower plants, lower concentrations of lemongrass leaf extract can be used to enhance growth.

Sample	R ²	R value	co-efficient	intercept	Significance level
		Control	·		
R/S length	0.02	0.141	0.01	12.26	Ns
R/S FW	0.21^{*}	0.447	0.32	-0.61	P < 0.1
R/S DW	0.02	0.141	-0.06	0.33	Ns
Sl/LA	0.14	0.374	0.19	14.26	Ns
Sl/LFW	0.09	0.3	0.002	1.99	Ns
		0.5%			
R/S length	0.271^*	0.520	0.14	3.66	P < 0.1
R/S FW	0.082	0.286	0.23	-0.14	Ns
R/S DW	0.047	0.216	0.26	0.50	Ns
Sl/LA	0.831**	0.911	1.62	-60.40	P < 0.05
Sl/LFW	0.05	0.223	0.01	1.12	Ns
		1%			
R/S length	0.032	0.178	0.09	5.10	Ns
R/S FW	0.194	0.440	0.22	-0.28	Ns
R/S DW	0.014	0.118	0.01	0.33	Ns
Sl/LA	0.333^{*}	0.574	0.54	-8.12	P < 0.1
Sl/LFW	0.01	0.1	-0.001	3.16	Ns
		2%			
R/S length	0.101	0.317	0.10	1.27	Ns
R/S FW	0.000	0.00	0.002	0.72	Ns
R/S DW	0.014	0.118	0.09	0.10	Ns
Sl/LA	0.18	0.424	0.37	-5.80	Ns
Sl/LFW	0.11	0.331	-0.01	1.80	Ns
		3%			
R/S length	0.182	0.426	0.22	-0.87	Ns
R/S FW	0.222	0.471	0.06	-0.44	Ns
R/S DW	0.026	0.161	0.02	0.10	Ns
Sl/LA	0.371^{*}	0.608	0.33	-6.84	P < 0.1
Sl/LFW	0.01	0.1	-0.00005	0.72	Ns

 Table 3. Summary statistics of regression analysis in treatments.

Note: RI = Root length, SI = Shoot length, LA = Leaf area, FW = Fresh weight, DW = Dry weight, *= Significant (weak), ** = Significant

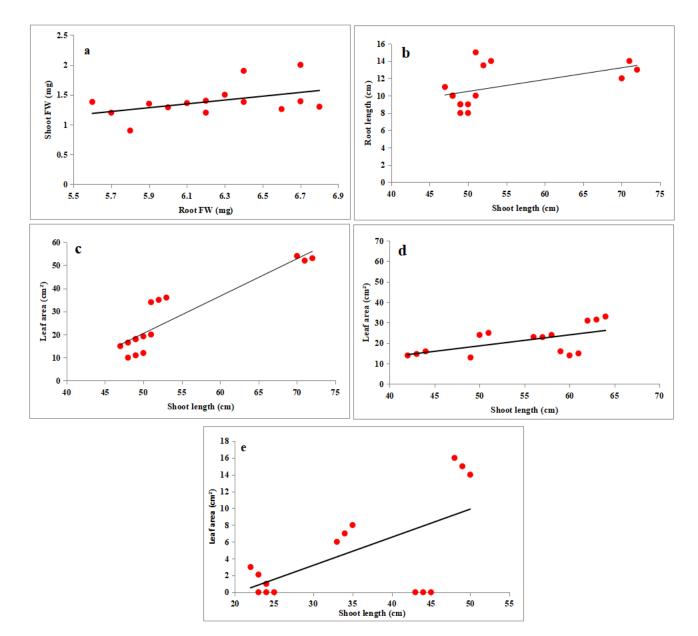


Fig. 2a. Regression between root and shoot fresh weight (FW) of control samples; Fig. 2b and c. Regression analysis between shoot/root length and shoot length/leaf area of 0.5% samples respectively; Fig. 2d. Regression between shoot length and leaf area of 1% samples; Fig. 2d. Regression between shoot length and leaf area of 3% samples.

Conclusion

Effectiveness of foliar spray of lemon grass aqueous leaf extract seems to be productive for the growth of sunflower in lower concentrations. It is recommended that use of organic products is more beneficial for agricultural use as observed in our experiment.

Acknowledgments

We are warm-heartedly thankful for the kind assistance provided by Dr. Rafat Saeed and Dr. Sumera Moin in the lab and field experiment.

References

Agarwal, P.R.I. Gupta and K. Gill. 2018. Importance of biofertilizers in agriculture biotechnology. Ann. Biol. Res., 9(3): 1-3.

- Avery, D. 1996. From saving the planet with pesticides. In: *The True State of the Planet* (Ed.): Baley, R.. The Fee Press: New York, NY USA.
- Azza, A., E. El-Din and S.F. Hendaway. 2010. Effect of dry yeast and compost tea on growth and oil content of *Borago* officinalis plant. *Res. J. Agri. Biol. Sci.*, 6: 424-430.
- Badgley, C.J., E. Moghtader, E. Quintero and J. Zakern. 2007. Organic agriculture and the global food supply. *Renew. Agri. Food Sys.*, 22: 86-108.
- Beckett, R.P. and J.V. Staden. 1989. The effect of seaweed concentrate on the growth and yield of potassium stressed wheat. *Plant Soil.*, 116: 29-36.
- Borrelli, F. and A.A. Izzo. 2000. The plant kingdom as a source of anti-ulcer remedies. *Phytotheraph. Res.*, 14(8): 581-591.
- Cheel, I., C. Theoduloz, J. Rodríguez and G.S. Hirschmann. 2005. Free radical scavengers and antioxidants from lemongrass (*Cymbopogon citratus* (DC.) Stapf). J. Agri. Food Chem., 53(7): 2511-2517.
- Hankins, S.D. and H.P. Hockey. 1990. The effect of a liquid seaweed extract from Ascophyllum nodosum (Fucales,

Phaeophyta) on the two- spotted red spider mite *Tetranychus urticae*. *Hydrobio*., 204: 555-559.

- Jang, S.J. and Y.I. Kuk. 2019. Growth promotion effects of plant extracts on various leafy vegetable crops. *Hort. Sci. Tec.*, 322-336.
- Khan, A., M. Ahmed and S.S. Shaukat. 2018. Allelopathy: An overview. *FUUAST. J. Biol.*, 8(2): 331-350.
- Khan, A., M. Ahmed, S.S. Shaukat, T.A. Rao. 2014. A study of the growth of Soybean: Production of nodules and the extent of soil improvements. *FUUAST. J. Biol.*, 4(1): 89-92.
- Khan, M.A. and I.A. Ungar. 1997. Effect of thermoperiod on recovery of seed germination of halophytes from saline conditions. *Amer. J. Bot.*, 84: 279-283.
- Khandakar, A.L. and J.W. Bradbeer. 1983. *Jute seed quality*. Dhaka, Bangladesh Agricultural Research Council Dhaka, Bangladesh.
- Kirchmann, H., L. Bergström, T. Kätterer, O. Andrén and R. Andersson. 2008. Can organic crop production feed the world? In Organic Crop Production-Ambitions and Limitations. (Eds.): Kirchmann, H., L. Bergström. Springer: Doordrecht, The Netherlands, 39-74
- Leite, J.R., M.D.L.V. Seabra and E. Maluf. 1986. Pharmacology of lemongrass (*Cymbopogon citratus Stapf*). III. Assessment of eventual toxic, hypnotic and anxiolytic effects on humans. J. Ethnopharmacol., 17(1): 75-83.
- Nardi, A.E., F.L. Lopes, R.C. Freire, A.B. Veras and I. Nascimento. 2009. Panic disorder and social anxiety disorder types in a caffeine challenge test. *Psychiat. Sub. Res.*, 169(2): 149-53.
- Norrie, J and J.P. Keathley. 2006. Benefits of Ascophyllum nodosum marine-plant extract applications to 'Thompson seedless' grape prod uction. (of the X Proceedings International Symposium on Plant Bioregulators in Fruit Production, 2005). Acta Hort., 727: 243-247.

- Noshad, S. and A. Khan. 2019. Recovery in growth of Solanum melongena. L from adverse effects of waste water effluents. *FUUAST. J. Biol.*, 9(1): 33-39.
- Pimentel, D. 1996. Green revolution agriculture and chemical hazards. *Sci. Total. Environ.*, 188: 86-98.
- Rashid, I., A. Shahbaz and M.A. Malik. 2002. Sunflower summer legumes intercropping system under rainfed conditions: yield and yield components. *Pak. J. Agri. Res.*, 17(3): 231-236 retrieved from http://www.pjar.org.pk
- Runnie, I., M.N. Salleh, S. Mohamed, R.J. Head and M.Y. Abeywardena. 2004. Vasorelaxation induced by common edible tropical plant extracts in isolated rat aorta and mesenteric vascular bed. J. Ethnopharmacol., 92(2-3): 311-316.
- Seufert, V.N. and A.E. Foley. 2012. Comparing the yields of organic and conventional agriculture. *Nature Ramankutty.*, 485: 229-232.
- Shah, N.A., K.M. Aujla, M. Ishaq and A. Farooq. 2013. Trends in sunflower production and its potential in increasing domestic edible oil production in Punjab, Pakistan. Sarhad. J. Agric., 29(1): 7-14.
- Sousa, S.M., P.S. Silva and L.F. Viccini. 2010. Cytogenotoxicity of Cymbopogon citratus (DC) Stapf (lemongrass) aqueous extracts in vegetal test system. Ann. Brazil. Acad. Sci., 82(2): 305-311.
- Tilman, D., K.G. Cassman, P.A. Matson, R. Naylor and S. Polasky. 2002. Agricultural sustainability and intensive production practices. *Nature*, 418: 671-677.
- Timsina, J. 2018. Can organic materials supply enough nutrients to achieve food security? J. Agri. Forest. Uni., 2: 9-21.
- Ullah, G., E.A. Khan and A.A. Khakwani. 2018. Physiological response of spring wheat to seeding and potassium application rates. *Pak. J. Bot.*, 50(3): 937-947.
- Woodstock, L.W. 1976. Progress report on the seed vigor testing handbook. Newsletter of the Association of official seed analysis., 50(2): 1-78.

(Received for publication 26 April 2020)