



## Brief Article

### Determination Sodium Hypochlorite (NaClO) Application on Quantitative Parameters of Wheat (*Triticum aestivum* L.) Seed and Correlation Between Measured traits

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#### RESEARCH ARTICLE

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#### ABSTRACT

To assessment effect of concentration and times of disinfection wheat seeds, infected by *Aspergillus* fungus a research was conducted based on Factorial experiment according randomized complete block design with four replications. The factors included four levels of sodium hypochlorite (2, 4, 6, and 8%) and four times of seed disinfection (2, 5, 7, and 10 minutes). Result of analysis of variance indicated interaction effect of treatments on germination percentage ( $p \leq 0.05$ ), Radicle length ( $p \leq 0.05$ ), plumule length ( $p \leq 0.05$ ), germination rate ( $p \leq 0.05$ ), seed dry matter ( $p \leq 0.01$ ), speed of germination ( $p \leq 0.05$ ) and seedling dry matter ( $p \leq 0.01$ ) was significant, but on mean of germination time, radicle dry weight, plumule dry weight and abnormal bud was not significant. Germination rate as important trait had significant relation with seedling dry matter (0.992\*\*), radicle length (0.990\*\*), germination percentage (0.983\*\*), plumule length (0.982\*\*), speed of germination (0.981\*\*), seed dry matter (0.924\*\*) and abnormal bud (-0.767\*). Treatment of 6% concentrations of sodium hypochlorite along 7 minute had highest amount of Seed dry matter (0.0899 gr), speed of germination (38.991 day), seedling dry matter (0.1899 gr), germination percentage (98.87 %), radicle length (11.40 cm), plumule length (10.91 cm), germination rate (39.98 day), so it possible to advised for improve wheat seed germination parameters.

**Keywords:** *Aspergillus flavus*, *Chamran cultivar*.

#### INTRODUCTION

The overarching goal of crop establishment is to achieve rapid and uniform germination (Ghodrat and Roustae, 2012). Seed-borne diseases have been found to affect the growth and productivity of crop plants. A seed borne pathogen present externally or internally or associated with the seed as contaminant may cause seed abortion, seed rot, seed necrosis, reduction or elimination of germination capacity as well

as seedling damage resulting in development of disease at later stages of plant growth by systemic or local infection (Ishfaq *et al.*, 2016). Sodium hypochlorite is effective as a disinfecting and sterilizing agent against a broad range of bacteria, viruses, and fungi. Disinfecting seeds with strong solutions of NaOCl such as 5.25% for 1 to several min does not reduce germination inability.

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However, this compound has a strong oxidizing property which makes it highly reactive with amino acids, nucleic acids, amines, and amides. The general reaction between amino acids and NaOCl produces respective aldehyde,  $\text{NH}_4\text{Cl}$  and  $\text{CO}_2$  (Mendes De-Jesus *et al.*, 2016). This research was conducted to determine the most appropriate concentration and duration of application sodium hypochlorite on germination factors of seeds infected by *Aspergillus* fungus.

## MATERIALS AND METHODS

### Treatment Information

In order to evaluate effect of different concentrations of sodium hypochlorite along several times on quantitative parameters of wheat seeds (Chamran cultivar) infected by *Aspergillus flavus* fungus a factorial experiment was carried out according randomized complete block design with four replications. The treatments included four concentrations of sodium hypochlorite ( $C_1= 2\%$ ,  $C_2= 4\%$ ,  $C_3= 6\%$ , and  $C_4= 8\%$ ) and four application times ( $T_1= 2$ ,  $T_2= 5$ ,  $T_3= 7$ , and  $T_4=10$  minutes).

### Trait Measurement

The following formulae were used in order to measure the desired parameters (Pernezny *et al.*, 2002):

**Equ 1.** Germination % = (Number of germinated seed/ total number of tested seeds in each treatment)  $\times 100$ .

**Equ 2.** Germination rate = Number of germinated seeds in the second day/2 + number of germinated seeds in the eighth day/8).

**Equ 3.** Mean germination time=  

$$\frac{\sum (nt)}{\sum n} - 3.$$

**n:** number of germinated seeds per each day and **t** is the days after planting. When the germination stops, in order to determine the lengths of radicle and plumule 10 seedlings were selected randomly from each treatment and the lengths of radicle and plumule were measured with a ruler. To test the seedling growth and the dry weights of the radicle, plumule, and seedling, the radicle, plumule, and seeds were

separated from the 10 selected seedlings and were separately placed in the oven at  $72^\circ\text{C}$  for 48 hours, then their dry weight was measured using a sensitive scale.

### Statistical analysis

Analysis of variance was done by SAS software (Ver.8) and mean comparison was done by Duncan test ( $p \leq 0.05$ ). Determine correlation between traits was performed by Minitab software (Ver.13).

## RESULTS AND DISCUSSION

### Seed dry matter

Analysis of variance result indicated effect of different concentration and several time of application of sodium hypochlorite and interaction effect of treatments on seed dry matter was significant at 5% and 1% probability level, respectively. According result of mean comparison maximum seed dry matter (0.0899 gr) was observed for 6% concentrations of sodium hypochlorite along 7 minute and minimum of that (0.0761 gr) was for 2% concentrations of sodium hypochlorite along 2 minute (Table 1). Miche and Balandreau (2001) and Ajdukovia and Vasia (2005) have pointed similar results.

### Speed of germination

Result of analysis of variance revealed effect of different concentration and several time of application of sodium hypochlorite and interaction effect of treatments on speed of germination was significant at 5% probability level. Mean comparison result showed maximum and minimum amount of speed of germination belonged to 6% concentrations of sodium hypochlorite along 7 minute (38.991 day) and 2% concentrations of sodium hypochlorite along 2 minute (34.153 day) (Table 1). Musgrave and Ding (1998) found same results.

### Seedling dry matter

According result of ANOVA different concentration, time and interaction effect of treatments on seedling dry matter was significant at 1% probability level (Table 1).

**Table 1.** Mean values of the traits as affected by concentration and time of application sodium hypochlorite

Treatments		Radicle dry weight (gr.)	Plumule dry weight (gr.)	Seed dry matter (gr.)	Speed of germination (day)	Seedling dry matter (gr.)	Abnormal bud (%)
2 Minute	2%	0.035 <sup>a</sup>	0.06 <sup>a</sup>	0.0761 <sup>d</sup>	34.153 <sup>d</sup>	0.1519 <sup>d</sup>	1.99 <sup>a</sup>
	4%	0.037 <sup>a</sup>	0.062 <sup>a</sup>	0.0765 <sup>d</sup>	34.250 <sup>cd</sup>	0.1535 <sup>d</sup>	1.98 <sup>a</sup>
	6%	0.038 <sup>a</sup>	0.066 <sup>a</sup>	0.0767 <sup>cd</sup>	34.280 <sup>cd</sup>	0.1565 <sup>cd</sup>	1.97 <sup>a</sup>
	8%	0.038 <sup>a</sup>	0.077 <sup>a</sup>	0.0770 <sup>cd</sup>	34.330 <sup>c</sup>	0.1579 <sup>cd</sup>	1.96 <sup>a</sup>
5 Minute	2%	0.039 <sup>a</sup>	0.067 <sup>a</sup>	0.0775 <sup>c</sup>	35.440 <sup>c</sup>	0.1615 <sup>c</sup>	1.90 <sup>a</sup>
	4%	0.04 <sup>a</sup>	0.068 <sup>a</sup>	0.0778 <sup>bc</sup>	35.789 <sup>bc</sup>	0.1625 <sup>c</sup>	1.88 <sup>a</sup>
	6%	0.04 <sup>a</sup>	0.069 <sup>a</sup>	0.0783 <sup>bc</sup>	35.834 <sup>bc</sup>	0.1639 <sup>bc</sup>	1.87 <sup>a</sup>
	8%	0.038 <sup>a</sup>	0.069 <sup>a</sup>	0.0781 <sup>c</sup>	35.980 <sup>bc</sup>	0.1649 <sup>bc</sup>	1.86 <sup>a</sup>
7 Minute	2%	0.039 <sup>b</sup>	0.065 <sup>a</sup>	0.0822 <sup>ab</sup>	37.088 <sup>b</sup>	0.1801 <sup>ab</sup>	1.81 <sup>a</sup>
	4%	0.04 <sup>a</sup>	0.066 <sup>a</sup>	0.0842 <sup>ab</sup>	37.550 <sup>b</sup>	0.1835 <sup>ab</sup>	1.80 <sup>a</sup>
	6%	0.039 <sup>a</sup>	0.066 <sup>a</sup>	0.0899 <sup>a</sup>	38.991 <sup>a</sup>	0.1899 <sup>a</sup>	1.79 <sup>a</sup>
	8%	0.042 <sup>a</sup>	0.07 <sup>a</sup>	0.0881 <sup>ab</sup>	38.790 <sup>ab</sup>	0.1880 <sup>ab</sup>	1.78 <sup>a</sup>
10 Minute	2%	0.049 <sup>a</sup>	0.07 <sup>a</sup>	0.0801 <sup>ab</sup>	36.210 <sup>b</sup>	0.1715 <sup>b</sup>	1.71 <sup>a</sup>
	4%	0.024 <sup>a</sup>	0.069 <sup>a</sup>	0.0805 <sup>b</sup>	36.303 <sup>b</sup>	0.1750 <sup>b</sup>	1.70 <sup>a</sup>
	6%	0.041 <sup>a</sup>	0.069 <sup>a</sup>	0.0809 <sup>b</sup>	37.450 <sup>ab</sup>	0.1770 <sup>b</sup>	1.69 <sup>a</sup>
	8%	0.039 <sup>a</sup>	0.068 <sup>a</sup>	0.0811 <sup>b</sup>	37.555 <sup>ab</sup>	0.1785 <sup>ab</sup>	1.66 <sup>a</sup>
Concentration(C)		ns	ns	*	*	**	ns
Time (T)		ns	ns	*	*	**	ns
C * T		ns	ns	**	*	**	ns
CV (%)		3.25	1.98	3.68	2.54	3.99	2.78

ns, \*, \*\*: non significant, significant at 0.05 and 0.01 probability levels, respectively. Same letters in columns are not significantly different at  $p \leq 0.05$ .

Maximum seedling dry matter (0.1899 gr) was obtained for 6% concentrations of sodium hypochlorite along 7 minute and minimum of that (0.1519 gr) was for 2% concentrations of sodium hypochlorite along 2 minute (Table 1). Carisse *et al.*, 2000 confirmed that result.

### Germination percentage

Result of analysis of variance revealed effect of different concentration of sodium hypochlorite on germination percentage was not significant but of effect of several time of application of sodium hypochlorite and interaction effect of treatments was significant at 5% probability level (Table 1). According result of mean comparison the maximum germination percentage (98.87%) was observed for the 6% concentrations of sodium hypochlorite along 7 minute and minimum of that (91.54%) was for 2% concentrations of sodium hypochlorite along 2 minute (Table 1). Miche and Balandreau (2001) have pointed similar results in this regard.

### Radicle length

Result of analysis of variance indicated effect of different concentration and several time of application of sodium hypochlorite and interaction effect of treatments on radicle length was significant at 5% probability level (Table 1). Maximum seedling dry matter (11.40 cm) was obtained for 6% concentrations of sodium hypochlorite along 7 minute and minimum of that (7.10 cm) was for 2% concentrations of sodium hypochlorite along 2 Min (Table 1). Other researchers confirmed that result (Hasan *et al.*, 2013).

### Plumule length

According result of analysis of variance effect of effect of different concentration and several time of application of sodium hypochlorite and interaction effect of treatments on plumule length was significant at 5% probability level (Table 1). Mean comparison result revealed maximum plumule length (10.91 cm) was observed for 6% concentrations of sodium

**Continued Table 1.** Mean values of the traits as affected by concentration and time of application sodium hypochlorite

Treatment		Germination percentage (%)	Radicle length (cm)	Plumule length (cm)	Germination rate (day)	Mean of germination time (day)
2 Minute	2%	91.54 <sup>e</sup>	7.10 <sup>d</sup>	8.09 <sup>d</sup>	36.24 <sup>d</sup>	5.1 <sup>a</sup>
	4%	91.87 <sup>de</sup>	7.20 <sup>d</sup>	8.21 <sup>cd</sup>	36.55 <sup>d</sup>	5.1 <sup>a</sup>
	6%	91.96 <sup>d</sup>	7.51 <sup>d</sup>	8.39 <sup>cd</sup>	36.73 <sup>cd</sup>	5.3 <sup>a</sup>
	8%	92.19 <sup>d</sup>	7.72 <sup>cd</sup>	8.58 <sup>cd</sup>	36.97 <sup>cd</sup>	5.2 <sup>a</sup>
5 Minute	2%	93.12 <sup>d</sup>	8.21 <sup>c</sup>	8.61 <sup>c</sup>	37.22 <sup>c</sup>	5.4 <sup>a</sup>
	4%	93.57 <sup>cd</sup>	8.41 <sup>bc</sup>	8.87 <sup>c</sup>	37.49 <sup>c</sup>	5.2 <sup>a</sup>
	6%	93.87 <sup>cd</sup>	8.55 <sup>bc</sup>	8.91 <sup>bc</sup>	37.69 <sup>bc</sup>	5.2 <sup>a</sup>
	8%	93.99 <sup>c</sup>	8.96 <sup>bc</sup>	8.99 <sup>bc</sup>	37.96 <sup>bc</sup>	5.2 <sup>a</sup>
7 Minute	2%	97.07 <sup>ab</sup>	10.91 <sup>b</sup>	10.31 <sup>ab</sup>	39.11 <sup>ab</sup>	4.9 <sup>a</sup>
	4%	97.24 <sup>ab</sup>	10.98 <sup>b</sup>	10.55 <sup>ab</sup>	39.25 <sup>ab</sup>	5.2 <sup>a</sup>
	6%	98.87 <sup>a</sup>	11.40 <sup>a</sup>	10.91 <sup>a</sup>	39.98 <sup>a</sup>	5.1 <sup>a</sup>
	8%	98.11 <sup>ab</sup>	11.31 <sup>ab</sup>	10.70 <sup>ab</sup>	39.66 <sup>ab</sup>	4.65 <sup>a</sup>
10 Minute	2%	94.11 <sup>b</sup>	9.35 <sup>b</sup>	9.11 <sup>b</sup>	38.11 <sup>b</sup>	5.1 <sup>a</sup>
	4%	94.36 <sup>b</sup>	9.55 <sup>b</sup>	9.25 <sup>b</sup>	38.29 <sup>b</sup>	5.0 <sup>a</sup>
	6%	95.49 <sup>b</sup>	9.78 <sup>b</sup>	9.67 <sup>b</sup>	38.72 <sup>b</sup>	5.2 <sup>a</sup>
	8%	95.66 <sup>b</sup>	9.89 <sup>b</sup>	9.88 <sup>ab</sup>	38.96 <sup>b</sup>	5.44 <sup>a</sup>
Concentration (C)		ns	*	*	*	ns
Time (T)		*	*	*	*	ns
C* T		*	*	*	*	ns
CV (%)		7.6	4.55	8.1	5.65	6.28

ns, \*, \*\*: non significant, significant at 0.05 and 0.01 probability levels, respectively. Same letters in columns are not significantly different at  $p \leq 0.05$ .

hypochlorite along 7 minute and minimum of that (8.09 cm) was for 2% concentrations of sodium hypochlorite along 2 minute (Table 1). Soltani *et al.* (2001) have pointed similar results in this regard.

### Germination rate

Assessment result of analysis of variance indicated that effect of different concentration and several time of application of sodium hypochlorite and interaction effect of treatments on germination rate was significant at 5% probability level (Table 1). Maximum germination rate (39.98 day) was obtained for 6% concentrations of sodium hypochlorite along 7 minute and minimum of that (36.24 day) was for 2% concentrations of sodium hypochlorite along 2 minute (Table 1).

### Correlation between measured traits

Germination rate as important trait had significant relation with seedling dry matter (0.99<sup>\*\*</sup>), radicle length (0.99<sup>\*\*</sup>), germination percentage (0.98<sup>\*\*</sup>), plumule length

(0.98<sup>\*\*</sup>), speed of germination (0.98<sup>\*\*</sup>), seed dry matter (0.92<sup>\*\*</sup>) and abnormal bud (-0.76<sup>\*</sup>). Hasan *et al.*, 2013; Razavi, 2007 confirmed mentioned result.

### CONCLUSION

Treatment of 6% concentrations of sodium hypochlorite along 7 minute had highest amount of seed dry matter (0.089 gr), speed of germination (38.99 day), seedling dry matter (0.189 gr), germination percentage (98.87 %), Radicle length (11.40 cm), plumule length (10.91 cm), germination rate (39.98 day), so it possible to advised for improve seed germination parameters.

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**Table 2.** The correlation coefficient between measured traits

Traits	GP	RL	PL	GR	MGT	RDW	PDW	SDM-1	SG	SDM-2
RL	0.985**									
PL	0.993**	0.985**								
GR	0.983**	0.990**	0.982**							
MGT	-0.426 <sup>ns</sup>	-0.436 <sup>ns</sup>	-0.408 <sup>ns</sup>	-0.369 <sup>ns</sup>						
RDW	0.206 <sup>ns</sup>	0.184 <sup>ns</sup>	0.197 <sup>ns</sup>	0.192 <sup>ns</sup>	0.037 <sup>ns</sup>					
PDW	0.075 <sup>ns</sup>	0.136 <sup>ns</sup>	0.109 <sup>ns</sup>	0.180 <sup>ns</sup>	0.019 <sup>ns</sup>	0.164 <sup>ns</sup>				
SDM-1	0.954**	0.923**	0.947**	0.924**	-0.499 <sup>ns</sup>	0.174 <sup>ns</sup>	0.069 <sup>ns</sup>			
SG	0.972**	0.961**	0.959**	0.981**	-0.356 <sup>ns</sup>	0.224 <sup>ns</sup>	0.127 <sup>ns</sup>	0.930**		
SDM-2	0.976**	0.989**	0.979**	0.992**	-0.400 <sup>ns</sup>	0.157 <sup>ns</sup>	0.161 <sup>ns</sup>	0.936**	0.975**	
AB	-0.654*	-0.731*	-0.658*	-0.767*	0.116 <sup>ns</sup>	-0.103 <sup>ns</sup>	-0.271 <sup>ns</sup>	-0.565*	-0.761*	-0.780**

<sup>ns</sup>, \* and \*\*: no significant, significant at 5% and 1% probability level, respectively. **GP**: Germination percentage, **RL**: Radicle length, **PL**: Plumule length, **GR**: Germination rate, **MGT**: Mean of germination time, **RDW**: Radicle dry weight, **PDW**: Plumule dry weight, **SDM-1**: Seed dry matter, **SG**: Speed of germination, **SDM-2**: Seedling dry matter, **AB**: Abnormal bud.

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