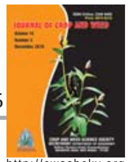
Journal of Crop and Weed, 16(2): 46-51 (2020)

ISSN- O : 2349 9400 ; P : 0974 6315



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Assessing the role of ameliorants based on physiological traits in sesame under waterlogged condition

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Received : 04.07.2020 ; Revised : 16.07.2020 ; Accepted : 20.07.2020

DOI : https://doi.org/10.22271/09746315.2020.v16.i2.1313

ABSTRACT

A pot culture experiment was conducted to understand physiological changes of sesame plants under waterlogging and to study the role of some foliar and seed treatments on stress mitigation. Waterlogging was imposed at 20 days after sowing fora period of 72 hr. Urea, Potassium nitrate, 1- naphthaleneacetic acid, calcium nitrate, tricyclazole and salicylic acid were given as foliar treatments whereas Pseudomonas fluorescens was applied both as foliar spray and seed priming agent. Treatments were applied 2 days before waterlogging. Waterlogging affected the plant survival due to hampered root and shoot growth. The scavenging of free radicals was affected with higher lipid peroxidation and lower enzymatic activity. Ameliorants, especially KNO_3*, salicylic acid and Psuedomonas fluorescens, were found to alleviate the inhibitory effect of stress in terms of highersurvival per cent, root and shoot growth and photosynthetic parameters. Plants applied with these treatments were found to be producing lower melondialdehide and higher catalase enzymeactivity and chlorophyll content thanuntreatedwaterlogged plants. Field evaluationrevealed 23 per cent reduction in yield per plant with 72 hr of waterlogging. Application of Pseudomonas fluorescens,* KNO_3 *and salicylic acid recorded 16.52, 13.73 and 7.93 per cent improvement in yield per plant compared to untreated waterlogged plants respectively.*

Keywords**:** Ameliorants, potassium nitrate, *Pseudomonas fluorescens*, salicylic acid and waterlogging

Changing climatic conditions imposes multiple stresses to plants. Among them waterlogging affects normal plant growth and survival. Excessive rain fall creates a hypoxic condition in the root zone of plants which further advances to anoxia as time progresses. This causes photoxidative damage to leaves by increased generation of reactive oxygen species (ROS) (Yordanova *et al*., 2004) which leads to leaf chlorosis and senescence. In susceptible plants which are unable to withstand low oxygen conditions in root zone, drastic yield loss will occur. Sesame is an ancient oilseed crop known for its high quality unsaturated fatty acids. It is a drought tolerant plant but susceptible to excessive moisture in soil. Excess moisture stress depending on duration and crop stage can affect growth and yield. It has been reported that yield loss in sesame due to waterlogging may vary between 15% and 80% based on the soil type and duration of stress (Athul, 2016).

Under water logged conditions, ROS scavenging is carried out by a number of well-characterized enzymes, primarily superoxide dismutase, ascorbate peroxidase and catalase (Irfan *et al*., 2010). It has been identified that high levels of these scavenging enzymes are critical for the survival of many crop plants such as sesame (Wei *et al*., 2013), rice (Ushimaro *et al*., 1992) and mungbean (Ahmed *et al*., 2002). The malondialdehyde (MDA) produced as a byproduct of lipid peroxidation by ROS has been suggested as a general indicator for water logging tolerance (Wu *et al*., 2003). The MDA content in the susceptible sesame variety ezhi-2 was reported to increase with waterlogging whereas it was unaffected in the tolerant variety ZZM2541 (Wei *et al*., 2013).

Since unpredictable excessive rainfall cannot be prevented, adopting mitigation strategies for imparting tolerance with ameliorants can be a better strategy for reducing the impact of water logging stress on crops. According to Steffens *et al*. (2005), nutrient deficiency is the major reason for poor plant growth in waterlogged soil. Canola plants under waterlogging reported a decrease in nitrogen (N), phosphorus (P), potassium (K) and calcium (Ca) uptake (Boem *et al*., 1996). Habibzadeh *et al*. (2013) reported that foliar spray of calcium nitrate, potassium nitrate, urea and tricyclazole before waterlogging was found to improve growth and reduce growth inhibition due to waterlogging in canola. He also observed an enhancement in root characters (vigour, length, volume and dry weight), activities of antioxidant enzymes and decreased MDA production in leaves. Pang *et al*. (2006) suggested that resistance to waterlogging in plants is imparted by avoiding loss of K^+ during hypoxia or anoxia. Salicylic acid is a phenolic compound involved in growth, developmental regulation of plant responses to biotic and abiotic stress factors, moreover it is a signaling molecule during stress (Khan *et al*., 2013). Foliar application of salicylic acid is

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reported to enhance chlorophyll and protein contents, shoot length and number of fruits in waterlogged tomato plants (Singh *et al*., 2017). In waterlogged barley plants, foliar application of 1- Naphthalene acetic acid (NAA) enhanced production of adventitious root formation (Pang *et al*., 2007).*Pseudomonas fluorescens* is well known for its ability to increase nutrient availability by solubilizing phosphatase and production of chelating substances like siderophores and also enhance activity of antioxidant enzymes enabling the plants to survive and maintain their growth under stress condition (Elekhtyar, 2015). The present study envisages to investigatethe ameliorative efficiency of different treatments on sesame plants subjected to waterlogged conditions.

MATERIALS AND METHODS

Pot culture study

Seeds of *Sesamum indicum* var. Thilak obtained from Onattukara Regional Agriculture Research Station, Kayamkulam, Kerala was grown in mud pots in 2019 at College of Horticulture, Vellanikkara. Waterlogging was imposed at 20 DAS (days after sowing) for 72 hrs. The ameliorative treatments were given two days before the imposition of waterlogging.T_1- Urea (1500 mg L^{-1}), T_2- Potassium nitrate (KNO_3) (5000 mg L^{-1}), T_3- 1-Naphthalene acetic acid (NAA) (5 µg in 200µL), T_4- Calcium nitrate ($Ca(NO_3)_2$)(4100 mg L^{-1}), T_5- *Pseudomonas fluorescens*, T_6- Tricyclazole (50 mg L^{-1}), T_7-Salicylic acid (100ppm), T_{8} – Control 1 (Waterlogged control or WC), T_9- Control 2 (no waterlogging and no ameliorants or NC). For the treatments, T_1, T_2, T_3, T_4, T_6, T_7- Foliar spray at 2 days before water logging. T_5 – Seed priming of *Pseudomonas fluorescens* talc formulation (10 g L^{-1}) at the time of sowing followed by foliar spray of culture broth (30 m L L^{-1}) at 2 days before waterlogging. Leaf samples were collected on the fourth day *i.e*., 3 days after imposing waterlogging and following observations were recorded.

MDA content was measured following the method of De Vos *et al*. (1991), while catalase enzyme activity was measured by the method of Maehly(1954).Total chlorophyll was estimated by the DMSO method suggested by Hiscox and Israelstam (1979). Total soluble protein content and nitrate reductase enzyme activity in the leaf was estimated by the method suggested by Lowry *et al*. (1951) and Hageman and Flesher (1960) respectively. Photosynthetic rate, transpiration rate and stomatal conductance were measured at the end of waterlogging period using infrared gas analyzer (Model LI-6400 of Licor Inc. Lincoln, Nebraska, USA). Survival per cent was measured at the end of waterlogging as the ratio of seedlings survived to the total number of seedlings.Morphological observations such as plant height, shoot dry weight, root dry weight and root number were taken.

Field evaluation

Three best performing ameliorants from the pot culture study were evaluated in farmer's upland field in Alappuzha district in 2019. The variety used was Thilak. The experiment was laid out in Randomized Block Design with 5 treatments and 4replication. The treatments selected were as follows.T_1: Potassium nitrate (KNO_3) (5000 mg L^{-1}), T_2: *Pseudomonas fluorescens* (10 g L^{-1}), T_3 : salicylic acid (100 ppm), T_4 : waterlogged control (WC), T_5 : No waterlogging and no ameliorants (NC).Treatment application was similar to the pot culture experiment. Waterlogging was imposed in the field for 72 hrs at 20DAS. Morphological observations and yield parameters such as number of branches per plant, number of capsule per plant, number of seeds per capsule, and seed yield per plant were calculated after harvest. The data was analyzed using WASP 2.0 developed by ICAR, GOA. Results of pot culture study were analyzed in completely randomized design and field data in randomized block design.

RESULTS AND DISCUSSION

Effect of waterlogging on sesame

Investigations reveal that waterlogging affected normal physiological and biochemical processes of sesame plants. Increased production of ROS in response to waterlogging is evidenced by the enhanced MDA production in waterlogged plants (control 1 in Table 1). Enhanced MDA content indicate lipid peroxidation and reduction of membrane integrity. Das and Choudhury (2014) have opined that a balance between ROS production and ROS scavenging activity decides the fate of cell under waterlogging. In control 1 high MDA content and low catalase activity implies an unbalanced system of ROS production and scavenging (Table 1). Under waterlogged condition nitrate availability is a major problem which is evident in the reduced nitrate reductase enzyme activity (Table 1) of sesame plants under waterlogged condition (control 1). Waterlogging resulted in decrease in transpiration rate of sesame plants (Table 2). This reduction is attributed by the hampered root permeability under low oxygen level leading to stomatal closure (Table 2). Reduced chlorophyll content (Table 1), stomatal closure (Table 2) and oxidative stress (Table 1) resulted in decline in photosynthetic rate under waterlogging (Table 2). Increased oxidative stress, reduced availability of nitrate and altered photosynthetic process affected the survival of sesame plants (Table 3). The lower survival of plants during waterlogging was

Table 1: Effect of treatments on biochemical parameters of sesame under waterlogging in pot study

Treatments	Catalase enzyme activity (µ moles of H_2O_2 utilized $g^{-1}min^{-1}$)	MDA content (ìmol. g^{-1})	Total chlorophyll content (m g g^{-1})	Nitrate reductase enzyme activity (µ moles of NO_2^- formed g^{-1} fresh weight hr^{-1}	Soluble protein content (m g g^{-1})
T_1: Urea	48.56^{d}	6.02^{c}	0.71^{cd}	283.33^{b}	14.37^{bc}
T_2: KNO_3	64.08^{b}	5.22^{d}	0.81^{bc}	206.66^{c}	18.12^{b}
T_3: NAA	55.83^{c}	6.70^{b}	0.71^{cd}	100.00^{f}	14.87^{bc}
T_4: $Ca(NO_3)_2$	59.08^{c}	6.22^{bc}	0.91^{b}	200.00^{cd}	18.37^{b}
T_5 : *P. fluorescens*	70.44^{a}	4.48^{e}	0.90^{b}	210.00^{c}	27.25^{a}
T_6: Tricyclazole	50.33^{d}	6.30^{bc}	0.55^{d}	195.00^{d}	14.50^{bc}
T_7: Salicylic acid	70.76^{a}	5.28^{d}	0.78^{bc}	203.33^{cd}	19.00^{b}
T_8: Control 1 (WC)	19.35^{e}	7.73^{a}	0.39^{e}	180.00^{e}	17.50^{b}
T_9: Control 2 (NC)	22.74^{e}	3.63^{f}	1.19^{a}	393.33^{a}	11.87^{c}
SEm (±)	**6.76**	**0.16**	**0.05**	**37.04**	**8.96**
LSD (0.05)	**4.46**	**0.69**	**0.16**	**10.44**	**5.14**

Note: T_1- (1500 mg L^{-1}, T_2- 5000 mg L^{-1}, T_3- 5 µg in 200µL, T_4- 4100 mg L^{-1}, T_5- Pseudomonas talc formulation (10 g L^{-1}) for seed priming and culture broth for foliar spray (30 m L L^{-1}) , T_6- 50 mg L^{-1}, T_7-100ppm. Means with the same superscript do not differ significantly at 5% level of probability using Duncan's Multiple Range Test (DMRT)

Table 2: Effect of treatments on photosynthetic parameters of sesame under waterlogging in pot study

Treatments	Photosynthetic rate (µ mole CO_2 $m^{-2}s^{-1}$)	Stomatal conductance (µmol $m^{-2}s^{-1}$)	Transpiration rate (µmol H_2O $m^{-2}s^{-1}$)
T_1: Urea	6.75^{d}	0.134^{c}	3.427^{f}
T_2: KNO_3	8.78^{b}	0.229^{b}	5.207^{c}
T_3: NAA	2.07^{f}	0.136^{c}	2.880^{g}
T_4: $Ca(NO_3)_2$	1.43^{h}	0.083^{d}	2.253^{h}
T_5 : *P. fluorescens*	7.15^{c}	0.159^{c}	4.697^{d}
T_6: Tricyclazole	1.79^{g}	0.143^{c}	3.463^{e}
T_7: Salicylic acid	6.55^{e}	0.209^{b}	5.530^{b}
T_8: Control 1 (WC)	1.12^{i}	0.047^{d}	1.333^{i}
T_9: Control 2 (NC)	9.74^{a}	7.487^{a}	14.800^{a}
SEm (±)	**0.002**	**0.001**	**0.001**
LSD (0.05)	**0.07**	**0.04**	**0.01**

also contributed by the poor root parameters (Table 3). Ability of plant roots to take up and transport mineral nutrients was significantly impaired under waterlogging stress (Malik *et al*. 2001), making nutrient delivery to the shoot inefficient. This resulted in poor shoot growth as evident from the lower shoot height and shoot dry weight (Table 3) of waterlogged (control 1) plants.

Mitigation of waterlogging stress in sesame

Mitigation treatments should inhibit the physiological and biochemical alterations resulted from water logging. Hence, same parameters were estimated for the plants treated with mitigation treatments or ameliorants. All the ameliorants were able to decrease the MDA production (Table 1) while enhancing catalase enzyme activity (Table 1) and chlorophyll content (Table 1). All the treatments except NAA enhanced the nitrate reductase activity where the application of urea as foliar spray resulted in highest improvement (Table 1). Only the application of *P. fluorescens* improved the soluble protein content (Table 1) compared to waterlogged control (control 1). All the treatments improved photosynthesis, especially *P. fluorescens*, KNO_3, salicylic acid and urea resulted in highest photosynthetic rate and

Table 3: Effect of treatments on morphological parameters of sesame under waterlogging in pot study

Treatments	Survival per cent (%)	Plant height (cm)	Shoot dry weight (g)	Root dry weight (g)	Root length (cm)	Root number per plant
T_1: Urea	51.66^{cd}	13.57^{bcd}	0.253^{d}	0.034^{d}	5.00^{bcd}	41.66^{b}
T_2: KNO_3	55.75^{bc}	17.67^{b}	0.354^{b}	0.042^{bc}	6.50^{b}	50.66^{a}
T_3: NAA	48.66^{de}	10.67^{cd}	0.157^{e}	0.040^{c}	3.30^{d}	31.33^{c}
T_4: $Ca(NO_3)_2$	49.75^{de}	14.33^{bc}	0.307^{c}	0.023^{f}	4.70^{bcd}	39.66^{b}
T_5 : *P. fluorescens*	59.66^{b}	15.33^{b}	0.345^{bc}	0.045^{a}	6.00^{bc}	50.30^{a}
T_6: Tricyclazole	52.50^{cd}	13.50^{bcd}	0.250^{d}	0.045^{a}	4.20^{cd}	55.00^{a}
T_7: Salicylic acid	57.66^{b}	16.00^{b}	0.363^{b}	0.044^{ab}	6.30^{b}	55.00^{a}
T_8: Control 1 (WC)	45.38^{e}	9.13^{d}	0.240^{d}	0.015^{g}	4.00^{d}	41.33^{b}
T_9: Control 2 (NC)	100.00^{a}	25.83^{a}	0.560^{a}	0.027^{e}	9.50^{a}	37.33^{bc}
SEm (±)	**7.82**	**6.82**	**0.001**	**0.001**	**1.28**	**13.37**
LSD (0.05)	**4.80**	**4.48**	**0.05**	**0.003**	**1.94**	**6.27**

Table 4: Effect of treatments on morphological parameters of sesame in field experiment

Treatments	Survival per cent (%)	Plant heightat harvest (cm)	Shoot dry weight at harvest (g)	Root dry weight at harvest (g)
T_1: KNO_3	90.80^{b}	122.50^{bc}	25.76^{b}	3.34^{c}
T_2: *P. fluorescens*	84.74^{c}	125.17^{b}	26.00^{b}	4.13^{b}
T_3: Salicylic acid	81.42^{d}	119.50^{c}	24.00^{c}	3.50^{c}
T_4: Control 1 (WC)	68.66^{e}	111.33^{d}	19.33^{d}	2.46^{d}
T_5: Control 2 (NC)	100.00^{a}	137.33^{a}	30.23^{a}	4.66^{a}
SEm (±)	**0.99**	**1.80**	**0.63**	**0.05**
LSD (0.05)	**3.28**	**5.3**	**1.49**	**0.41**

stomatal conductance (Table 2). The reduction of biochemical and photosynthetic alterations during waterlogging with five treatments such as *P. fluorescens*, salicylic acid, KNO_3, tricyclazole and urea resulted in improving the survival under waterlogging (Table 3). *P. fluorescens*, salicylic acid, KNO_3 and $Ca(NO_3)_2$ improved shoot dry weight and height (Table 3). Root dry weight was improved by all treatments (Table 3). Adventitious root production enhanced by application of salicylic acid, tricyclazole, *P. fluorescens* and KNO_3 resulted in increased root number (Table 3). Salicylic acid, *P. fluorescens* and KNO_3 also improved the root length (Table 3).

Application of NAA recorded the lowest shoot characters which was even lower than waterlogged control or WC (Table 3). Moreover, the leaves showed symptoms of scorching. However, it improved the root dry weight (Table 3). It also improved the antioxidant system in waterlogged plant, which was evident from lower MDA production (Table 1). Enhancement in photosynthesis (Table 2) resulted from increased chlorophyll content (Table 1) and increased root growth (Table 3). Survival rate was lower in NAA treatment (Table 3).

$Ca(NO_3)_2$application reduced lipid peroxidation and improved catalase activity (Table 1). The increased photosynthesis (Table 2) with Ca $(NO_3)_2$ application was a result of enhanced nitrate reductase activity, chlorophyll content (Table 1) and root dry weight (Table 3) which also promoted shoot growth (Table 3). Application of urea resulted in increased nitrate reductase activity (Table 1). This also contributed to increased chlorophyll content (Table 1) and photosynthetic parameters (Table 2) and higher plant survival(Table 3). Tricyclazole was efficient in improving the root growth parameters such as dry weight and number (Table 3). It also enhanced the antioxidant system (Table 1) and photosynthetic parameters (Table 2) and improved plant survival (Table 3). Alleviation of waterlogging stress by the application of nitrogen compounds ($Ca(NO_3)_2$, Urea, KNO_3) and tricyclazole in canola was reported by Habibzadeh *et al*. (2013). Salicylic acid and KNO_3 was effective in mitigating the stress induced alterations of photosynthesis (Table 2), antioxidant system (Table 1)

Table 5: Effect of treatments on yield and yield attributes of sesame in field experiment

Treatments	No. branches plant^{-1}	No. capsules plant^{-1}	No. seeds capsule^{-1}	Yield plant^{-1} (g)	Yield (t ha^{-1})
T_1: KNO_3	5.00^b	66.00^{bc}	54.00^{bc}	5.30^b	0.795^b
T_2: *Psuedomonas fluorescens*	5.33^b	67.66^b	56.66^{ab}	5.43^b	0.815^b
T_3: Salicylic acid	5.00^b	64.00^c	53.33^c	5.03^c	0.755^c
T_4: Control 1 (WC)	4.00^c	58.66^d	50.00^d	4.66^d	0.70^d
T_5: Control 2 (NC)	6.67^a	77.33^a	59.00^a	6.06^a	0.91^a
SEm (±)	**0.117**	**2.883**	**2.617**	**0.006**	**0.008**
LSD (0.05)	**0.643**	**3.197**	**3.046**	**0.150**	**0.022**

and shoot and root growth (Table 3). *Pseudomonas fluorescens* application resulted in enhancement of all parameters studied including the photosynthetic, biochemical and morphological observations.

Evaluation of mitigation treatments in field

From the pot culture study it was evident that application of *P. fluorescens*, KNO_3 and salicylic acid before flooding imparted better tolerance to the plants when they experience waterlogged condition during early vegetative stage *i.e*., 20 DAS as compared other treatments.

Plant survival in field condition also differed significantly with respect to the treatments (Table 4). Among the ameliorants higher survival percent was observed in KNO_3 followed by *P. fluorescens* and salicylic acid. Under waterlogging, plant height at harvest was significantly reduced (Table 4). Highest plant height was recorded in control 2 plot. Among ameliorants, *P. fluorescens* and KNO_3 recorded highest plant height. Shoot and root dry weight (Table 4) also significantly improved by these treatments.

Amelioration treatments resulted in improvement of physiological (Table 2) and biochemical parameters (Table 1) which resulted in better growth and enhanced yield (Table 5) compared to the untreated waterlogged plants (control 1). Application of *P. fluorescens*, KNO_3 and salicylic acid before waterlogging stress resulted in enhanced production of branches, capsules per plant and seeds per capsules (Table 5) compared to the untreated control (control 1). Control 1recorded 4.66 g yield per plant which resulted in 23 per cent yield reduction compared to control 2. Sarkar *et al*. (2016) reported 51.67% and 58.24% yield decline in two sesame varieties *viz*., BARI Til 2 and BARI Til 3 under 36 hrs of continuous waterlogging. The reduction in yield was minimized by the application of *P. fluorescens*, KNO_3 and salicylic acid which recorded 16.52, 13.73 and 7.93 per cent improvement in yield compared to control 1 respectively. An improvement of 7.85 to 17.14 per cent in yield per hectare under waterlogging was obtained by the use of these ameliorants in sesame variety Thilak (Table 5).

The result presented here reveals that a waterlogging stress of 72hr at vegetative growth stage had a marked inhibitory effect on vegetative and reproductive growth of sesame.The ameliorants have an influence on the antioxidant system of waterlogged sesame plant. Foliar application of KNO_3 and foliar application and seed priming of *P. fluorescens* have the highest ameliorative potential to reduce the growth inhibitory effect of waterlogging thereby promoting the yield enhancement.

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