

**MORPHOLOGY AND YIELD OF MUSTARD AS INFLUENCED
BY DIFFERENT SOWING TIMES AND BIOSTIMULATORS**

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BY DIFFERENT SOWING TIMES AND BIOSTIMULATORS**

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CERTIFICATE

*This is to certify that the thesis entitled '**MORPHOLOGY AND YIELD OF MUSTARD AS INFLUENCED BY DIFFERENT SOWING TIMES AND BIOSTIMULATORS**' submitted to the Department of Agricultural Botany, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (M.S.) in AGRICULTURAL BOTANY**, embodies the results of a piece of bonafide research work carried out by **MD. ZAKIR HOSSAIN**, Registration No.12-04777 under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

I further certify that any help or source of information, received during the course of this investigation has been duly acknowledged.

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*DEDICATED
TO
MY BELOVED PARENTS*

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MORPHOLOGY AND YIELD OF MUSTARD AS INFLUENCED BY DIFFERENT SOWING TIMES AND BIOSTIMULATORS

ABSTRACT

A field experiment was carried out at Agricultural Farm, Sher-e-Bangla Agricultural University, Dhaka during the period from November 2017 to February 2018 to morphology and yield of mustard as influenced by different sowing times and biostimulators. Salicylic acid and chitosan were used as biostimulators. The experiment consisted of two factors as four different sowing times *viz.*, 03 November (S₁), 08 November (S₂), 13 November (S₃), 18 November (S₄) and four biostimulators *viz.*, Control (B₀), 0.2 mM salicylic acid (B₁), 40 ppm chitosan (B₂) and 0.2 mM salicylic acid + 40 ppm chitosan (B₃). The total treatment combinations were sixteen and the planting material was BARI sarisha 14. The experiment was laid out in Randomized Complete Block design (RCBD) with three replications. Experimental results showed that different sowing times had significant effect on morphology, yield contributing characters and yield parameters of mustard. The highest yield (1.48 ton ha⁻¹) was obtained from the 13 November sowing (S₃) and the lowest (0.91 ton ha⁻¹) was from 8 November sowing (S₂). These yield data were consistent with the following morphological and yield contributing characters of mustard such as number of branch (6.90), number of siliqua (62) and seed number per siliqua (23.17). As sowing times biostimulators also significantly influenced the morphological, yield contributing characters and yield parameters of mustard. The results revealed that biostimulators 0.2 mM salicylic acid along with 40 ppm chitosan (B₃) produced the highest yield (1.42 ton hectare⁻¹) and lowest (0.83 ton hectare⁻¹) was from control condition (B₀). The morphological and yield contributing characters like number of branch (6.32), number of siliqua (56.83), siliqua length (5.47 cm) and seed number per siliqua (22.62) was also higher at 0.2 mM salicylic acid and 40 ppm chitosan and these also consistent with the seed yield of mustard. In case of interaction effect between sowing times and biostimulators, the highest yield (1.66 ton ha⁻¹) was observed with 13 November sowing and 0.2 mM salicylic acid along with 40 ppm chitosan treatment combination and the lowest seed yield (0.90 ton /ha) was observed with 8 November sowing along with control condition. The seed yield from the lowest to highest are consistent with the several parameters of both morphological and yield contributing characters of mustard to different sowing times and biostimulators.

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CHAPTER I

INTRODUCTION

Mustard (*Brassica spp.*) has a remarkable demand for edible oil in Bangladesh and it is one of the most important oilseed crops throughout the world after soyabean. In Bangladesh Mustard is the principal oilseed crop, which plays a significant role in the national economy. It occupies first position of the list in respect of area and production among the oilseed crops. Mustard seeds contain 40-45% oil and 20-25% protein. Mustard plant belongs to the genus *Brassica* under the family Brassicaceae. It is a cold loving crop and grows during *rabi* season (October-February) with low input support. There is an ever increasing demand of edible oil in the country and the local production can meet up only one third of the requirement. Every year, it needs to import oil and oilseeds to meet up the deficit. The cultivation of mustard has to compete with other food grain crops have shifted to marginal lands of poor productivity. With increasing growth rate of population, the demand of edible oil is increasing day by day. It is, therefore, highly accepted that the production of edible oil should be increased considerably to fulfill the demand of the country.

There is very little scope of expansion for mustard and other oilseed area in the country, due to various reason such as arable land decreasing, interested in cereal crops (rice, pulses, maize) growing, lack of management practices including-find suitable date of sowing, lack of proper doses of fertilizer application according to soil health, lack of suitable genotypes, no knowledge about the use of techniques of Climate-Smart Agriculture (CSA) in crop production.

It is generally established that proper sowing/ planting time is an important factor for the development of crop production including mustard. Previous authors showed that, about 20% yield of mustard reduced due to unplanned sowing. Climatic change may be the major cause for yield reduction in winter crops. Moreover, the rise of global temperatures as predicted between 1.10C and 6.40C during 21st century might aggravate the situation. On the current trends, the average global temperature will rise by 2-30C within next 50 years (Stern, 2006). Temperature is increasing day by day which hampers the growth of mustard and reduced yield. Mustard is cultivated after harvesting of *T.aman* rice in

Bangladesh. Sometimes it may affect growth and yield of mustard mainly due to rising temperature.

Climate-Smart Agriculture (CSA) is an approach for developing agricultural structure to secure sustainable food security under climate change. Different biostimulators such as salicylic acid and chitosan use in CSA. Salicylic acid acts as a plant biostimulators and plays an important role for growth and development of Mustard. It is a potent signaling molecule in plants is involved in defense mechanisms by regulating physiological and biochemical functions and has diverse effects on tolerance to biotic and abiotic stress factors (Nazar *et al.*, 2011). (Khan *et al.*, 2003) and others(2015) stated that Salicylic acid is an endogenous bio stimulators of phenolic nature, which participates in the regulation of physiological processes in plant, such as stomatal movement, ion uptake, inhibition of ethylene biosynthesis, transpiration and stress tolerance. In addition to that ,exogenous application of SA enhanced the growth and photosynthetic rate in crops under water stress, and increases photosynthetic activity and stomatal conductance under drought stress (Habibi, 2012). It has been also found that plants treated with SA generally exhibited better resistance to drought stress. It increased the number of siliqua, pods/siliqua and seed yield of mustard (Gutierrez-Coronado *et al.*, 1998). It also plays a significant role in plant water relations (Barkosky and Einhelling, 1993), photosynthesis, growth and stomatal regulation under abiotic stress conditions (Khan *et al.*, 2003). Therefore, it is suggesting that salicylic acid improve morphology, development and yield of mustard.

However to my knowledge no study has conducted to find the roles of SA on change the morphological, yield contributing characters and yield of mustard under SAU edaphic and climatic condition with the changing environment.

Another biostimulators chitosan is the N-deacetylated derivative of chitin. It is a natural polysaccharide, which can be produced after the alkaline deacetylation of chitin. The chitosan is largely used to mimic biotic and abiotic stresses in crop. The first study of using chitosan as an antipathogen in plants was reported by Allan and Hadwiger, 1979 and they also found, the fungicidal effects of chitosan on different cell wall compositions of fungi. The improvement of the defense system after applying chitosan, both in

monocotyledon and dicotyledons, oil producing crops such as mustard, vegetables such as tomato is the center of addressing this biopolymer in multi-research area. It has been a bio-fungicide, bio-bactericide, and bio-virucide, which spurs plant defense system against the pathogen, thus inducing the immune system of crops and vegetables specially in tomato. Furthermore, the growing demand for food also stimulated the increased use of industrial fertilizer, which causes serious environmental unbalance and is having catastrophic effects on human health. Therefore, the use of it as a biofertilizer is considered. It has been reported to have a positive effect on rhizobacteria growth, where it possesses a symbiotic relation with growth promoting rhizobacteria, thus triggered germination rate and improving plant nutrient uptake.

However to my knowledge no study has conducted to find the roles of chitosan on changes the morphological, yield contributing characters and yield of mustard under SAU edaphic and climatic condition with the changing environment.

In this observation the present study was therefore carried out to study the morphological, yield contributing characters, yield of mustard by different sowing times, Salicylic acid and Chitosan with the following objectives:

- I. To find out the independent effects of sowing times on morphological, yield contributing characters, and yield of mustard.
- II. To find out the independent effect of sole or together use of salicylic acid and/or chitosan on morphological, yield contributing characters and yield of mustard.
- III. To determine the suitable combination of sowing times, sole or together use of Salicylic acid and/or Chitosan on morphological, yield contributing characters and higher yield of Mustard.

CHAPTER II

REVIEW OF LITERATURE

Effect of sowing time:

A field experiment was conducted by Muhal *et al.* (2014) to evaluate the effect of planting duration and salicylic acid application on yield, quality and nutrient uptake of *Brassica* species. The result revealed that foliar application of salicylic acid produced significantly longest plant and higher seed yield at different days after sowing compared to water spray.

A field experiment was conducted by Godara *et al.* (2016) to evaluate the effect of sowing time, varieties and salicylic acid (SA) application on different physiological parameters (i.e. relative water content, photosynthetic rate, transpiration rate, stomata conductance, leaf temperature, Membrane stability index, chlorophyll stability index, heat susceptibility index) of Indian mustard. The experiment consisted of three sowing date and four levels of Salicylic acid. Physiological traits like relative water content, photosynthetic rate transpiration rate, stomata conductance, leaf temperature, chlorophyll stability index Content, heat susceptibility index and membrane stability index are directly correlate with heat stress tolerance in crop plant. Results were revealed that effect of different sowing time, varieties and concentration of SA has shown significant effect on all tested physiological parameters of Indian mustard and those are associated with high temperature stress tolerance.

A field experiment was conducted by Muhal and Solanki (2015) at Udaipur to evaluate the effect of seeding dates and salicylic acid (SA) application on growth attributes, phenology and agro-meteorological indices of *Brassica* species and observed that number of days taken to attain physiological maturity, number of siliqua per plant and seed yield was significantly higher under 100 ppm SA foliar spray compared to water spray.

Sarmah (1996) *et al.* conducted a field experiment during three consecutive *rabi* seasons of 1992-93 to 1994-95 using indian mustard variety TM 2 under four sowing dates viz., 25th October, 9th November, 24th November and 9th December. The plant height was higher in earlier sowings than later sowings.

Reddy and Kumar (1997) reported that 4th October sown crop gave significantly higher plant height (138 cm) over 5th November sown crop in mustard.

Shahidullah *et al.* (1997) reported that late sowing mustard faced higher temperature during seed filling period and caused forced maturity which resulted into reduced growth period of mustard.

Ghosh (1998) reported from West Bengal that rapeseed crop sown in the middle of October resulted into significant increase in plant height and number of branches plant-1 as compared to the crop sown at later dates.

Thakur and Singh (1998) reported that early sowing date gave higher plant height and number of primary and secondary branches per plant than later sowing of *Brassica* species.

Butter and Aulakh (1999) reported that plant height was higher with early sowing (25th October) at India.

Singh *et al.* (1999) reported that dry matter accumulation in various plant parts decreased with delayed sowing at Hisar.

Khichar *et al.* (2000) at Assam reported that higher plant height of mustard was recorded under 20th October sowing (185 cm).

Singh *et al.* (2001) observed that Indian mustard (*Brassica juncea*) sown in second week of October recorded significantly higher plant height (188 cm) as compared to November first week (170 cm) at Jodhpur.

Kurmi (2002) reported that greater plant height was recorded with 17th November sowing (97 cm) as compared to 14th December sowing (77 cm) in mustard.

Singh and Singh (2002) reported that mustard crop sown on 14th and 29th October produced significantly better growth characters viz., plant height, leaves per plant and leaf area index at 60 days after sowing, dry matter accumulation per plant and number of primary and secondary branches per plant. Both the sowing dates were significantly superior to 13th and 28th November sowings. They further recorded higher plant height with 14th October sowing as compared to 29th October, 13th November and 28th November sowings.

Singh *et al.* (2002) observed that the accumulation of dry matter and its allocation to different plant parts was significantly reduced with delay in sowing owing to the prevalence of low day (11 to 18°C) and night (0 to 7°C) temperatures, high evening (66 to 95%) and morning (85 to 97%) RH and poor sunshine (0 to 6 hrs) during the active vegetative growth phase led to poor growth and development of crop.

Sihag *et al.* (2003) reported that higher dry matter accumulation at 90 days of crop growth (31.07 g/plant) and at harvest (42.40 g/plant) was obtained with 15th October sown crop as compared to delayed sown crop.

Panda *et al.* (2004) reported that plant height, dry matter accumulation and number of branches per plant of mustard decreased significantly with delayed sowing after 16th October at New Delhi.

Khushu and Singh (2005) reported that mustard crop sown on 24th October recorded higher total dry matter production as compared to 8th November sown crop.

Kaur and Hundal (2006) reported that highest dry matter (12992 kg ha⁻¹) was obtained when *Brassica juncea* (Cv. RL-1359) was sown on early October as compared to late October (8852 kg /ha) and mid-November (951 kg /ha).

Neog *et al.* (2006) reported that mustard cultivar Pusa Jaikisan and Varuna produced more biomass (1230 and 1172 g m⁻²) on normal sowing as compared to early (1117 and 996 g m⁻²), late sowing (858 and 757 g m⁻²) and very late (531 and 458 g m⁻²) sowing of crop.

Roy and Chakravarty (2007) at IARI, New Delhi reported that delay in sowings resulted in reduction of the biomass. Dhaliwal *et al.* (2008) reported that 5th October sown Indian mustard registered significantly higher plant height (158 cm) over 25th November sown crop.

Saha and Khan (2008) at West Bengal indicated that total dry matter and plant height of mustard decreased significantly in delayed sowing after 5th November sowing.

Abolfazl *et al.* (2009) reported that delay in sowing led to decreased above ground dry matter and leaf area index of mustard.

Somayeh *et al.* (2011) reported that maximum plant height (138.3 cm) was recorded in 30th August sown crop which was significantly different from the all other planting dates.

Kumari *et al.* (2012) reported that 10th October sown crop resulted in significantly higher plant height, number of primary and secondary branches of mustard over 30th October sown crop.

Ansari *et al.* (1990) reported that mustard sown during 1st October produced significantly higher seed yield (987.37 kg /ha) followed by 1st November (900.0 kg /ha) and 15th October (845.4 kg /ha) sown crop.

Solanki *et al.* (1990) while working at Udaipur, India reported that number of siliquae plant-1, seed siliqua-1, test weight and seed yield plant-1 was significantly reduced when toria crop was sown on 30th September as compared to 15th September. They also observed that seed and stover yield was significantly higher in 15th September sown crop over 31st August and 30th September sown crop. However, sowing dates had no significant effect on harvest index.

Sarmah (1996) reported that sowing on 25th October remaining comparable with 9th November sowing resulted in substantial increase in seed yield as compared with delayed sowing on 24th November and 9th December. Delayed sowing on 24th November and 9th December resulted in 24.8 and 51.6 per cent yield reduction in comparison with sowing on 24th October, respectively.

Surekha and Reddy (1996) evaluated four Indian mustard varieties viz. Vardan, TM-4, Pusabasant and Pusabahaar under four sowing dates viz., 5th October, 20th October, 5th November and 20th November in sandy clay loam soil at Rajendranagar, Hyderabad during *rabi* 1993-94. Sowing mustard on 5th October gave significantly higher seed yield than later sowings. Every successive fortnight delay in sowing beyond 5th October resulted in significant yield reduction which was to the tune of 34.6, 67.6 and 88.4 per cent with 20th October, 5th November and 20th November sowings, respectively. They also reported that sowing mustard on 5th October had more branching, higher number of primary, secondary and tertiary branches, siliquae plant-1 and seeds siliqua-1 as compared with later sown crop.

Reddy and Kumar (1997) observed that seed yield of mustard was higher with 4th October sowing (646 kg /ha) as compared to 5th November sowing (486 kg/ha). Similar trend was observed in respect to stover yield.

Mondal *et al.* (1999) reported that early planting of mustard produced the highest number of siliquae plant-1 and reduced in the late sowings. Sowing of mustard on 5th October gave significantly higher seed and stover yield as well as higher harvest index than 20th October and 4th November sowing due to cooler temperature regime prevalent during its reproductive period (Annual Progress Report, 2000).

Chand *et al.* (2000) revealed that sowing of mustard in the first week of October recorded significantly higher yield (11.06 q ha⁻¹) as compared to sowing on 23rd October and 8th November (8.60 and 6.46 q ha⁻¹, respectively).

Panwar *et al.* (2000) reported that yield of mustard decreased when sown on 5th November (11.7 q ha⁻¹) as compared to 5th and 20th October (17.0 and 17.7 q ha⁻¹, respectively).

Khan and Tak (2002) conducted a field experiment on a clay loam and suggested that the seed yield was higher under 20th October sown crop than 5th October and 5th November sown crop.

Shivani and Kumar (2002) reported that mustard sown on 25th September and 5th October registered higher 1000-seed weight and number of seeds siliqua-1 as compared to 15th October, 25th October and 4th November sowing.

Singh and Singh (2002) at Faizabad, Uttar Pradesh observed that number of siliquae plant-1, seed siliqua-1, test weight and seed yield plant-1 decreased significantly when the Indian mustard sown after 29th October. However, 14th October and 29th October sowings were at par in this respect. Similar trend was also observed in seed, stover and biological yields of Indian mustard.

Tomar *et al.* (2002) reported that early sowing (4th week of September) of mustard recorded significantly higher test weight and seed yield over 1st, 2nd and 3rd week of October sown crop.

Sihag *et al.* (2003) reported that the higher mustard seed yield (21.50 q ha⁻¹) was obtained in 15th October sowing.

Kumar *et al.* (2004) reported that more seed yield (29.80 q ha⁻¹) of mustard was obtained under 21st October sown crop by 6.5 and 3.5 per cent over 7th and 14th October sown crop, respectively.

Panda *et al.* (2004) observed that number of siliquae plant⁻¹, seeds siliqua⁻¹, test weight, biomass yield and harvest index of Indian mustard decreased significantly with the delay in sowing beyond 16th October.

Khushu and Singh (2005) reported that 24th October sown crop recorded higher seed yield over 8th November sown mustard.

Srivastav *et al.* (2005) reported that the seed yield during 2004-05 was highest under early sowing dates (before 15th October).

Kaur and Hundal (2006) reported that highest seed yield (1486 kg ha⁻¹) was obtained by *Brassica juncea* (Cv. RL-1359) when crop was sown during late October as compare to early October (1409 kg /ha) and mid-November (951 kg /ha).

Prasad and Kumari (2006) reported that mustard produced more seed yield (1338 kg /ha) when crop was sown during last week of October as compare to 1st week of November (1250 kg ha⁻¹). Mustard crop gave maximum yield of 19.42 q ha⁻¹ when sowing temperature was around 26.20 C. The seed yield significantly decreased by 1.14 and 5.69 q ha⁻¹ when the sowing was delayed and the temperature were lower by 2.2 and 4.90 C, respectively as compared to normal sowing date (5th October).

Dhaliwal *et al.* (2008) reported that Indian mustard (*Brassica juncea*) sown on 5th October recorded significantly higher yield attributes and seed yield (1898 kg /ha) as compared to 30th October and 25th November sown crop.

Khushu *et al.* (2008) reported that grain and biomass yield was more in 6th October sown crop (1533 kg /ha and 7419.6 kg /ha) followed by 21st October and 5th November sown crops which may be attributed to the more radiation absorbed by 6th October sown crop.

Saha and Khan (2008) reported that mustard sown on 22nd October and 29th October gave significantly higher siliquae plant-1 (54.0 and 52.2), 1000-seeds weight (2.6 and 2.4 g) and seed yield (786.9, 734.1 kg /ha) over 5th November, 12th November and 19th November sown crop.

Abolfazl *et al.* (2009) reported that there was a strong negative relationship between seed yield and air temperature during reproductive stages of Mustard (*Brassica napus*). They further reported that delay in sowing led to decrease in harvest index and seed yield.

Saikia *et al.* (2009) reported that Indian cultivar Binoy (B-9) of *Brassica campestris* var. yellow sarson was sown at five different dates at weekly interval starting from 13th October and they observed that the highest seed yield (999.7 kg /ha) was obtained under 13th October sown crop and the lowest yield (603.3 kg /ha) was observed under 27th October sown crop.

Rao *et al.* (2011) reported that mustard yield were less than 2000 kg /ha when maximum temperature was less than 30⁰ C during 1-6 week after sowing and minimum temperature was less

than 12⁰ C during 1-5 week after sowing. Higher yields (2400 to 3000 kg /ha) were obtained when minimum temperature during 16-20⁰ C was less than 6⁰ C.

Somayeh *et al.* (2011) reported that the different sowing dates had highly significant effect on various yield attributes of mustard. The crop sown on 30th August recorded

significantly higher number of seeds siliqua-1, 1000-seed weight, siliquae plant-1 and seed yield (3034 kg /ha) of mustard as compared to 27th January sown crop.

Vashisth *et al.* (2011) reported that yield was more in 15th October sown crop as compared to 30th October and 15th November sown mustard crop.

Yadav *et al.* (2011) reported that the seed yield was significantly higher in 20th October (2049.7 kg ha⁻¹) than 10th November (1437.3 kg /ha) and 30th November (915.1 kg /ha) sown mustard.

Bora and Sharma (2012) reported that maximum seed yield (754 kg ha⁻¹) obtain when rapeseed was sown 15th November as compare to 25 November (651 kg /ha), 5th December (456 kg /ha) and 15th December (259 kg /ha) sown crop.

Kumari *et al.* (2012) reported that yield attributes and seed yield of mustard were significantly higher in 10th October sowing compared to delayed sowings. The decrease in seed yield due to sowing on 20th October and 30th October over 10th October was to the tune of 8.7 and 29.2 per cent during first year and 10.1 and 29.3 per cent during second year, respectively.

Kushwaha *et al.* (2009) reported *Brassica campestris* took less days to attained physiological maturity (87-90 days) as compared to *Brassica juncea* in all dates of sowing viz., 8th September, 18th September, 28th September and 9th October.

Effect of Salicylic acid:

Salicylic acid (C₇H₆O₃) is an endogenous biostimulators of phenolic nature, which participates in the regulation of physiological processes in plant, such as stomata movement, ion uptake, inhibition of ethylene biosynthesis, transpiration and stress tolerance (Khan *et al.*, 2003 and Shakirove *et al.*, 2003).

Foliar application of salicylic acid exerted a significant effect on plant growth metabolism when applied at physiological concentration. Salicylic acid increased the number of flowers, pods/plant and seed yield of mustard (Gutierrez-Coronado *et al.*, 1998). It also plays a significant role in plant water relations (Barkosky and Einhelling, 1993), photosynthesis, growth and stomatal regulation under abiotic stress conditions (Khan *et al.*, 2003; Arfan *et al.*, 2007).

Field study was conducted by Sharma *et al.* (2013) on an assembly of 25 Indian mustard genotypes to test the efficacy of salicylic acid (SA) on yield attributes, seed filling and seed yield and further to visualize the extent of genotypic variations in mitigating the yield losses with SA due to terminal heat stress under late sown conditions and revealed that foliar application of SA improved growth parameters as well as plant height of mustard and yield parameter like 1000 seed weight, number of siliqua on main shoot compared to the application of water.

Mustard crop is very sensitive to temperature stress during reproductive stage. Heat stress due to high ambient temperatures is a serious threat to crop production all over worldwide (Hall, 2001).

Among the internal factors, biostimulators play a vital role in regulation of growth and development. SA is important biostimulators and expressed positively in biotic and abiotic stress. It also enhanced photosynthesis efficiency, metabolism and growth of mustard plant under elevated temperatures (Chhabra *et al.*, 2013).

Salicylic acid activates a novel gene BjDREB1B encoding a DRE (dehydration responsive element) binding protein, leading to elevated level of proline thereby provided tolerance to the plants against harmful effects of temperature stress. Exogenous application of SA reverses the effect of heat stress on *Brassica juncea*. Salicylic acid in *Brassica juncea* plants exposed to temperature stress was reported by Cong *et al.* (2008).

The induction of chalcone synthase and phenylalanine ammonialyase by salicylic acid application which results in the synthesis of certain phenolic compounds that play an important role in conferring resistance against various abiotic stresses including temperature stress (Campos *et al.*, 2003.)

At the sub-cellular level major modifications occur in the chloroplast, leading to significant changes in photosynthesis. For example, heat stress reduced photosynthesis by changing the structural organization of thylakoids. Such changes result in the formation of antenna-depleted photosystem-II (PSII) and hence reduced photosynthetic and respiratory activities (Kaur *et al.*, 2009).

The activities of superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) and the level of proline exhibited increases in response to SA or heat stress application. Heat stress also induces oxidative stress. For example, generation and reaction of activated oxygen species (AOS), which causes the autocatalytic peroxidation of membrane lipids and pigments subsequently leads to membrane permeability and modification of its functions (Xu, *et al.*, 2006).

The effect of Salicylic acid on heat tolerance showed that the heat tolerance of mustard plants was improved by spraying with Salicylic acid. This effect was concentration-dependent, as SA exhibited a protective effect only at low concentrations (0.01-0.1 mM). Both treatment with 0.01 mM SA and hardening at 45⁰ C for 1 h led to an increase in the H₂O₂ level and a reduction in catalyses activity (Dat *et al.* 1998a).

Dat *et al.* (1998b) reported that spraying mustard (*Sinapis alba* L.) seedlings with salicylic acid (SA) solutions between 10 and 500 mM significantly improved their tolerance to a subsequent heat shock at 55⁰ C for 1.5 h. The effects of SA were concentration dependent, with higher concentrations failing to induce thermo tolerance. The time course of thermo tolerance induced by 100 mM SA was similar to that obtained with seedlings acclimated at 45⁰ C for 1 h.

Salicylic acid plays a key role in providing tolerance against temperature stress. A foliar spray of lower concentrations of salicylic acid conferred heat tolerance to mustard. Further this treatment, accompanied with hardening at 45 °C for 1 h enhanced the H₂O₂ level and also reduced the CAT activity, thereby increasing the potential of plants to withstand the heat stress (Dat *et al.*, 1998a). A similar response was observed in potato plantlets, raised from the cultures, supplemented with lower concentrations of acetyl salicylic acid (Lopez-Delgado *et al.*, 1998).

Salicylic acid enhanced the thermo tolerance of tobacco plants when applied at low concentration (10 µmol L⁻¹), whereas at ten times this concentration it had no protective effect against heat stress (Dat *et al.* 2000).

Tomato and bean plants, 0.1 and 0.5 mM SA or acetyl-SA increased drought tolerance when imbibed by the seeds for 1 day or when applied to 2-week old plants by soil drenching 1 week before drought stress (Senaratna *et al.* 2000).

When salicylic acid was applied by soaking the grains before sowing, it improved the drought tolerance of wheat plants. Soaking wheat grains with 100 ppm acetyl-SA had an alleviating effect on the injury of plants subjected to drought stress (Al-Hakimi and Hamada 2001). Pretreatment of 2-week-old maize plants with 0.5 mM SA for 1 day decreased their drought tolerance, although it increased the polyamine content of the plants (Nemeth *et al.* 2002).

Fariduddin *et al.* (2003) reported that the dry matter accumulation was significantly enhanced in *Brassica juncea*, at concentrations of 10⁻⁵ M salicylic acid were sprayed but the concentration above that inhibitory. The application of 0.05 mM salicylic acid in wheat seedlings also improved plant growth after salt stress and caused the accumulation of ABA and prolines.

Spraying wheat leaves with 1 mM SA increased antioxidant enzyme activities, chlorophyll and relative water content, and the membrane stability index, and decreased H₂O₂ and lipid peroxide levels under moderate water stress (Agarwal *et al.* 2005).

The exogenous application of SA at an optimal concentration of 0.1 mM induced the synthesis of Hsp70 and Hsp17.6, which belongs to the class I cytosolic family of small plants and has protein-refolding activity, parallel with an increase in the heat tolerance of pea (Pan *et al.* 2006).

In cucumber plants (*Cucumis sativa* L.), foliar spraying with 1 mM SA induced heat tolerance, as shown by the lower electrolyte leakage parameter, lower H₂O₂ and lipid peroxide levels, and higher Fv/Fm chlorophyll a fluorescence value, whereas the hydroponic application of the same concentration had the opposite effect (Shi *et al.* 2006).

Horvath *et al.* (2007) strongly argue that SA could be a very promising compound for the reduction of the abiotic stress sensitivity of crops, because under certain conditions it has been found to mitigate the damaging effects of various stress factors in plants. Several methods of application (soaking the seeds prior to sowing, adding to the hydroponic solution, irrigating, or spraying with SA solution) have been shown to protect various plant species against abiotic stress factors by inducing a wide range of processes involved in stress tolerance mechanisms. They also reported that the application of exogenous SA could provide protection against several types of stresses such as high or low temperature, heavy metals, and so on.

Hayat *et al.* (2009) studied on pots holding 7 day-old seedlings of Indian mustard (*Brassica juncea* L.) were subjected to differential temperature stress by exposing plants to 30 or 40 °C for 24 hour. Seedlings were sprayed with double distilled water (DDW) or 10⁻⁵ M salicylic acid (SA) at the 8-day stage and were sampled at 30 DAS. The plants exposed to temperature stress exhibited a significant decline in growth, and in levels of chlorophyll, nitrate reductase and carbonic anhydrase activities and photosynthetic

parameters. A follow-up treatment with SA protected against the stress generated by temperature and significantly improved the above parameters. Antioxidative enzymes and levels of proline significantly increased in response to SA as well as to temperature stress.

Kaur *et al.* (2009) reported that *Brassica* crop sown early encounters high temperature stress, which causes a great yield lost. They observed the effects of heat shock, heat acclimation and SA in four genotypes of Brassica, TL15, PBT37, RL1359 and PBR210. Heat acclimation for 3 h at sublethal temperature and SA pretreatments at 10 and 20 μM for 2 h prior to heat shock at 40-55°C were found to be effective in imparting thermo protection at 4 day old seedling stage. These pretreatments helped seedlings to recover from heat stress by increasing seedling length, reduced electrolyte leakage and conferring membrane protection. Increased level of total soluble sugars, fresh/dry weight, and also increase in enzymatic activities of invertase, CAT, POX conferred thermo tolerance.

Hussain *et al.* (2011) reported that SA could be used as a potential biostimulators to improve salt tolerance in plants. Yusuf *et al.* (2012) reported that in Indian mustard (*Brassica juncea*) plants exposed to SA (10^{-5} M) significantly had more content of proline in the leaves over the water sprayed control plants. However, the combination of Ni (100 mg kg⁻¹) and NaCl (150 mM) followed by SA (10^{-5} M) spray generated maximum value for the proline content among all the treatments by 78.20 per cent over the control. The control plants showed the least value for the proline content.

Bidabadi *et al.* (2012) reported that salicylic acid (SA) induces biotic and abiotic stress tolerance in crops. The results indicated that with increasing levels of PEG, proliferation rate, fresh weight increase, relative water content and chlorophyll concentrations were significantly decreased. The SA concentrations improved shoot tips performance by increasing proliferation rate, fresh weight increase and relative water content. SA treatments also enhanced plant tolerance against oxidative stress. This was observed through significant reduction in H₂O₂ and MDA contents of SA treated shoot tips under

water stress conditions. The results revealed that exogenous application of SA helped to reduce the harmful effects of water deficit on banana regenerates in vitro.

SA alone or with thiourea treatment using either salicylic acid or its interactions (SA+Th1 or SA+TH2) had induced an abrupt increase in shoots morphology of wheat (e.g. shoot height, shoot fresh and dry weights). In addition, the area of flag leaf had subsequently increased in the heading and anthesis stages. The spike length, spike number, spike weight plant⁻¹, grains number plant⁻¹, grain weight plant⁻¹ and 1000 grains weight were up-regulated in response to grain priming using SA and with, particularly, the interaction of SA+ thiourea. Yield production was up regulated upon applications of SA+ thiourea in drought-stressed wheat plants. The biological yield in drought-stressed wheat plants was 9.83 tons feddan⁻¹, crop index was 28.08 and harvest index was 39.04 per cent (Hassanein *et al.*, 2012).

Effect of Chitosan:

Karimi *et al.* (2012) reported Chitosan is an anti-transpirant compound that has proved to be effective in many crops and was used to protect plants against oxidative stress and to stimulate plant growth. Chitosan is a natural, low toxic and inexpensive compound that is biodegradable and environmentally friendly with various applications in agriculture.

It was found that foliar applications with chitosan resulted in higher vegetative growth and improvement in fruit quality of different crops (Farouk *et al.*, 2008).

Bittelli *et al.* (2001) reported that foliar application of chitosan decreased transpiration in plants, and reduced water use while maintaining biomass production and yield. Chitosan enhanced crops shoot and root length, fresh and dry weights of shoots, root and leaf area as well as the level of chlorophylls.

Foliar spraying of chitosan in most cases resulted in a significant increase in plant growth parameters under normal or stressed conditions, more pronounced at the intermediate concentration (200 mg per liter) of chitosan (Ghoname *et al.*, 2010).

The stimulating effect of chitosan on plant growth may be attributed to an increase in the availability and uptake of water and essential nutrients through adjusting cell osmotic pressure, and reducing the accumulation of harmful free radicals by increasing antioxidants and enzyme activities (Guan *et al.*, 2009).

The positive effect of chitosan on plant growth may be due to its effect on increasing nutrient uptake in increase elements content such as nitrogen, phosphorous, and potassium. Phosphorous and potassium is an essential nutrient playing an important role in the biosynthesis and translocation of carbohydrates, and necessary for stimulating cell division, cell turgor and forming DNA and RNA (Farouk and Amany, 2012).

Doares *et al.* (1995) reported the mechanisms of chitosan in counteracting the harmful effect of water stress are not well understood and there are a few reports in the literature. Transcriptional activation, induced by chitosan and jasmonate, of genes encoding phenylalanine ammonia lyase and protease inhibitors, suggests that chitosan may influence pathways involving jasmonic acid which plays a key role in the regulation of water use by plants. The reported effects of chitosan on stomatal aperture suggest the possibility that it might be a valuable anti-transpirant with useful agricultural applications.

Guan *et al.* (2009) reported the application of chitosan significantly decreased lipid peroxidation by stimulating antioxidant enzymes, leading to decreased membrane permeability and improved function.

The increase in crops yield due to chitosan application may be due to its effects in stimulating physiological processes, improving vegetative growth, followed by active

translocation of photoassimilates from source to sink tissues. The increases in plant biomass may be due to improving photosynthetic machinery (Khan *et al.*, 2002).

Ghoname *et al.* (2010) observed that foliar application of chitosan on sweet pepper increased significantly the number of fruits per plant and the mean weight of fruit, as well as quality characteristics of the fruit. The role of chitosan in alleviating the harmful effect of water stress on yield may be due to an increase in stomatal conductance and net photosynthetic CO₂-fixation activity under water stress (Khan *et al.*, 2002), and to its role in reducing transpiration to save water.

Abu-Muriefah, (2013) reported foliar-applied chitosan, in particular 200 mg/l, increased plant growth, yield and its quality as well as physiological constituents in plant shoot under stressed or non-stressed conditions as compared to chitosan untreated plants. It is suggested that chitosan could be a promising material used to reduce the harmful effect of water stress on the growth and yield of plants.

Al-Hetar *et al.* (2011) reported that, when chitosan used in plants, it can increase the yield, reduce transpiration and induce a range of metabolic changes as a result of which, plants become more resistant to viral, bacterial and fungal infections.

Pongprayoon *et al.* (2013) reported plants treated with chitosan may be less prone to stress evoked by unfavorable conditions, such as drought, salinity, low or high temperature. Chitosan stimulates vital processes of plants on every level of biological organization, from single cells and tissues, through physiological and biochemical processes, to changes on the molecular level related to expression of genes.

On an industrial scale, chitosan is obtained as a result of chemical or enzymatic chitin deacetylation. Chitin occurs mostly in crustacean armours. Chitosan parameters are mostly determined by three parameters: morphological structure, the degree of deacetylation and the molecular weight (Aranaz *et al.* 2009).

Some of the aforementioned properties of chitosan may specifically stimulate plant reactions and the impact on microorganisms and the molecular weight seems to be one of the most important factors affecting the biological activity of this biopolymer (Li *et al.*, 2011).

Dzung *et al.* (2011) report that as a result of spraying of coffee seedlings with chitosan solutions of the molecular weight of 2 kDa, the content of chlorophylls and carotenoids in leaves increased by 15.36% in plants grown in the field and by 46.38–73.5% in plants grown in the greenhouse in comparison to the control.

Nguyen *et al.* (2013) reported the increase of the chlorophyll content as a result of application of chitosan may be caused by plants enhanced uptake of nutrients on coffee seedlings. The authors demonstrated that after spraying the seedlings with chitosan of the molecular weight of 600 kDa three times, an increase of the content of nitrate, phosphorus and potassium in leaves by respectively 9.8–27.4%, 17.3–30.4% and 30–45% was observed. The experiment also proved that seedlings sprayed with a 10–50 ppm chitosan solution were characterized by increased intensity of net photosynthesis.

Limpanavech *et al.* (2008) reported applying chitosan of various physicochemical properties in growing of *Den-drobium* ‘Eiskul’, plants treated with chitosan of the molecular weight of 45 kDa and deacetylation degree of >90% flowered earlier and produced more inflorescences. The research results showed that chitosan did not affect the length of the inflorescence shoot.

Pospieszny and Atabekov (1989) reported that chitosan may exhibit antiviral activity in plants depending on the species and environmental conditions.

CHAPTER III

MATERIALS AND METHODS

The experiment was conducted at the Agricultural Farm, Sher-e-Bangla Agricultural University, Dhaka-1207 during the period from November 2017 to February 2018.

3.1 Site Description

3.1.1 Geographical Location

The experimental area was situated at 23°77'N latitude and 90°33'E longitude at an altitude of 8.6 meter above the sea level.

3.1.2 Agro-Ecological Region

The experimental field belongs to the Agro-ecological zone of “The Modhupur Tract”, AEZ-28. This was a region of complex relief and soils developed over the Modhupur clay, where floodplain sediments buried the dissected edges of the Modhupur Tract leaving small hillocks of red soils as ‘islands’ surrounded by floodplain. The experimental site was shown in the map of AEZ of Bangladesh in Appendix I.

3.1.3 Climate

The area has sub-tropical climate, characterized by high temperature, high relative humidity and heavy rainfall with occasional gusty winds in Kharif season (April-September) and scanty rainfall associated with moderately low temperature during the Rabi season (October-March). Weather information regarding temperature, relative humidity and rainfall prevailed at the experimental site during the study period was presented in Appendix III.

3.1.4 Soil

The soil of the experimental site belongs to the general soil type, Shallow Red Brown Terrace Soils under Tejgaon Series. Top soils were clay loam in texture, olive-gray with common fine to medium distinct dark yellowish brown mottles. Soil pH ranged

from 6.1-6.5 and had organic matter 1.29%. The experimental area was flat having available irrigation and drainage system and above flood level.

3.2 Details of the Experiment

3.2.1 Treatments

Two sets of treatments included in the experiment were as follows:

(A) Sowing times: 4 (S)

- i. $S_1 = 03$ November, 2017
- ii. $S_2 = 08$ November, 2017
- iii. $S_3 = 13$ November, 2017
- iv. $S_4 = 18$ November, 2017

(B) Biostimulators: 4 (B)

- i. $B_0 =$ Control
- ii. $B_1 = 0.2$ mM Salicylic acid
- iii. $B_2 = 40$ ppm Chitosan
- iv. $B_3 = 0.2$ mM Salicylic acid + 40 ppm Chitosa

3.2.2 Experimental Design

The experiment was laid out in a Randomized Control Block Design (RCBD) with three replications having two factors. There were 16 treatment combinations. The total numbers of unit plots were 48. The size of unit plot was 1.8 m by 1.25 m (2.25 m²). The distances between each plots were .50 m and replications were 1 m.

3.3 Crop/Planting Material

BARI Sarisha14 was used as plant material.

3.3.1 Description of Variety

BARI Sarisha14 is a Mustard variety, released in 2006, suitable for cultivation *rabi* season. Short duration variety, Crop duration is 75-80 days. plant height 75-85 cm, leaf light green, smooth, siliqua/plant 80-102, two chambers are present in pod but as like as four chambers. Seed/siliqua 22-26, seed color pink, 1000 seed weight 3.5-3.8 g. Yield 1.4-1.6 t/ha

3.4 Crop Management

3.4.1.1 Seed Collection

Seeds of BARI Sarisha14 were collected from Genetic Resource and Seed Division, BARI, Joydebpur, Gazipur, Bangladesh by the recommendation of the supervisor.

3.4.1.2 Preparation of Experimental Land

The experimental field was first ploughed on 18 October, 2017 with the help of a tractor, later on October 30, 2017 the land was prepared by three successive ploughings and cross ploughings with a tractor drawn plough and subsequently leveled by laddering. All weeds and other plant residues of previous crop were removed from the field. Manure and fertilizers were applied as per recommendation of Bangladesh Agricultural Research Institute (BARI). Cowdung @ 15 t ha⁻¹, TSP @ 250 kg ha⁻¹ and MoP @ 150 kg ha⁻¹ were applied during final land preparation. Immediately after final land preparation, the field layout was made on November 01, 2017 according to experimental specification. Furadan 10 G (an insecticide) was also applied during final land preparation to control soil insects. Individual plots were cleaned and finally leveled with the help of wooden plank.

3.4.1.3 Seed sowing

The first time seeds of mustard were sown on 03 November 2017 in rows in the furrows having a depth of 2-3 cm. Then another three sowing is done as per design date.

3.4.2 Intercultural operations

3.4.2.1 Thinning

Seeds germination started after four different DAS. Thinning was done two times; first thinning was done at 8 DAS and second was done at 15 DAS to maintain optimum plant population in each plot as per the treatment of plant density.

3.4.2.2 Irrigation and weeding

Irrigation was provided for three times after seed sowing, before flowering and during pod development to all experimental plots equally. The crop field was weeded before providing irrigation.

3.4.2.3 Protection against insect and pest

At early stage of growth few worms (*Agrotis ipsilon*) infested the young plants and at later stage of growth pod borer (*Maruca testulalis*) attacked the plants. Ripcord 10 EC was sprayed at the rate of 1 ml with 1 litre water for two times at 15 days interval after seedlings germination to control the insects.

3.5 Application of biostimulators

3.5.1 Application of Salicylic Acid (SA)

0.03 g Salicylic Acid(SA) and 1ml of Ethanol Dissolved in H₂O and make the 0.2 mM of SA solution. Tween-20 detergent was used as surfactant to prevent dropout of salicylic acid solution from leaves and it was applied as treatment combinations at 30, 45 and 55 days after sowing (DAS) by a hand sprayer.

3.5.1 Application of Chitosan (CHT)

40 mg of Chitosan (CHT) dissolved in water. Tween-20 detergent was used as surfactant to prevent dropout of Chitosan solution from leaves and it was applied as treatment combinations at 30, 45 and 55 days after sowing (DAS) by a hand sprayer.

3.6 Harvesting

Harvesting was done when 90% of the siliqua became brown in color which was estimated by eye observation. The matured pods were collected by hand picking from each plot.

3.7 Data collection

Five plants were allotted for each treatment in each plot for each replication. Data was collected from each sample plant and mean value was calculated.

The following data were recorded.

- i. Plant height(cm)
- ii. Number of leaves plant⁻¹
- iii. Leaf length(cm)
- iv. Leaf breadth (cm)
- v. Number of branches plant⁻¹
- vi. Number of siliqua plant⁻¹
- vii. Length of siliqua (cm)
- viii. Total number of seed siliqua⁻¹
- ix. 1000 seeds weight(g)
- x. Seed yield(g/ plot⁻¹)
- xi. Seed yield (ton/ha)

3.7.1 Plant height

Plant height was measured from the sample plants in centimeter from the ground level to the tip of the leaf and mean value was calculated. Plant height was recorded at 40, 55 and 70 days after sowing to observe the growth rate.

3.7.2 Leaves plant⁻¹

Number of leaves was counted from the sample plants and means value was calculated. Number of leaves was recorded 40 and 55 days after sowing to observe the growth rate.

$$\text{Leaf plant}^{-1} = \frac{\text{Total number of leafs from five sample plants}}{5}$$

3.7.3 Leaf length

Leaf length was measured from the 10 sample leaf and means value was calculated. Leaf length was recorded 40 and 55 days after sowing to observe the growth rate.

$$\text{Leaf length} = \frac{\text{Total leaf length from ten sample leaves}}{10}$$

3.7.4 Leaf breadth

Leaf breadth was measured from the 10 sample leaf and means value was calculated. Leaf breadth was recorded 40 and 55 days after sowing to observe the growth rate.

$$\text{Leaf breadth} = \frac{\text{Total leaf breadth from ten sample leaves}}{10}$$

3.7.5 Number of branches plant⁻¹

Total number of branches was counted from all sample plants and mean was calculated by the following formula:

$$\text{Branches plant}^{-1} = \frac{\text{Total number of branches from five sample plants}}{5}$$

3.7.6 Number of siliqua per plant

Numbers of total siliqua of selected plants from each plot were counted and the mean numbers were expressed as per plant basis. Data were recorded as the average of 5 plants selected at random from the inner rows of each plot.

3.7.7 Length of siliqua

Length of siliqua was taken from randomly selected ten siliqua and the mean length was expressed on per siliqua basis.

3.7.8 Number of seeds per siliqua

The number of seeds per siliqua was recorded from randomly selected 10 siliqua at the time of harvest. Data were recorded as the average and express in sedds per siliqua.

3.7.9 1000 seeds weight (g)

One thousand cleaned, dried seeds of mustard were counted from each harvest sample and weighed by using a digital electronic balance and weight was expressed in gram (g).

3.7.10 Seed yield per plot

The seeds collected from (1 m × 1 m) square meter area of each plot were sun dried properly, weighted and data were recorded. Then it was converted to plot size.

3.7.11 Seed yield (ton/ha)

The seeds collected from (1 m × 1 m) 1 meter square area of each plot and sun dried properly, weighted and data were recorded. Then it was converted to ton per ha.

3.8 Statistical analysis

All the collected data were analyzed following the analysis of variance (ANOVA) technique using MSTAT package and the mean difference were adjusted by LSD technique. (Gomez and Gomez, 1984).

CHAPTER IV

RESULTS AND DISCUSSION

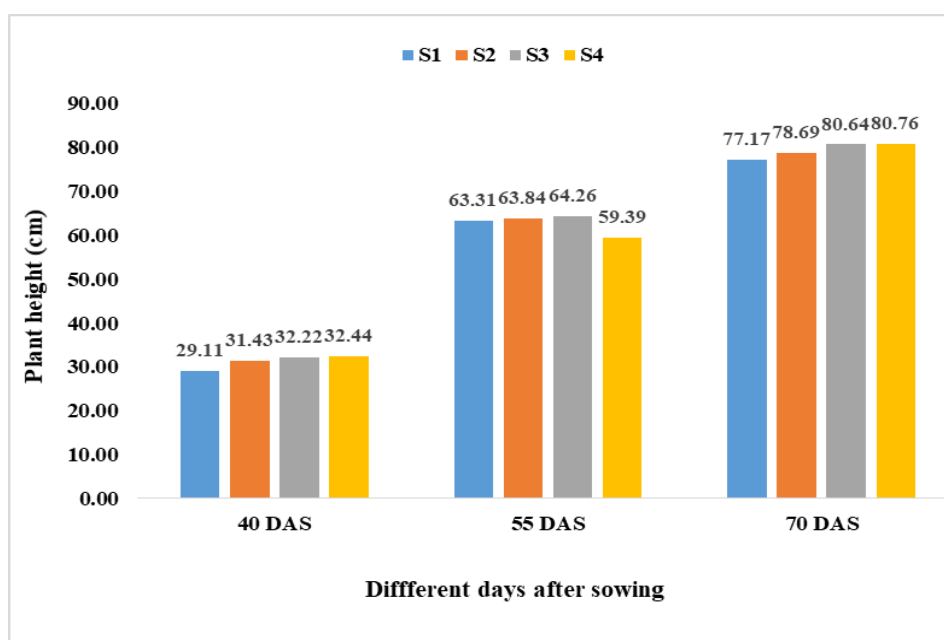
Results obtained from the present study regarding the effects of Salicylic acid, Chitosan and their interactions on the growth and yield components of mustard and have been presented, discussed and compared in this chapter. The analytical results have been presented in Table 1 to 7, Figure 1 to 22 and Appendix IV to X.

4.1 Crop growth parameters

4.1.1 Plant height

i. Effect of sowing times

The plant height of mustard was significantly influenced by different sowing times at 40, 55 and 70 days after seeding (DAS) (Appendix IV and Figure 1).



S₁ = 03 November, S₂ = 08 November, S₃ = 13 November and S₄ = 18 November.

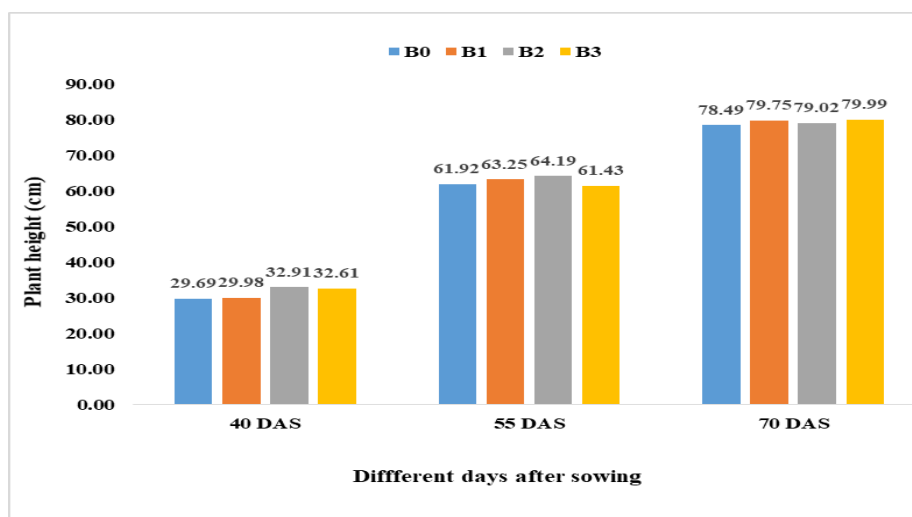
Figure 1. Influence of sowing times on plant height of mustard, BARI sarisha 14 at different days after sowing (LSD value of 5% level of significant 0.94, 8.09 and 1.63 for 40, 55 and 70 DAS respectively)

The result revealed that at 40, 55 and 70 DAS, 13 November sowing (S₃) produced the tallest plant 29.96, 58.50 and 71.75 cm, respectively. The lowest plant height

was observed in 8 November sowing time at 40, 55 and 70 DAS 17.00, 39.58 and 46.00 cm, respectively. The trend of plant height was very slow in the initial stage of growth, and then the rapid increase of plant height was observed from 55 to 70 DAS. Similar results were found Kurmi (2001) who mentioned that greater plant height was recorded with 17th November sowing of mustard. Bora and Sharma (2012) reported that plant height of mustard was higher when sowing of seed in 15 November. In this study, it found that sudden rainfall affect the moist and at after 7 November 2017. Thus the growth of plant which were sown on 8 November 2017 is reduced. Therefore these experimental study showed higher plant height on 13 November 2017. These findings also correlated with many published results.

ii. Effect of biostimulators

Significant variation of plant height was found due to different concentration of biostimulators: Salicylic Acid and Chitosan at 40, 55, 70 DAS (Appendix IV and Figure 2).



B₀ = Control, B₁ = 0.2 mM Salicylic, B₂ = 40 ppm Chitosan and B₃ = 0.2 mM Salicylic acid + 40 ppm Chitosan.

Figure 2. Influence of biostimulators on plant height of mustard, BARI sarisha 14 at different days after sowing (LSD value of 5% level of significant 0.94, 8.09 and 1.63 for 40, 55 and 70 DAS respectively)

Experimental study showed that 0.2 mM salicylic acid and 40 ppm of chitosan increase the height (26.92 cm) of mustard plant at 40 DAS. The lowest plant height (20.17 cm) was observed at control condition. At 55 and 70 DAS numerically the highest plant height (55.50 cm and 65.42 cm) was observed at 0.2 mM salicylic acid

and 40 ppm chitosan; the lowest plant height was observed control level at 55 DAS and 70 DAS. The increased length of an internode has been variously ascribed to increases in numbers of cells, increases in length of cells and to both. Hasanah and Sembiring (2018) published the similar result. They observed that spraying of salicylic acid and chitosan increased plant height. El-Gawad and Bondok (2015) reported that application of mixture of chitosan and salicylic acid gave maximum height of plant. This experimental results also consistent with above mentioned researchers finding. These results support that the together application of salicylic acid and chitosan showed better performance to increase the plant height of mustard than sole application of salicylic acid and chitosan.

Table 1. Interaction effect between sowing times and biostimulators on plant height of mustard, BARI sarisha 14 at different days after sowing

Treatments	Plant height (cm) at different days after sowing		
	40 DAS	55 DAS	70 DAS
S ₁ B ₀	16.50 h	38.33 j	44.67 gh
S ₁ B ₁	19.67 fg	40.33 hij	49.67 f
S ₁ B ₂	27.00 cde	45.33 fg	55.00 e
S ₁ B ₃	26.33 de	51.33 de	60.33 cd
S ₂ B ₀	15.50 h	34.33 k	41.00 h
S ₂ B ₁	15.33 h	39.00 ij	43.00 h
S ₂ B ₂	17.50 gh	42.67 gh	48.00 fg
S ₂ B ₃	19.67 fg	42.33 ghi	52.00 ef
S ₃ B ₀	28.33 bcde	49.67 e	63.67 c
S ₃ B ₁	29.00 bcd	55.33 c	69.00 b
S ₃ B ₂	30.33 ab	61.33 b	75.00 a
S ₃ B ₃	32.17 a	67.67 a	79.33 a
S ₄ B ₀	20.33 f	42.33 ghi	51.67 ef
S ₄ B ₁	22.33 f	48.00 ef	55.67 de
S ₄ B ₂	26.00 e	53.33 cd	63.67 c
S ₄ B ₃	29.50 abc	60.67 b	70.00 b

LSD _(0.05)	2.68	2.39	4.78
CV (%)	6.85	4.17	4.97

S₁ = 03 November, S₂ = 08 November, S₃ = 13 November and S₄ = 18 November, B₀ = Control, B₁ = 0.2 mM Salicylic, B₂ = 40 ppm Chitosan and B₃ = 0.2 mM Salicylic acid + 40 ppm Chitosan

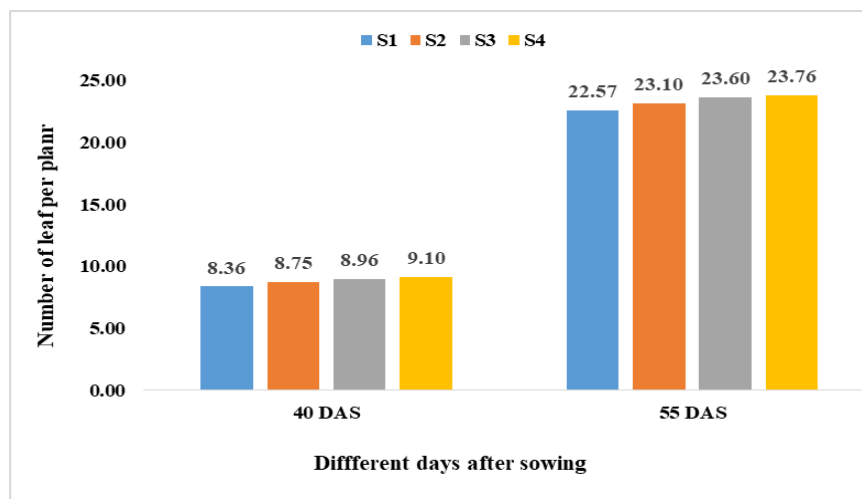
iii. Interaction effect between sowing times and biostimulators

Significant interaction effect between the sowing time and biostimulators was observed at 40, 55 and 70 DAS (Appendix IV and Table 1). The tallest plant 32.17, 67.67 cm and 79.33 cm respectively was obtained from 13 November sowing date along with together application of .2 mM salicylic acid and 40 ppm chitosan treatment combination at 55 and 70 DAS. The lowest plant height 34.33 cm and 41 cm was observed with 8 November sowing and control condition at 55 and 70 DAS. The lowest plant height (41 cm) at 70 DAS was statistically similar with 8 November sowing date along with together application of 0.2 mM salicylic acid treatment condition. In this study the plant height of mustard exhibitional various responses to different date of sowing along with sole or together application of salicylic acid chitosan. Muhal *et al.* (2014) evaluate the effect of planting duration and salicylic acid application on yield, quality and nutrient uptake of *Brassica* species. Therefore these experimental results suggest that sowing of middle November is better than other sowing along with salicylic acid and chitosan to get the highest plant height of mustard.

4.1.2 Number of leaf plant⁻¹ at different days after sowing

i. Effect of sowing times

The production of total number of leaf of mustard was significantly influenced by different sowing times at 40 and 55 DAS (Appendix V and Figure 3).



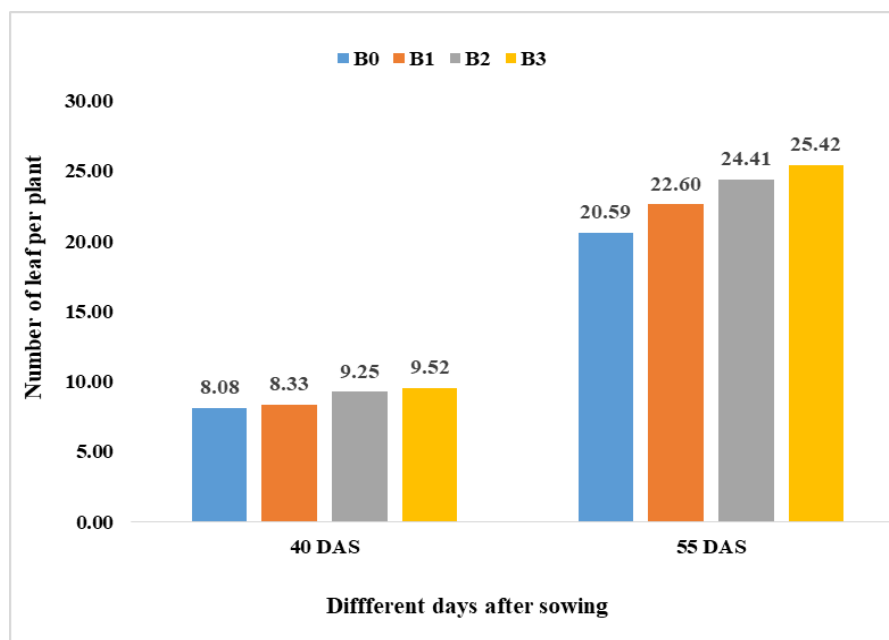
S₁ = 03 November, S₂ = 08 November, S₃ = 13 November and S₄ = 18 November.

Figure 3. Influence of sowing times on number of leaf per plant of mustard, Bari sarisha 14 at different days after sowing (LSD value of 5% level of significant 0.34 and 0.61 for 40 and 55 DAS respectively)

The highest number of leaf was observed in 13 November sowing 9.17 and 21.33 respectively at 40 and 55 DAS. In mustard plant leaf number is increased with the increase of growth duration until 55 DAS. After 55 DAS the leaf number is decreased because of plants produces fruits in that stage.

ii. Effect of biostimulators

The production of total number of leaf plant⁻¹ was significantly influenced by different salicylic acid and chitosan at 40 and 55 DAS (Appendix V and Figure 4). These results support that the together application of salicylic acid and chitosan showed better performance to increase the number of leaf of mustard than sole application of salicylic acid and chitosan.



B₀ = Control, B₁ = 0.2 mM Salicylic, B₂ = 40 ppm Chitosan and B₃ = 0.2 mM Salicylic acid + 40 ppm Chitosan.

Figure 4. Influence of biostimulators on number of leaf per plant of mustard, BARI sarisha 14 at different days after sowing (LSD value of 5% level of significant 0.34 and 0.61 for 40 and 55 DAS respectively)

The highest number of leaf plant⁻¹ was recorded from together application of 0.2 mM salicylic acid and 40 ppm chitosan 8.57 and 21.33 respectively at 40 and 55 DAS. This results was agreement with the findings of Metwally *et. al.*(2013), who reported that salicylic acid and chitosan increased the number of leaves per plant

Table 2. Interaction effect between sowing times and biostimulators on number of leaf per plant of mustard, BARI sarisha 14 at different days after sowing

Treatments	Number of leaf per plant at different days after sowing	
	40 DAS	70 DAS
S ₁ B ₀	5.63 ij	13.17 hi
S ₁ B ₁	7.00 g	13.67 ghi
S ₁ B ₂	7.60 fg	17.00 def
S ₁ B ₃	7.90 ef	20.00 c
S ₂ B ₀	5.17 j	12.50 i
S ₂ B ₁	5.40 j	13.00 hi
S ₂ B ₂	5.60 ij	14.67 fghi
S ₂ B ₃	6.20 hi	16.00 efg
S ₃ B ₀	6.90 gh	17.33 de
S ₃ B ₁	9.00 cd	19.00 cd
S ₃ B ₂	10.17 ab	22.67 b
S ₃ B ₃	10.60 a	26.33 a
S ₄ B ₀	6.23 hi	13.83 ghi
S ₄ B ₁	8.23 ef	15.00 efgh
S ₄ B ₂	8.60 de	18.67 cd
S ₄ B ₃	9.57 bc	23.00 b
LSD _(0.05)	.76	2.41
CV (%)	6.02	8.79

S₁ = 03 November, S₂ = 08 November, S₃ = 13 November and S₄ = 18 November, B₀ = Control, B₁ = 0.2mM Salicylic, B₂ = 40 ppm Chitosan and B₃ = 0.2mM Salicylic acid + 40 ppm Chitosan

iii. Interaction effect between sowing times and biostimulators

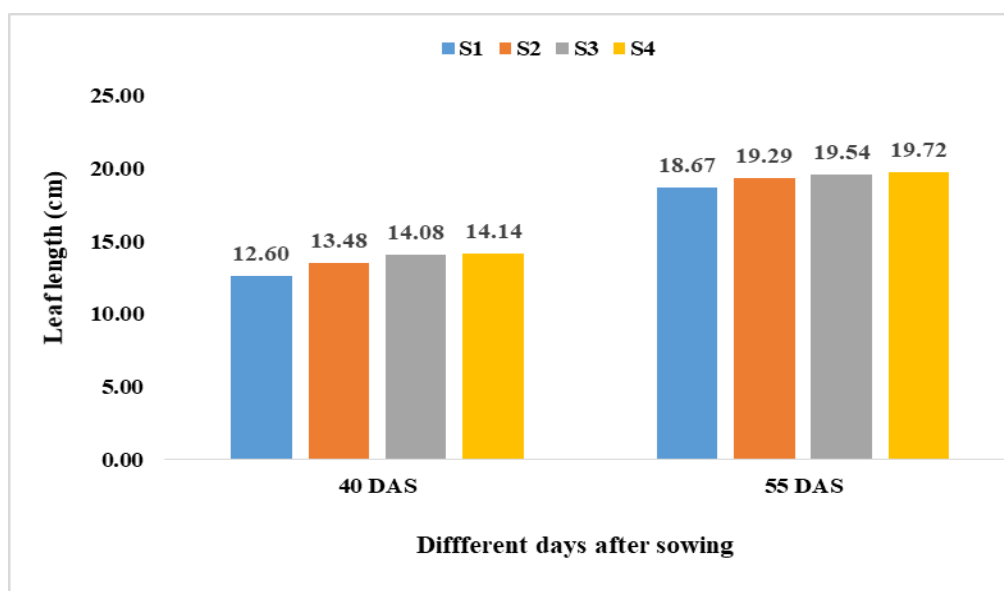
Interaction effect between sowing times and biostimulators significantly influenced the production of leaf number at 40 and 55 DAS (Appendix V and Table 2). The highest number of leaf plant⁻¹ was obtained from 13 November sowing date along with together application of 0.2 mM salicylic acid and 40 ppm chitosan at 40 and 55 DAS

10.60 and 26.33 respectively. The lowest number of leaf plant⁻¹ was found from 8 November sowing and control condition spray at 40 DAS (5.17) that was statistically similar with 8 November sowing date along with 0.2 mM salicylic acid condition. And the lowest number of leaf was also found from 8 November sowing date along with control condition at 55 DAS. Therefore these experimental results suggest that sowing of middle November is better than other sowing along with salicylic acid and chitosan to get the highest number of leaf of mustard.

4.1.3 Leaf length at different days after sowing

i. Effect of sowing times

The length of leaf of mustard was significantly influenced by different sowing times at 40 and 55 DAS (Appendix VI and Figure 5). The highest leaf length was observed in 18 November sowing (14.14 and 19.72 cm respectively) at 40 and 55 DAS that statistically similar with 13 November and 8 November sowing time at 40 DAS and lowest was observed in 3 November sowing time.

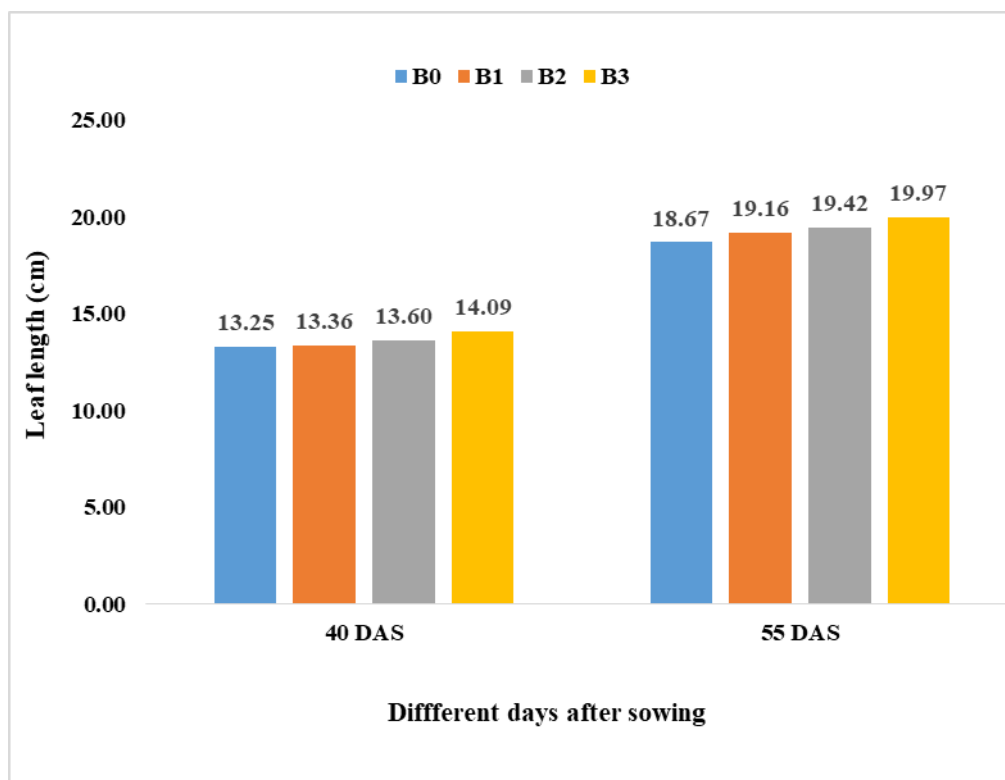


S₁ = 03 November, S₂ = 08 November, S₃ = 13 November and S₄ = 18 November.

Figure 5. Influence of sowing times on leaf length (cm) of mustard, BARI sarisha 14 at different days after sowing (LSD value of 5% level of significant 0.70 and 0.17 for 40 and 55 DAS respectively)

ii. Effect of biostimulators

The leaf length was significantly influenced by different salicylic acid and chitosan at 40 and 55 DAS (Appendix VI and Figure 6). The highest leaf length was recorded from mixture of 0.2 mM salicylic acid and 40 ppm chitosan (14.09 and 19.97 cm respectively) that statistically similar with 40 ppm chitosan spray at 40 DAS. The lowest leaf length was recorded in control level both 40 and 55 DAS that statistically similar with .2mM chitosan spray at 40 DAS.



B₀ = Control, B₁ = 0.2mM Salicylic, B₂ = 40 ppm Chitosan and B₃ = 0.2mM Salicylic acid + 40 ppm Chitosan.

Figure 6. Influence of biostimulators on leaf length (cm) of mustard BARI sarisha 14 at different days after sowing (LSD value of 5% level of significant 0.70 and 0.17 for 40 and 55 DAS respectively)

iii. Interaction effect between sowing times and biostimulators

Interaction effect between sowing times and biostimulators significantly influenced the leaf length at 40 and 55 DAS (Appendix VI and Table 3). The highest leaf length was obtained from 13 November sowing + mixture of 0.2 mM salicylic acid and 40 ppm chitosan at 40 DAS (14.63 cm) and 18 November sowing + mixture of 0.2 mM salicylic acid and 40 ppm chitosan at 55 DAS (20.62 cm). The lowest leaf length was

found from 3 November sowing + 0.2mM salicylic acid spray at 40 DAS and 3 November sowing + control at 55 DAS

Table 3. Interaction effect between sowing times and biostimulators on leaf length of mustard BARI sarisha 14 at different days after sowing

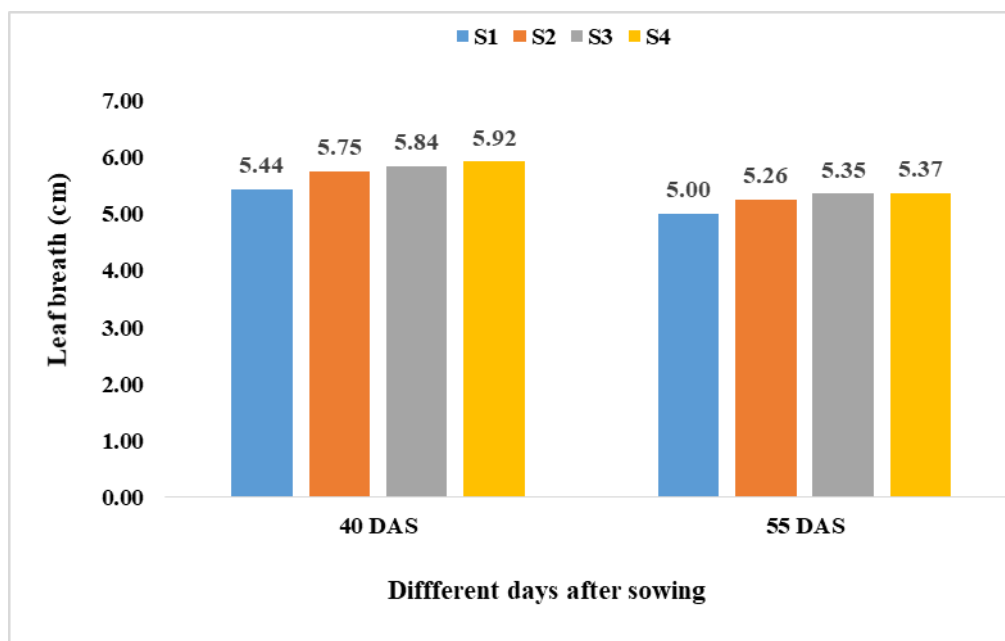
Treatments	Leaf length (cm) at different days after sowing	
	40 DAS	70 DAS
S ₁ B ₀	13.22 bcdefg	18.22 k
S ₁ B ₁	12.02 g	18.79 ij
S ₁ B ₂	12.14 fg	18.66 j
S ₁ B ₃	13.01 efg	19.01 hi
S ₂ B ₀	13.15 defg	18.56 jk
S ₂ B ₁	13.48 abcdef	19.10 ghi
S ₂ B ₂	13.18 cdefg	19.52 def
S ₂ B ₃	14.10 abcde	19.97 bc
S ₃ B ₀	13.28 abcdefg	18.88 ij
S ₃ B ₁	13.89 abcde	19.31 fgh
S ₃ B ₂	14.53 abcd	19.68 cde
S ₃ B ₃	14.63 a	20.27 b
S ₄ B ₀	13.35 abcdefg	19.03 hi
S ₄ B ₁	14.05 abcde	19.42 efg
S ₄ B ₂	14.56 abc	19.81 cd
S ₄ B ₃	14.60 ab	20.62 a
LSD _(0.05)	1.40	0.35
CV (%)	6.21	1.08

S₁ = 03 November, S₂ = 08 November, S₃ = 13 November and S₄ = 18 November, B₀ = Control, B₁ = 0.2 mM Salicylic, B₂ = 40 ppm Chitosan and B₃ = 0.2 mM Salicylic acid + 40 ppm Chitosan

4.1.4 Leaf breath at different days after sowing

i. Effect of sowing times

The breath of leaf of mustard was significantly influenced by different sowing times at 40 DAS but there is not any significant different at 55 DAS (Appendix VII and Figure 7).



S₁ = 03 November, S₂ = 08 November, S₃ = 13 November and S₄ = 18 November.

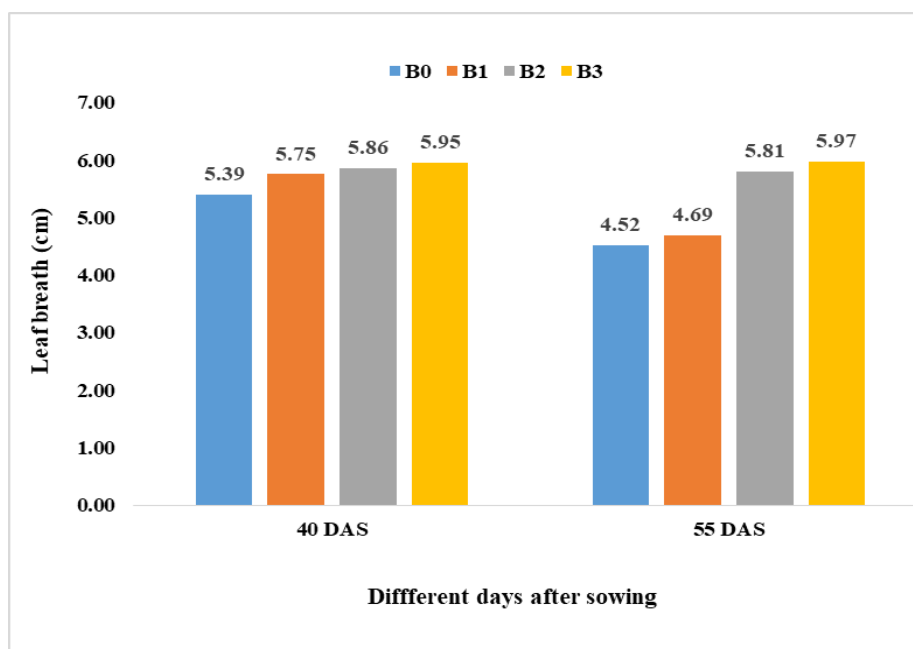
Figure 7. Influence of sowing times on leaf breath (cm) of mustard, BARI sarisha 14 at different days after sowing (LSD value of 5% level of significant 0.23 and 0.089 for 40 and 55 DAS respectively)

The highest leaf breath was observed in 18 November sowing (5.92 cm) at 40 DAS that statistically similar with 13 November and 8 November sowing time and the lowest was observed in 3 November sowing time. At 55 DAS numerically the highest leaf breath was found in 18 November sowing and lowest at 3 November sowing.

ii. Effect of biostimulators

The leaf breath was significantly influenced by different salicylic acid and chitosan at 40 and 55 DAS (Appendix VII and Figure 8). The highest leaf breath was recorded from mixture of 0.2mM salicylic acid and 40 ppm chitosan (5.95 and 5.97 cm respectively) that statistically similar with 40 ppm chitosan and 0.2 mM salicylic acid

spray at 40 DAS. The lowest leaf length was recorded in control level both 40 and 55 DAS.



B₀ = Control, B₁ = 0.2 mM Salicylic, B₂ = 40 ppm Chitosan and B₃ = 0.2 mM Salicylic acid + 40 ppm Chitosan.

Figure 8. Influence of biostimulators on leaf breath (cm) of mustard, BARI sarisha 14 at different days after sowing (LSD value of 5% level of significant 0.23 and 0.089 for 40 and 55 DAS respectively)

iii. Interaction effect between sowing times and biostimulators

Interaction effect between sowing times and biostimulators significantly influenced the leaf breath at 40 and 55 DAS (Appendix VII and Table 4). The highest leaf breath was obtained from 18 November sowing + mixture of 0.2 mM salicylic acid and 40 ppm chitosan at 40 DAS and 55 DAS (6.20 and 6.12 cm respectively) that statistically similar with 13 November sowing time + mixture of 0.2 mM salicylic acid and 40 ppm chitosan. The lowest leaf length was found from 8 November sowing + control spray at 40 DAS that statistically similar with 3 November sowing + control spray and 3 November sowing + 0.2 mM salicylic acid spray. At 55 DAS the lowest leaf breath was found at 3 November sowing + control spray that statistically similar with 3 November sowing + 0.2 mM salicylic acid spray.

Table 4. Interaction effect between sowing times and biostimulators on leaf breath of mustard, BARI sarisha 14 at different days after sowing

Treatments	Leaf breath (cm) at different days after sowing	
	40 DAS	70 DAS
S ₁ B ₀	5.32 e	4.33 h
S ₁ B ₁	5.32 e	4.40 gh
S ₁ B ₂	5.70 bcde	5.62 c
S ₁ B ₃	5.43 de	5.66 c
S ₂ B ₀	5.30 e	4.52 fg
S ₂ B ₁	5.85 abcd	4.71 de
S ₂ B ₂	5.83 abcd	5.84 b
S ₂ B ₃	6.03 ab	5.97 ab
S ₃ B ₀	5.42 de	4.59 ef
S ₃ B ₁	5.89 abc	4.80 d
S ₃ B ₂	5.92 abc	5.90 b
S ₃ B ₃	6.14 ab	6.12 a
S ₄ B ₀	5.51 cde	4.63 ef
S ₄ B ₁	5.95 abc	4.85 d
S ₄ B ₂	6.00 ab	5.87 b
S ₄ B ₃	6.20 a	6.12 a
LSD _(0.05)	0.45	0.16
CV (%)	4.65	1.86

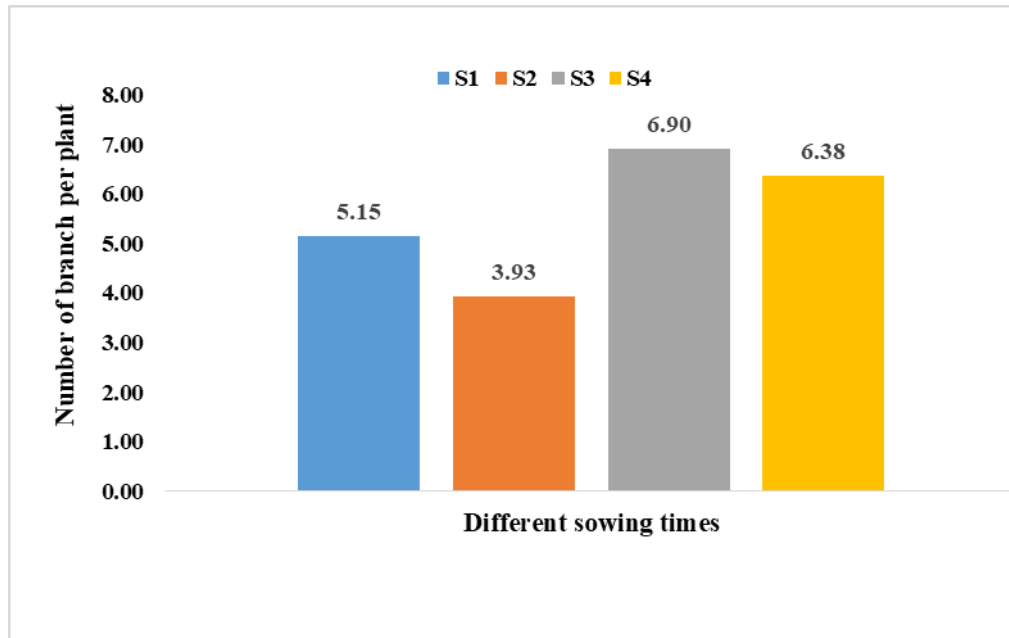
S₁ = 03 November, S₂ = 08 November, S₃ = 13 November and S₄ = 18 November, B₀ = Control, B₁ = 0.2 mM Salicylic, B₂ = 40 ppm Chitosan and B₃ = 0.2 mM Salicylic acid + 40 ppm Chitosan

4.1.5 Number of branches plant⁻¹

i. Effect of sowing times

Different sowing times significantly influenced the number of branches plant⁻¹ of mustard (Appendix VIII and Figure 9). It was observed that, the highest number of branches plant⁻¹ (6.90) was observed in 13 November sowing time and the lowest number of branches plant⁻¹ (3.93) was found in 8 November sowing time. These

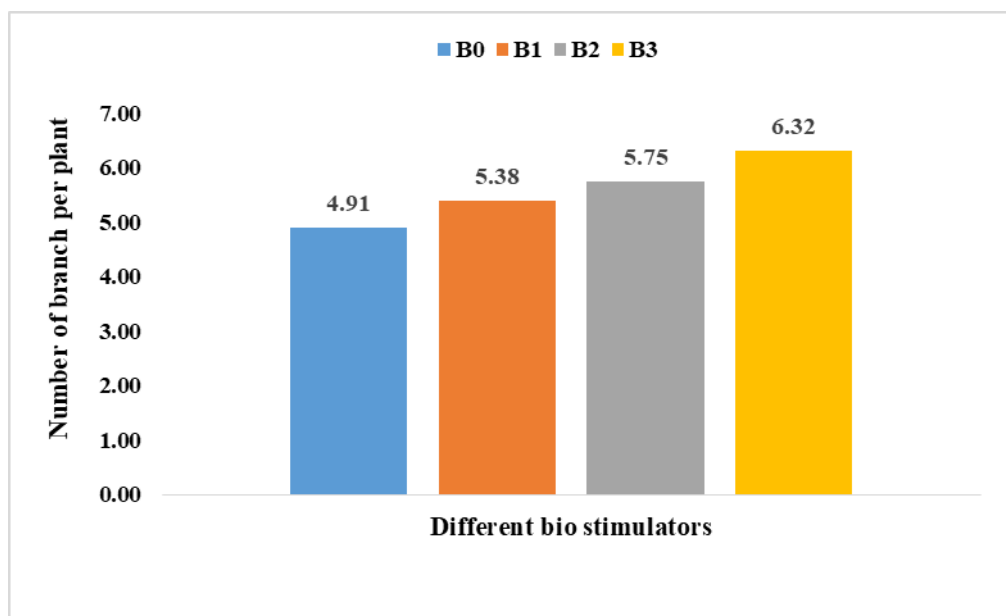
results are correlated with the other parameter of this study such as plant height (Figure no: 1), number of leaf per plant (Figure no: 3). Altogether, it is suggested that 13 November sowing and produced maximum number of branch per plant under the climatic and edaphic condition of SAU.



S₁ = 03 November, S₂ = 08 November, S₃ = 13 November and S₄ = 18 November.

Figure 9. Influence of sowing times on number of branch per plant of mustard, BARI sarisha 14 (LSD value of 5% level of significant 0.12)

Sarmah *et al.*, (1996) reported that 24 November sowing reduce the plant height of mustard and increase the number of branches per plant in mustard.



B₀ = Control, B₁ = 0.2mM Salicylic, B₂ = 40 ppm Chitosan and B₃ = 0.2mM Salicylic acid + 40 ppm Chitosan.

Figure 10. Influence of biostimulators on number of branch per plant of mustard, BARI sarisha 14 (LSD value of 5% level of significant 0.12)

ii. Effect of biostimulators

The total number of branches plant⁻¹ of mustard was significantly influenced by different salicylic acid and chitosan level (Appendix VIII and Figure 10). The highest number of branches (6.32) was recorded in mixture of 0.2 mM salicylic acid and 40 ppm chitosan and lowest (4.91) was found in control condition. This findings is agreed to result of Chhabra *et al.* (2013) and Khan *et al.* (2002). They found that plants sprayed with salicylic acid and chitosan showed significantly number of branches/plant. These results are also correlated with other parameter of this study such as plant height (Figure 2), number of leaf per plant (Figure 4). Altogether, it is suggested that combined application salicylic acid and chitosan in 13 November 2017 produced the maximum number of siliqua per plant under the climatic and edaphic condition of SAU.

Table 5. Interaction effect between sowing times and biostimulators on number of branch per plant, Siliqua per plant and length of siliqua of mustard, BARI sarisha 14

Treatments	Number of branch	Number of Siliqua	Siliqua length (cm)
S ₁ B ₀	4.17 i	38.67 f	3.63 i
S ₁ B ₁	4.97 h	41.33 f	4.20 h
S ₁ B ₂	5.47 g	47.93 e	4.80 fg
S ₁ B ₃	6.00 ef	52.67 d	5.00 ef
S ₂ B ₀	3.50 j	30.77 g	3.13 j
S ₂ B ₁	3.97 i	33.33 g	3.30 j
S ₂ B ₂	4.13 i	39.00 f	3.80 i
S ₂ B ₃	4.13 i	41.00 f	4.10 h
S ₃ B ₀	6.17 de	52.67 d	5.43 d
S ₃ B ₁	6.43 cd	58.67 c	6.03 bc
S ₃ B ₂	6.87 b	64.33 b	6.27 b
S ₃ B ₃	8.13 a	72.33 a	6.83 a
S ₄ B ₀	5.80 f	46.00 e	4.60 g
S ₄ B ₁	6.17 de	52.00 d	5.13 e
S ₄ B ₂	6.53 c	59.33 c	5.50 d
S ₄ B ₃	7.00 b	61.33 bc	5.93 c
LSD _(0.05)	.34	3.43	.26
CV (%)	3.54	4.16	3.19

S₁ = 03 November, S₂ = 08 November, S₃ = 13 November and S₄ = 18 November, B₀ = Control, B₁ = 0.2 mM Salicylic, B₂ = 40 ppm Chitosan and B₃ = 0.2 mM Salicylic acid + 40 ppm Chitosan

iii. Interaction effect between sowing times and biostimulators

Interaction effect between sowing times and biostimulators influenced the number of branches plant⁻¹ of mustard (Appendix VIII and Table 5). The highest number (8.13) was observed at 13 November sowing + mixture of .2mM salicylic acid and 40 ppm chitosan and lowest number (3.50) was found in 8 November sowing + control condition. These results are correlated with other parameter of this study such as plant

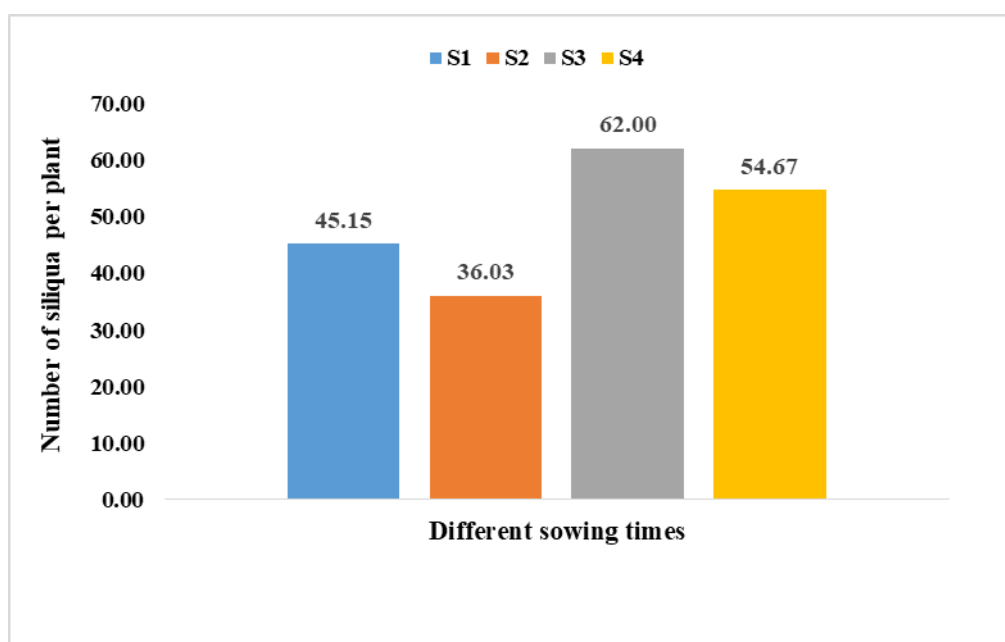
height (Table 1), number of leaf per plant (Table 3). Altogether, it is suggested that 13 November sowing+combined application salicylic acid and chitosan produce the maximum number of branch per plant under the climatic and edaphic condition of SAU.

4.2 Yield parameters

4.2.1 Number of siliqua plant⁻¹

i. Effect of sowing times

The number of siliqua plant⁻¹ significantly varied among the sowing time (Appendix VIII and Figure 11), where 13 November sowing (S₃) produces maximum number of siliqua (54.67), where as minimum



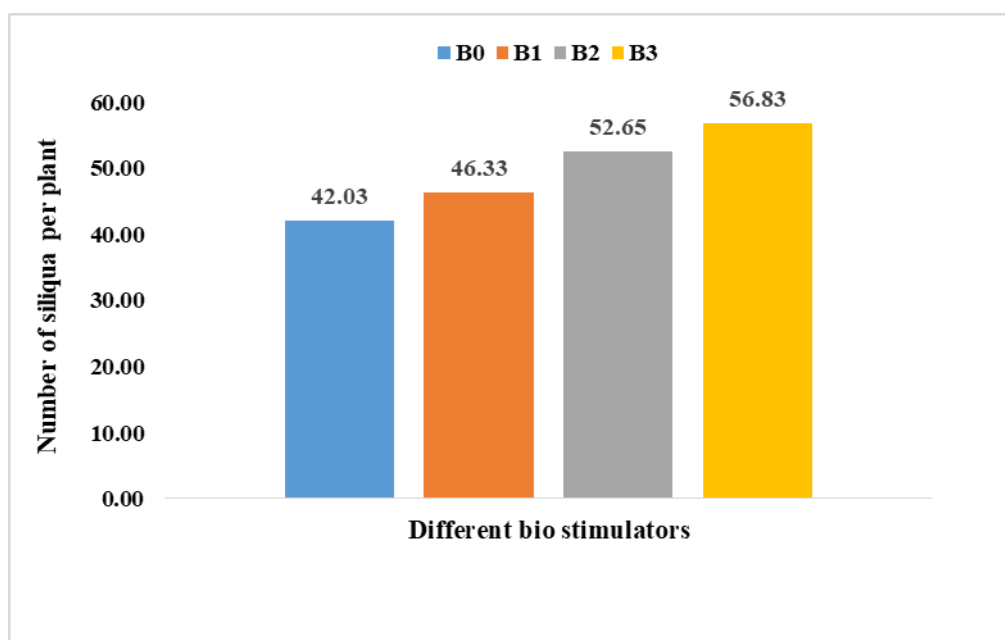
S₁ = 03 November, S₂ = 08 November, S₃ = 13 November and S₄ = 18 November.

Figure 11. Influence of sowing times on number of siliqua per plant of mustard,

BARI sarisha 14 (LSD value of 5% level of significant 2.25)

number of siliqua (36.03) of mustard found in 8 November 2017 (S₂). This result is supported by Somayeh *et al.*, (2011). These results are correlated with the other parameter of this study such as plant height (Figure no: 1), number of branch per plant (Figure no: 10). They reported that the different sowing dates had highly significant effect on various yield attributes of mustard. The crop sown on 13th November (S₃) recorded significantly higher number of siliqua plant⁻¹ of mustard as compared to late

January sown crop. Altogether, it is suggested that 13 November sowing and produced maximum number of siliqua per plant under the climatic and edaphic condition of SAU.



B₀ = Control, B₁ = 0.2 mM Salicylic, B₂ = 40 ppm Chitosan and B₃ = 0.2 mM Salicylic acid + 40 ppm Chitosan.

Figure 12. Influence of biostimulators on number of siliqua per plant of mustard, BARI sarisha 14 (LSD value of 5% level of significant 2.25)

ii. Effect of biostimulators

Different biostimulators: salicylic acid and chitosan level affected number of siliqua of mustard (Appendix VIII and Figure 12). The 0.2 mM salicylic acid and 40 ppm chitosan produces highest number of siliqua per plant (56.83). The lowest number of siliqua per plant (42.03) was observed at control condition. These results are correlated with other parameter of this study such as plant height (Figure 2), number of branch per plant (Figure 10). Bittelli *et al.* (2001) reported that foliar application of chitosan decreased transpiration in plants, and reduced water use while maintaining biomass production and yield. Chitosan enhanced mustard siliqua number as well as yield of plants. Altogether, it is suggested that combined application salicylic acid and chitosan of 13 November 2017 produce the maximum number of siliqua per plant under the climatic and edaphic condition of SAU.

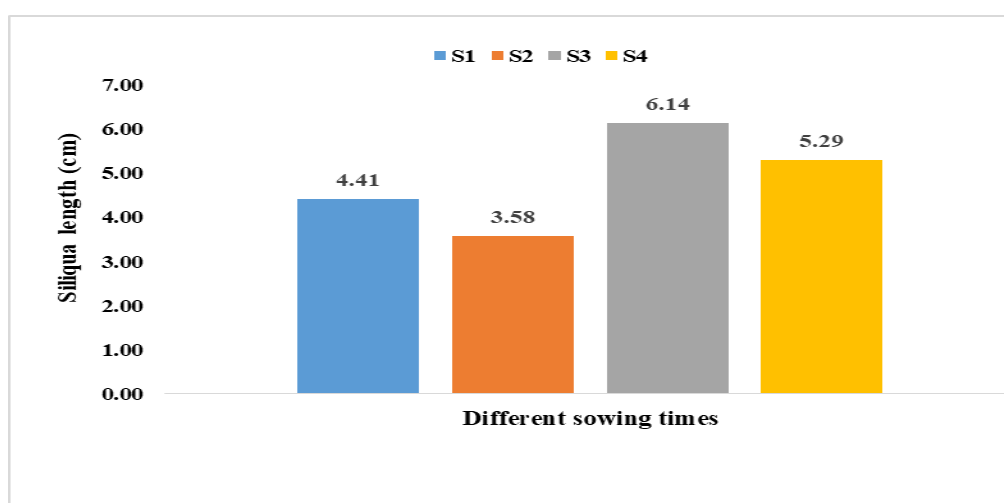
iii. Interaction effect between sowing times and biostimulators

Interaction effect between sowing times and biostimulators significantly influenced the number of siliqua per plant (Appendix VIII and Table 5). 13 November sowing + 0.2 mM salicylic acid and 40 ppm chitosan produces the highest number of siliqua per plant (72.33). The lowest number of siliqua per plant (30.77) was observed at 8 November sowing + control condition. These results are correlated with other parameter of this study such as plant height (Table 1), number of branch per plant (Table 5). Altogether, it is suggested that 13 November sowing+combined application salicylic acid and chitosan produce the maximum number of siliqua per plant under the climatic and edaphic condition of SAU.

4.2.2 Siliqua length

i. Effect of sowing times

The length of siliqua of mustard was significantly influenced by different sowing times (Appendix VIII and Figure 13). The highest siliqua length was observed in 13 November sowing (6.14 cm). The lowest siliqua length was observed at 8 November sowing time (3.58 cm). Altogether, it is suggested that 13 November sowing and produce maximum length of siliqua of mustard plant under the climatic and edaphic condition of SAU.

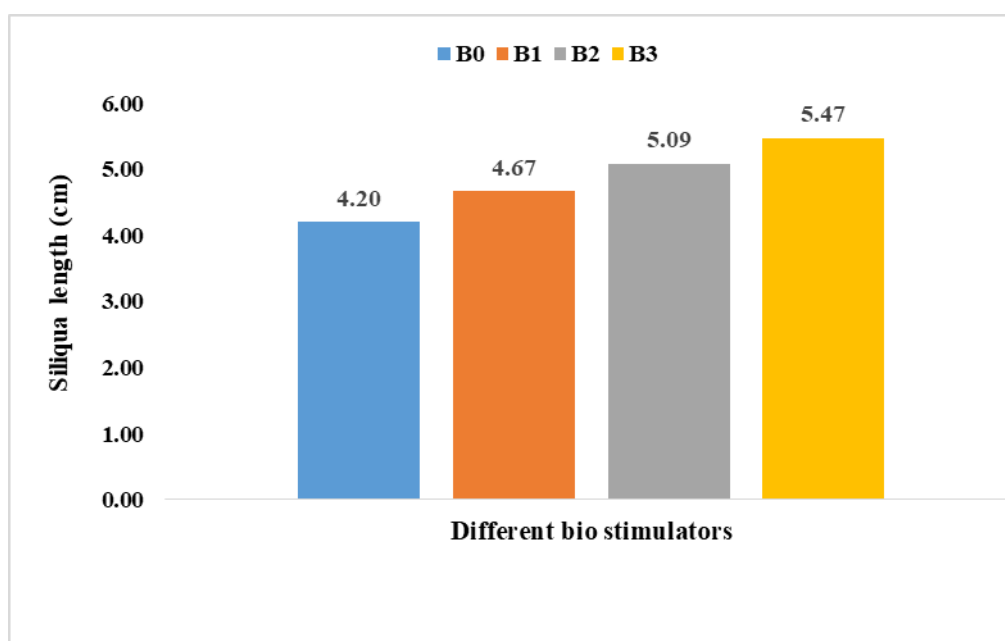


S₁ = 03 November, S₂ = 08 November, S₃ = 13 November and S₄ = 18 November.

Figure 13. Influence of sowing times on siliqua length (cm) of mustard, BARI sarisha 14 (LSD value of 5% level of significant 0.23)

ii. Effect of biostimulators

The siliqua length was significantly influenced by different biostimulators: salicylic acid and chitosan spray (Appendix VIII and Figure 14). The highest siliqua length (5.47) was recorded from treatment combination of 0.2mM salicylic acid and 40 ppm chitosan and lowest siliqua length (4.20 cm) was recorded in control treatment combination. . Altogether, it is suggested that combined application salicylic acid and chitosan of 13 November 2017 produce the maximum length of siliqua of mustard plant under the climatic and edaphic condition of SAU.



B₀ = Control, B₁ = 0.2 mM Salicylic, B₂ = 40 ppm Chitosan and B₃ = 0.2 mM Salicylic acid + 40 ppm Chitosan.

Figure 14. Influence of biostimulators on siliqua length (cm) of mustard, BARI sarisha 14 (LSD value of 5% level of significant 0.23)

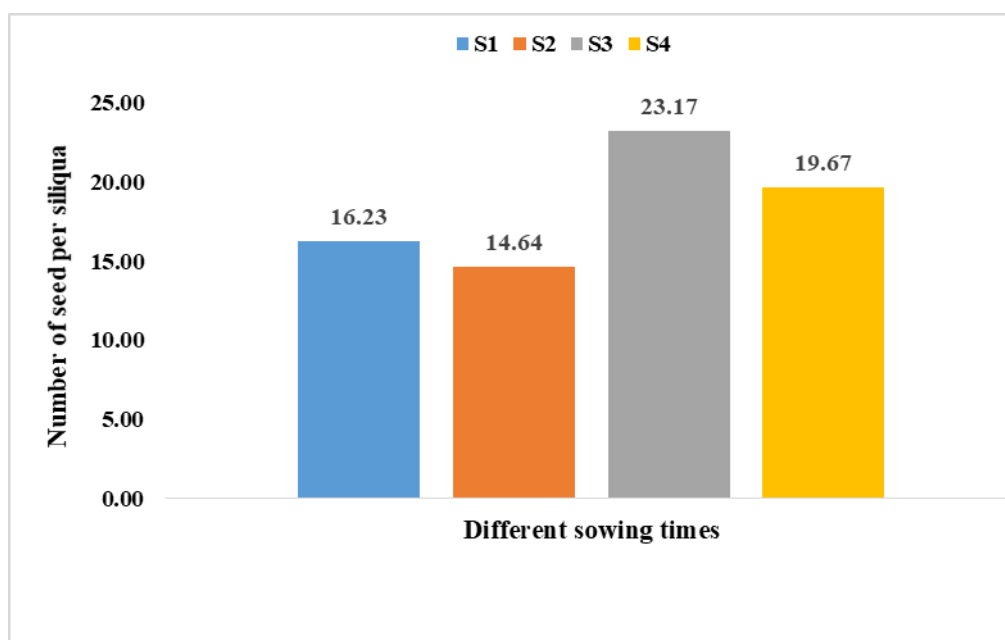
iii. Interaction effect between sowing times and biostimulators

Interaction effect between sowing times and biostimulators significantly influenced the siliqua length (Appendix VIII and Table 5). The highest siliqua length was obtained from 13 November sowing + mixture of 0.2 mM salicylic acid and 40 ppm chitosan (6.83 cm). The lowest siliqua length was found from 8 November sowing + control condition (3.13 cm) that statistically similar with 8 November sowing + 0.2 mM salicylic acid treatment condition. Altogether, it is suggested that 13 November sowing+combined application salicylic acid and chitosan produce the maximum length of siliqua of mustard plant under the climatic and edaphic condition of SAU.

4.2.3 Total number of seed siliqua⁻¹

i. Effect of sowing times

Different sowing times significantly effect on total number of seed per siliqua (Appendix IX and Figure 15).



S₁ = 03 November, S₂ = 08 November, S₃ = 13 November and S₄ = 18 November.

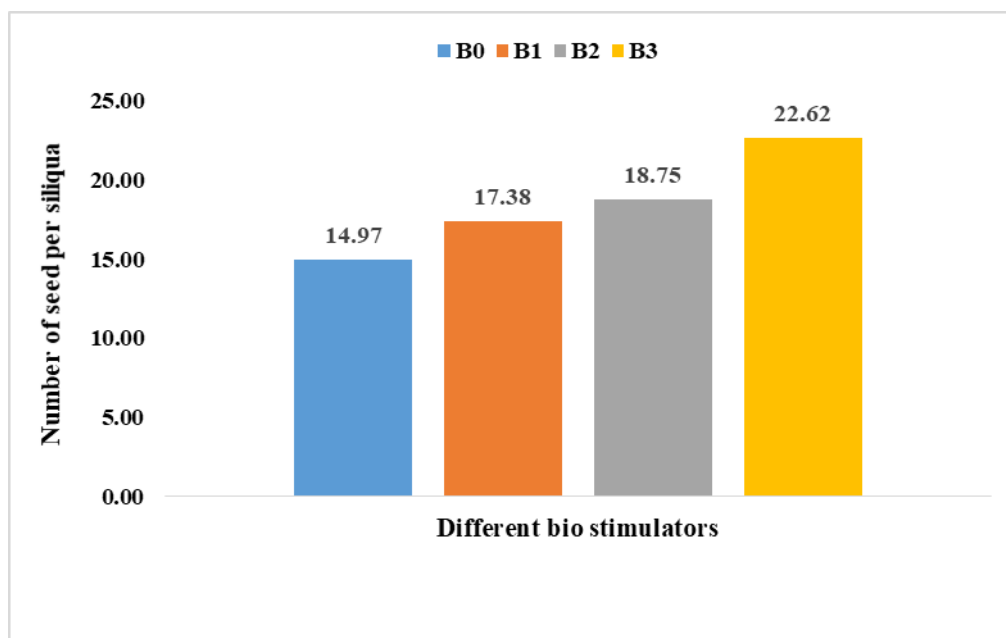
Figure 15. Influence of sowing times on number of seed per siliqua of mustard, BARI sarisha 14 (LSD value of 5% level of significant 2.01)

The highest number of seed per siliqua (23.17) was found from 13 November sowing. The lowest number of seed per siliqua (14.64) was found from 8 November sowing time. These results are correlated with the other parameter of this study such as

number of branch per plant (Figure 9), number of siliqua per plant (Figure 11), siliqua length (Figure 13). Dhaliwal *et al.* (2008) reported that Indian mustard (*Brassica juncea*) sown on 5th December recorded significantly higher yield attributes and seed per siliqua as compared to 30th October sown crop. Altogether, it is suggested that 13 November sowing and produced maximum no of seed per siliqua of mustard plant under the climatic and edaphic condition of SAU

ii. Effect of biostimulators

Total number of seed per siliqua also significantly influenced by different biostimulators: salicylic acid and chitosan spray (Appendix IX and Figure 16). The highest number of seed per siliqua (22.62) was found from combined application of 0.2 mM salicylic acid and 40 ppm chitosan and lowest number (14.79) was found in control condition. These results are also correlated with other parameters of this study such as number of branch per plant (Figure 10), no of siliqua (Figure 12), siliqua length (Figure 14). Ghoname *et al.* (2010) observed that foliar application of chitosan on sweet pepper increased significantly the number of fruits per plant and the mean weight of fruit, as well as quality characteristics of the fruit. The role of chitosan in alleviating the harmful effect of water stress on yield may be due to an increase in stomatal conductance and net photosynthetic CO₂-fixation activity under water stress and to its role in reducing transpiration to save water. Altogether, it is suggested that combined application salicylic acid and chitosan of 13 November 2017 produce the maximum number of seed per siliqua of mustard plant under the climatic and edaphic condition of SAU.



B₀ = Control, B₁ = 0.2 mM Salicylic, B₂ = 40 ppm Chitosan and B₃ = 0.2mM Salicylic acid + 40 ppm Chitosan

Figure 16. Influence of biostimulators on number of seed per siliqua of mustard, BARI sarisha 14 (LSD value of 5% level of significant 2.01)

iii. Interaction effect between sowing times and biostimulators

There was a significant effect with interaction between sowing times and biostimulators in respect of total number of seed per siliqua (Appendix IX and Table 6). The highest number of seed per siliqua (28.33) was recorded in 13 November sowing time + combined application of 0.2 mM salicylic acid and 40 ppm chitosan. The lowest number (13.10) was found in 8 November sowing time + control condition. These results are also correlated with other parameters of this study such as number of branch per plant (Table 5), no of siliqua/plant (Table 5), siliqua length (Table 5). Muhal *et al.*, (2014) reported combination effect between middle November sowing and Salicylic acid spray increase the number of seed per siliqua of Mustard. Altogether, it is suggested that 13 November sowing+treatment combination of salicylic acid and chitosan produce the maximum no of seed per siliqua of mustard plant under the climatic and edaphic condition of SAU.

Table 6. Interaction between sowing times and biostimulators on number of seed per siliqua and 1000 seed weight of mustard, BARI sarisha 14

Treatments	Number of seed per siliqua	1000 seed weight (g)
S ₁ B ₀	13.43 jk	4.00
S ₁ B ₁	15.17 hijk	4.00
S ₁ B ₂	16.00 ghi	4.00
S ₁ B ₃	20.33 cd	4.00
S ₂ B ₀	13.10 k	3.99
S ₂ B ₁	13.67 jk	4.00
S ₂ B ₂	14.67 ijk	4.03
S ₂ B ₃	17.13 ijk	4.04
S ₃ B ₀	18.00 efg	3.99
S ₃ B ₁	22.00 c	4.01
S ₃ B ₂	24.33 b	4.02
S ₃ B ₃	28.33 a	4.03
S ₄ B ₀	15.33 hij	3.99
S ₄ B ₁	18.67 def	4.01
S ₄ B ₂	20.00 cde	4.02
S ₄ B ₃	24.67 b	4.03
LSD _(0.05)	2.10	NS
CV (%)	6.86	8.95

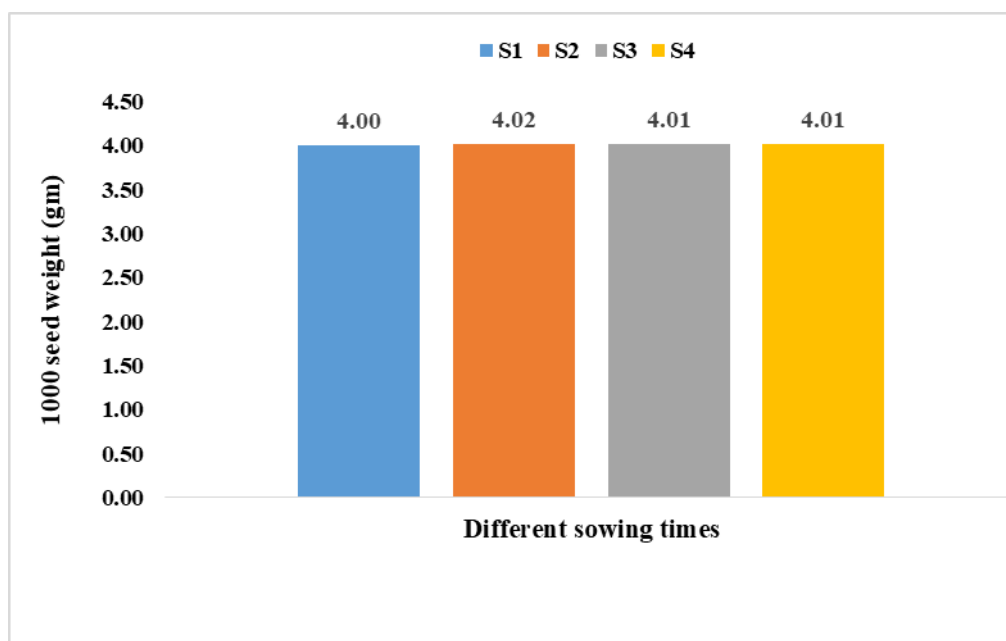
S₁ = 03 November, S₂ = 08 November, S₃ = 13 November and S₄ = 18 November, B₀ = Control, B₁ = 0.2mM Salicylic, B₂ = 40 ppm Chitosan, B₃ = 0.2mM Salicylic acid + 40 ppm Chitosan and NS= Not significant

4.2.4 Weight of 1000 seed

i. Effect of sowing times

Different sowing times showed no significant effect on 1000 seed weight of mustard (Appendix IX and Figure 17). Numerically the highest 1000 seed weight (4.02 gm) was found in 8 November sowing time and lowest was found in 3 November sowing

time. Lakra *et al.*, (2018) confirmed that in mustard there is not any significant difference on 1000 seed weight of same variety for different sowing times.

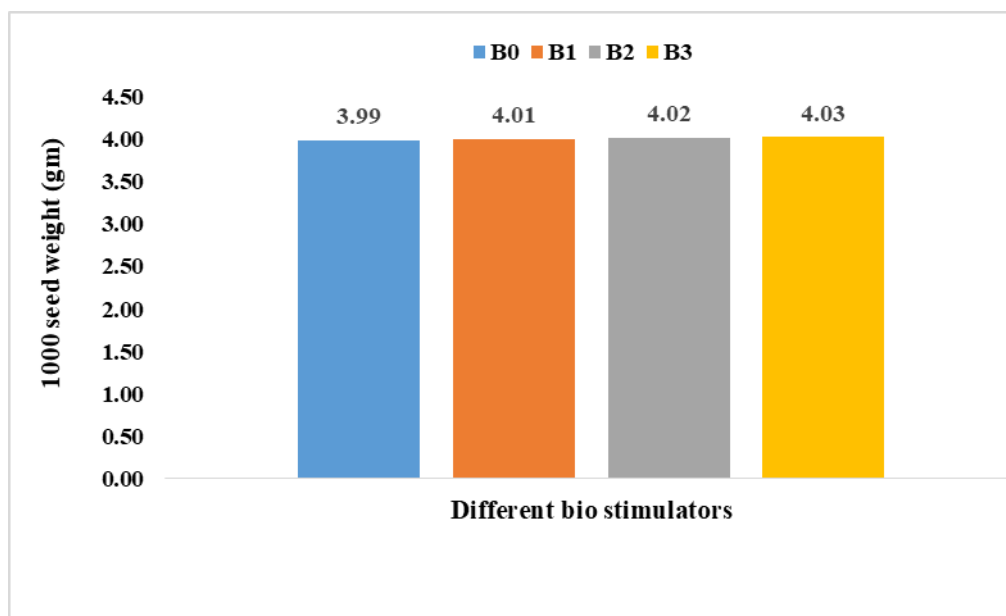


S₁ = 03 November, S₂ = 08 November, S₃ = 13 November and S₄ = 18 November.

Figure 17. Influence of sowing times on 1000 seed weight (gm) of mustard, BARI sarisha 14 (LSD value of 5% level of significant 1.23)

ii. Effect of biostimulators

Weight of 1000 seed weight was not also significantly influenced by different biostimulators: Salicylic acid and Chitosan spray (Appendix IX and Figure 18). The highest 1000 seed weight (4.03 gm) was found from combined application of 0.2 mM salicylic acid and 40 ppm chitosan and lowest was found in control condition.



B₀ = Control, B₁ = 0.2 mM Salicylic, B₂ = 40 ppm Chitosan and B₃ = 0.2 mM Salicylic acid + 40 ppm Chitosan

Figure 18. Influence of biostimulators on 1000 seed weight of mustard, BARI sarisha 14 (LSD value of 5% level of significant 1.23)

iii. Interaction effect between sowing times and biostimulators

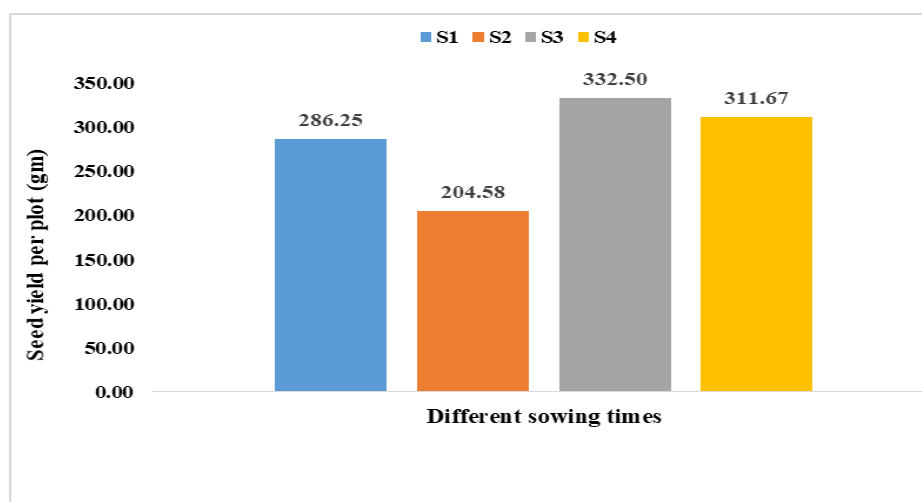
Interaction effect between sowing times and biostimulators was not found any significant in respect of 1000 seed weight of Mustard (Appendix IX and Table 6). The highest 1000 seed weight (4.04) was recorded in 8 November sowing time + mixture of 0.2 mM salicylic acid and 40 ppm chitosan and lowest weight was found in 8 November sowing time + control condition of biostimulators.

4.2.5 Seed yield per plot

i. Effect of sowing times

Seed yield was significantly influenced by the different sowing times (Appendix X and Figure 19). The highest seed yield per plot (332.50 g) was obtained from the 13 November sowing time. Seed yield is higher in 13 November sowing because in that date number of branch (Figure 9), number of siliqua (Figure 11), and number of seed per siliqua (Figure 15) were higher compare to other sowing time. The lowest seed yield per plot (204.58 gm) was found in 8 November sowing time. Singh and Singh (2002) reported the similar result. They found that middle November sowing of mustard can give highest number of seed per plant. Altogether, it is suggested that 13 November

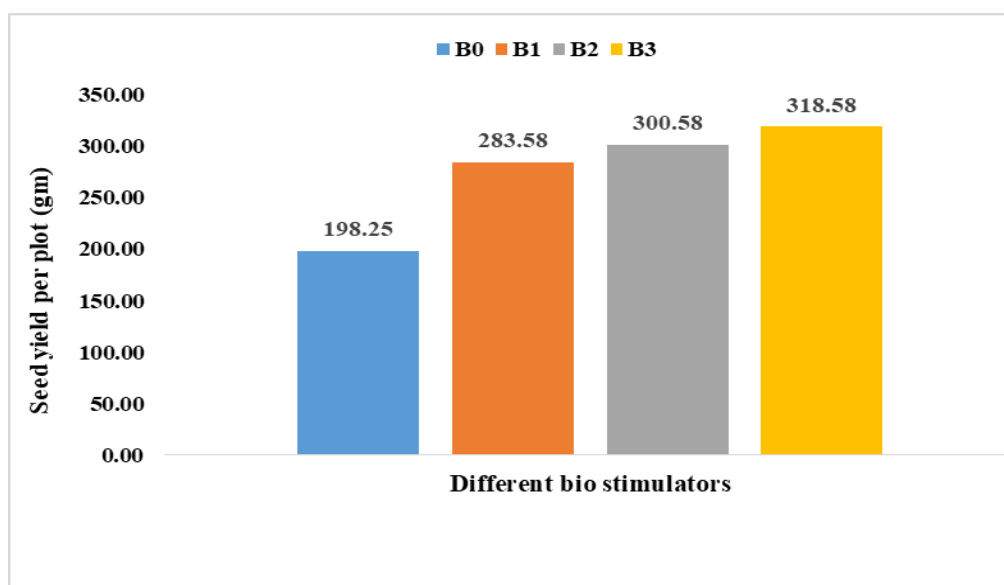
sowing and produced maximum seed yield/plot of mustard plant under the climatic and edaphic condition of SAU.



$S_1 = 03$ November, $S_2 = 08$ November, $S_3 = 13$ November and $S_4 = 18$ November.
Figure 19. Influence of sowing times on seed yield per plot of mustard, BARI sarisha 14 (LSD value of 5% level of significant 7.35)

ii. Effect of biostimulators

Different biostimulators: Salicylic acid and Chitosan has significant effect on seed yield per plot of Mustard (Appendix X and Figure 20).



$B_0 =$ Control, $B_1 = 0.2$ mM Salicylic, $B_2 = 40$ ppm Chitosan and $B_3 = 0.2$ mM Salicylic acid + 40 ppm Chitosan

Figure 20. Influence of biostimulators on seed yield per plot of BARI sarisha 14 (LSD value of 5% level of significant 7.35)

Combined application of 0.2 mM salicylic acid and 40 ppm chitosan produced significantly the highest seed yield per plot (318.58 g) and lowest seed yield per plot (198.25g) was found from control condition. These results are also correlated with other parameters of this study such as number of branch per plant (Figure 10), no of siliqua (Figure 12), siliqua length (Figure 14), number of seed per siliqua (Figure 16). Fariduddin *et al.* (2003) reported seed yield significantly enhanced in *Brassica juncea* at higher concentrations of salicylic acid sprayed. Ghoname *et al.* (2010) observed that foliar application of chitosan on crops increased significantly the number of seed per plant. Altogether, it is suggested that combined application salicylic acid and chitosan in 13 November 2017 produced the maximum seed/plot of mustard plant under the climatic and edaphic condition of SAU.

Table 7. Interaction effect between sowing times and biostimulators on seed yield per plot and yield (ton/ha) of BARI sarisha 14

Treatments	Seed yield per plot (g)	Yield (ton/ha)
S ₁ B ₀	255.00 g	1.13 g
S ₁ B ₁	290.00 e	1.29 e
S ₁ B ₂	300.00 de	1.33 de
S ₁ B ₃	300.00 de	1.33 de
S ₂ B ₀	202.67 i	0.90 i
S ₂ B ₁	215.00 i	0.96 i
S ₂ B ₂	238.33 h	1.06 h
S ₂ B ₃	254.33 g	1.13 g
S ₃ B ₀	291.67 e	1.30 e
S ₃ B ₁	321.67 c	1.43 c
S ₃ B ₂	343.33 b	1.53 b
S ₃ B ₃	373.33 a	1.66 a
S ₄ B ₀	271.67 f	1.21 f
S ₄ B ₁	307.67 cd	1.37 cd
S ₄ B ₂	320.67 c	1.43 c
S ₄ B ₃	346.67 b	1.54 b
LSD _(0.05)	15.39	.069
CV (%)	3.19	3.19

S₁=03November, S₂ = 08 November, S₃ = 13 November and S₄ = 18 November, B₀ = Control, B₁ = 0.2mM Salicylic, B₂ = 40 ppm Chitosan and B₃ = 0.2mM Salicylic acid + 40 ppm Chitosan

iii. Interaction effect between sowing times and biostimulators

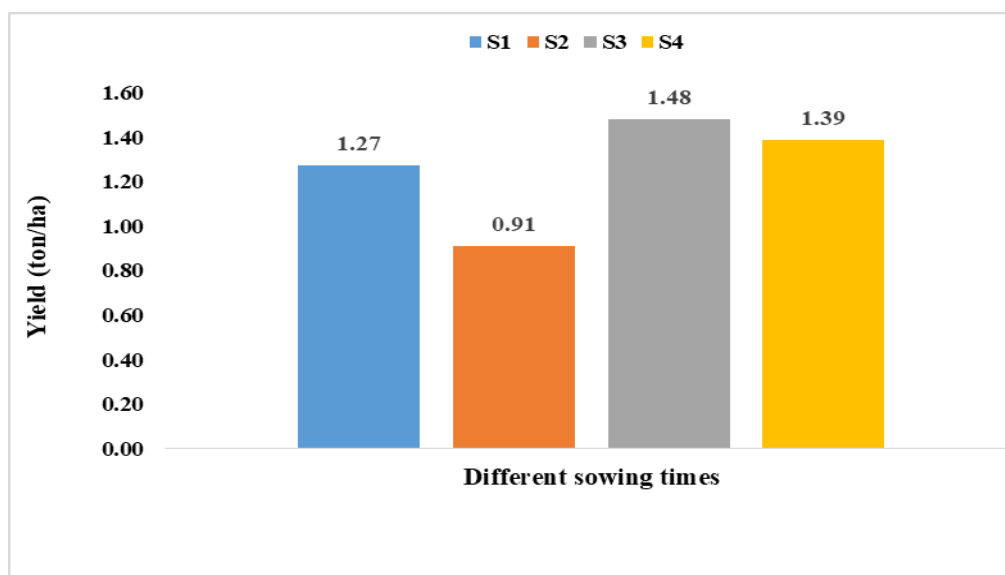
Interaction between sowing times and biostimulators played an important role for promoting the seed yield per plot. Yield was significantly influenced by the interaction effect (Appendix X and Table 7). Among the treatments, the highest seed yield per plot (373.33 g) was observed in 13 November sowing + mixture of .2 mM

salicylic acid and 40 ppm chitosan. The lowest yield (202.67 g) was observed in 8 November sowing + control condition that similar with 8 November sowing + 0.2 mM salicylic acid level. Muhal and Solanki (2015) at Udaipur to evaluate the effect of seeding dates and salicylic acid (SA) application on growth attributes, phenology and agro-meteorological indices of *Brassica* species and observed that number of days taken to attain physiological maturity, number of seed yield per plot was significantly higher under higher SA foliar spray compared to water spray. These results are also correlated with other parameters of this study such as number of branch per plant (Table 5), no of siliqua/plant(Table 5), siliqua length(Table 5), no of seed per siliqua(Table 6). Altogether, it is suggested that 13 November sowing+treatment combination of salicylic acid and chitosan produce the maximum yield per plot of mustard under the climatic and edaphic condition of SAU.

4.2.6 Seed yield ton hectare⁻¹

i. Effect of sowing times

Seed yield was significantly influenced by different sowing times (Appendix X and Figure 21).



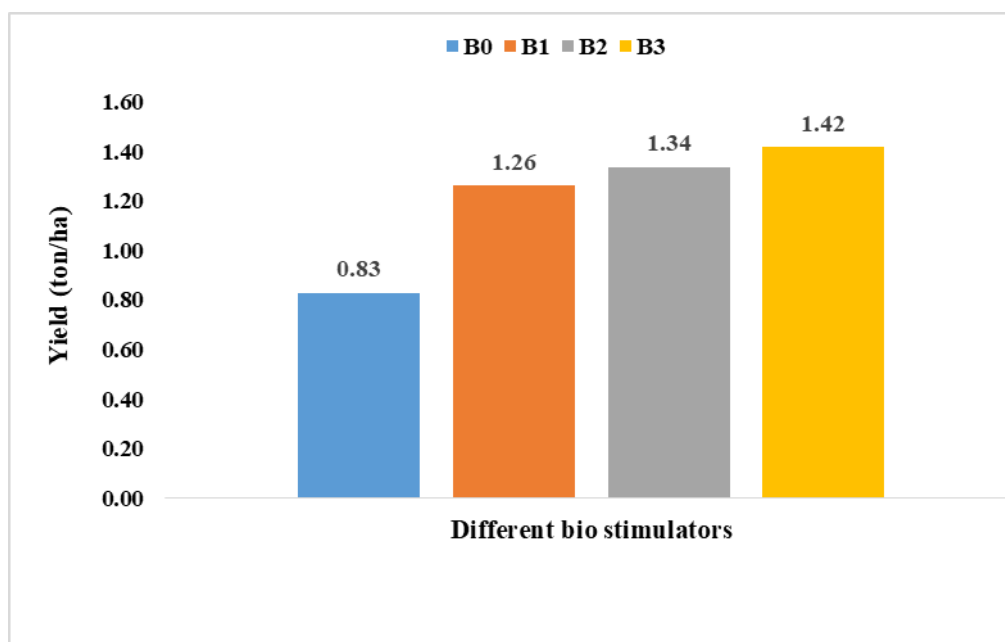
S₁ = 03 November, S₂ = 08 November, S₃ = 13 November and S₄ = 18 November.

Figure 21. Influence of sowing times on yield (ton/ha) of mustard, BARI sarisha 14 (LSD value of 5% level of significant 0.039)

The highest seed yield (1.48 ton/ha) was obtained from the 13 November sowing time and the lowest (0.91 ton/ha) was produced by 8 November sowing time. This result is the agreement of several findings. Shahidullah *et al.* (1997) reported that late sowing mustard faced more temperature and can give higher yield. Singh *et al.* (2002) observed that the accumulation of dry matter and its allocation of different plant parts was significantly reduced with early sowing owing to the prevalence of low day and night temperatures, high evening and morning RH and poor sunshine during the active vegetative growth phase led to poor growth and development of crop. These results are also correlated with the other parameters of this study such as number of branches per plant (Figure 9), number of siliqua per plant (Figure 11), siliqua length (Figure 13), number of seeds per siliqua (Figure 15), yield/plot (Figure 19). Altogether, it is suggested that 13 November sowing produces the maximum yield ton per hectare of mustard plant under the climatic and edaphic conditions of SAU.

ii. Effect of biostimulators

Different biostimulators: salicylic acid and chitosan had significant effects on the yield of mustard (Appendix X and Figure 22).



B₀ = Control, B₁ = 0.2 mM Salicylic, B₂ = 40 ppm Chitosan and B₃ = 0.2 mM Salicylic acid + 40 ppm Chitosan

Figure 22. Influence of biostimulators on yield (ton/ha) of mustard, BARI sarisha 14 (LSD value of 5% level of significant 0.039)

The highest yield (1.42 ton/ha) was found in combined application of 0.2 mM salicylic acid and 40 ppm chitosan and the lowest (0.83 ton/ha) yield was from control condition. Dat *et al.* (1998b) reported that spraying mustard (*Sinapis alba* L.) seedlings with salicylic acid (SA) solutions between significantly improved their tolerance to a subsequent heat shock and increase yield of seed. Bittelli *et al.* (2001) reported that foliar application of chitosan decreased transpiration in plants, and reduced water use while maintaining biomass production and yield. Chitosan enhance root and leaf area as well as the level of chlorophylls and improved seed yield. These results are also correlated with the other parameters of this study such as no of branch (Figure 10), number of siliqua (Figure 12), siliqua length (Figure 14), no of seed/siliqua (Figure 12), yield/plot (Figure 20). Altogether, it is suggested that combined application salicylic acid and chitosan of 13 November 2017 produce the maximum yield ton/ha of mustard under the climatic and edaphic condition of SAU.

iii. Interaction effect between sowing times and biostimulators

Interaction between sowing times and biostimulators played an important role for promoting the yield of mustard. Yield was significantly influenced by the interaction effect (Appendix X and Table 7). Among the treatments, the highest yield (1.66 ton/ha) was observed in 13 November sowing + combined application of 0.2 mM salicylic acid and 40 ppm chitosan. The lowest yield (0.90 ton/ha) was observed in 8 November sowing + control condition. A field experiment was conducted by Muhal *et al.* (2014) to evaluate the effect of planting duration and salicylic acid application on yield, quality and nutrient uptake of *Brassica* species. The result revealed that foliar application of salicylic acid produced significantly higher seed yield at middle sowing of November. These results are also correlated with other parameters of this study such as number of branch per plant (Table 5), number of siliqua/plant (Table 5), siliqua length (Table 5), number of seed per siliqua (Table 6), seed yield/plot (Table 7). Altogether, it is suggested that 13 November sowing+combined application of salicylic acid and chitosan produce the maximum yield ton per hectare of mustard under the climatic and edaphic condition of SAU.

CHAPTER V

SUMMARY AND CONCLUSION

The field experiment was conducted at the Agricultural Farm of Sher-e-Bangla Agricultural University (SAU), Dhaka, during the period from November 2017 to February 2018 to morphological, yield contributing characters and yield in mustard by observing growth and yield with different sowing times and biostimulators under the Modhupur Tract (AEZ-28). The experiment consisted of two factors as four different sowing times *viz.*, 03 November (S₁), 08 November (S₂), 13 November (S₃), 18 November (S₄) and four bio stimulators *viz.*, Control (B₀), 0.2 mM salicylic acid (B₁), 40 ppm chitosan (B₂) and 0.2 mM salicylic acid + 40 ppm chitosan (B₃). The experiment was laid out in Randomized Complete Block design (RCBD) with three replications.

The data on crop growth parameters like plant height, number of leaves plant⁻¹, leaf length, leaf breath and number of branch were recorded at different days after sowing. Yield parameters like total number of siliqua plant⁻¹, siliqua length, number of seed siliqua⁻¹, 1000 seed weight, seed yield plot⁻¹ and seed yield (ton/ha) were recorded at harvest. Data were analyzed using MSTAT-C package. The mean differences among the treatments were compared by least significant difference test at 5% level of significance.

Results showed that different sowing times significant effect on growth and yield parameters except weight of 1000 seed. The rapid increase of plant height was observed from 50 to 65 DAT of growth stages which was higher in the 13 November sowing time compared to the other time. However, at 40 and 70 DAS, 13 November sowing produced the tallest plant (29.96 cm and 71.75 cm respectively) and the lowest plant height was observed in 8 November sowing time (17.00 cm and 46.00 cm respectively). The higher number of leaf at all the growth stages was found in 13 November sowing. Again, the higher number of branches plant⁻¹ (6.90) was found also 13 November sowing and lowest number of of branches plant⁻¹ (3.93) was found at 8 November sowing. The highest number of siliqua per plant, siliqua length, seed yield per plot and yield (ton/ha) were obtained from 13 November sowing time and lowest were from 3 November sowing.

Biostimulators: salicylic acid and chitosan also significantly influenced growth and yield attributes except weight of 1000 seed. The results revealed that biostimulators 0.2 mM salicylic acid along with 40 ppm chitosan produced the tallest plant height, higher number of leaf per plant, leaf length and leaf breath at all the growth stages and control condition produced the smallest plant height, less number of leaf per plant, less leaf length and less leaf breath. The highest number of branches per plant, number of siliqua per plant, siliqua length, seed yield per plant and yield (ton/ha) were obtained from biostimulators 0.2 mM salicylic acid along with 40 ppm chitosan and lowest were from control condition.

Interaction effect between sowing times and biostimulators also significantly affected all parameters of growth and yield except 1000 seed weight. The tallest plant height was found in 13 November sowing and biostimulators 0.2 mM salicylic acid along with 40 ppm chitosan at 40, 55 and 70 DAS. The lowest plant height was observed in 8 November sowing and control condition at 40, 55 and 70 DAS. The highest number of leaf, leaf length and leaf breath were found from 13 November sowing and biostimulators 0.2 mM salicylic acid along with 40 ppm chitosan and lowest were 8 November sowing along with control condition for number of leaf at 40 and 55 DAS, leaf length at 55 DAS and leaf breath at all stage. The highest number of siliqua per plant was observed from 13 November sowing and biostimulators 0.2 mM salicylic acid along with 40 ppm chitosan and lowest from 8 November sowing along with control condition. The maximum number of branches per plant, siliqua length, seed yield per plot and yield (ton/ha) was found from 13 November sowing and biostimulators 0.2 mM salicylic acid and 40 ppm chitosan and lowest from 8 November sowing along with control condition for number of branch, siliqua length, seed yield per plot and yield (ton/ha). For number of seed per siliqua the lowest number was found from 8 November sowing along with control condition.

Based on the results of the present study, the following conclusions may be drawn-

- The highest number of seed and yield was observed in 13 November sowing time.

- The use of biostimulators: 0.2 mM salicylic acid along with 40 ppm chitosan gave the highest number of seed and yield.
- For interaction the highest seed yield and better yield contributing characters were observed in 13 November sowing time and application of 0.2 mM salicylic acid along with 40 ppm chitosan.

However, to reach a specific conclusion and recommendation the same experiment need to be repeated and the advanced research need to conduct to find the morphological mechanisms as a role of biostimulators for improving the seed yield of mustard to different sowing times.

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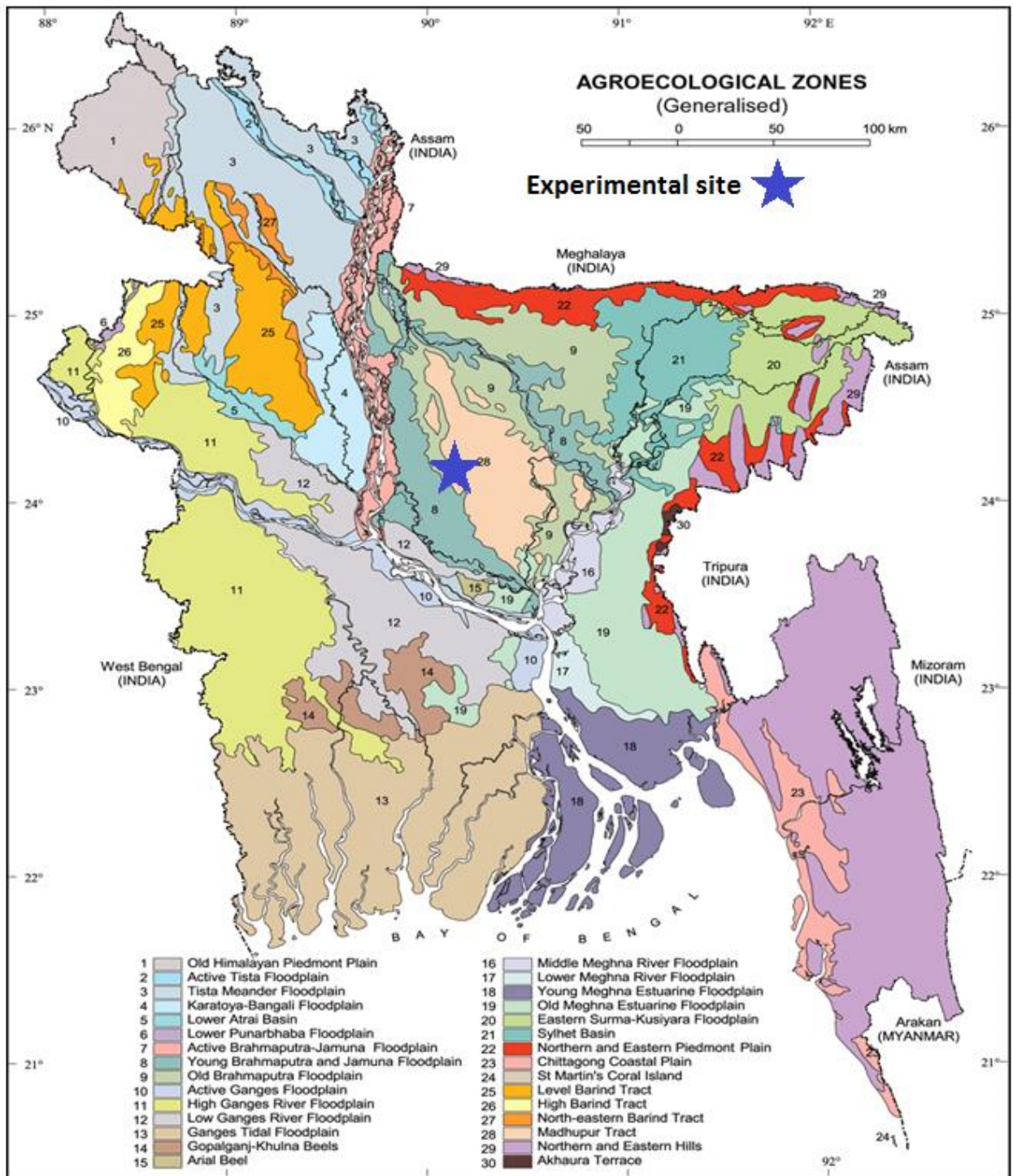
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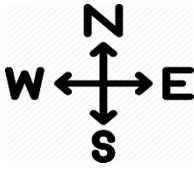
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APPENDICES

Appendix I. Map showing the experimental site under study



Appendix II. Layout of the Experiment



Plot size = $(1.8 \times 1.5) \text{ m}^2$
 = 2.25 m^2

Factors A:

(Sowing times)

$S_1=1^{\text{st}}$ sowing

$S_2=2^{\text{nd}}$ sowing

$S_3=3^{\text{rd}}$ sowing

$S_4=4^{\text{th}}$ sowing

Factors B:

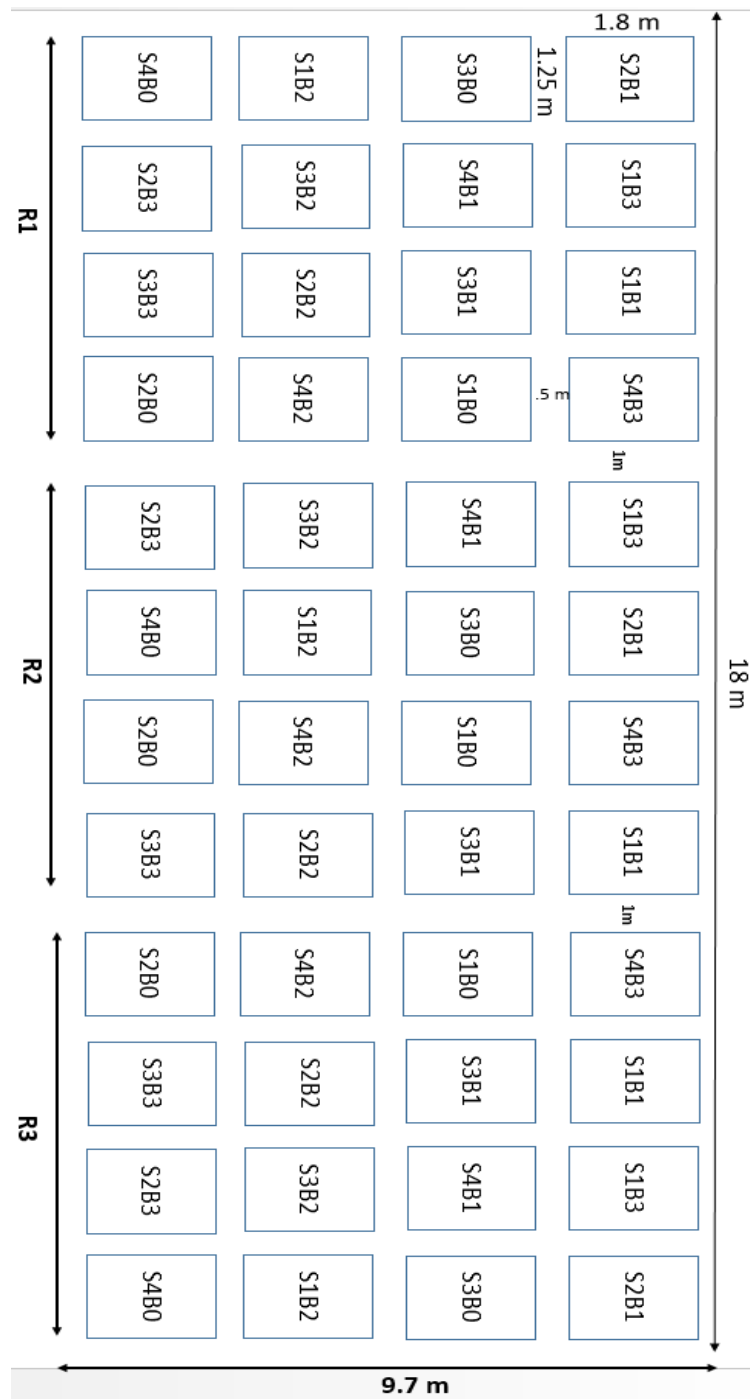
(Biostimulators)

B_0 =Control

$B_1=0.2\text{mM}$ Salicylic acid

$B_2=40$ ppm Chitosan

$B_3=0.2\text{mM}$ Salicylic acid
 + 40 ppm Chitosan



Appendix III. Monthly average of air temperature, relative humidity and total rainfall of the experimental site during the period from November 2017 to March 2018

Months	Maximum temperature (c ⁰)	Minimum temperature (c ⁰)	Relative humidity at 12 pm (%)	Rainfall (mm)
November 2017	28.60	8.52	56.75	14.40
December 2017	25.50	6.70	54.80	0.0
January 2018	23.80	11.70	46.20	0.0
February 2018	22.75	14.26	37.90	0.0
March 2018	35.20	21.00	52.44	20.4

Source: Bangladesh Meteorological Department (Climate and weather division) Agargaon, Dhaka

Appendix IV. Mean square values for Plant Height of mustard, BARI sarisha 14 at different days after sowing

Source of variation	Degrees of freedom	Plant Height of mustard, BARI sarisha 14 at		
		40 DAT	55 DAT	70 DAT
Replication	2	4.00	24.94	59.52
Sowing times	3	345.23*	830.83*	1474.69*
Biostimulators	3	117.49*	461.00*	534.91*
Interaction	9	10.56*	16.02*	6.15*
Error	30	2.58	4.05	8.21

*Significant at 5 % level

Appendix V. Mean square values for number of leaf of mustard, BARI sarisha 14 at different days after sowing

Source of variation	Degrees of freedom	Number of leaf of mustard, BARI sarisha 14 at	
		40 DAT	55 DAT
Replication	2	0.12	5.07
Sowing times	3	28.28*	115.10*
Biostimulators	3	14.75*	125.06*
Interaction	9	0.99*	4.19*
Error	30	0.20	2.09

*Significant at 5 % level

Appendix VI. Mean square values for leaf length of mustard, BARI sarisha 14 at different days after sowing

Source of variation	Degrees of freedom	Leaf length of mustard, BARI sarisha 14 at	
		40 DAT	55 DAT
Replication	2	3.103	4.848
Sowing times	3	6.152*	2.513*
Biostimulators	3	1.662*	3.491*
Interaction	9	0.740*	0.115*
Error	30	0.709	0.043

*Significant at 5 % level

Appendix VII. Mean square values for leaf breath of mustard, BARI sarisha 14 at different days after sowing

Source of variation	Degrees of freedom	Leaf breath of mustard, BARI sarisha 14 at	
		40 DAT	55 DAT
Replication	2	0.727	0.313
Sowing times	3	0.527*	0.344
Biostimulators	3	0.722*	6.693*
Interaction	9	0.063*	0.006*
Error	30	0.071	0.009

*Significant at 5 % level

Appendix VIII. Mean square values for number of branch, number of siliqua and length of siliqua of mustard, BARI sarisha 14

Source of variation	Degrees of freedom	Number of branch	Number of siliqua	Siliqua length
Replication	2	0.60	12.74	0.29
Sowing times	3	21.08*	1533.75*	14.65*
Biostimulators	3	4.24*	518.39*	3.58*
Interaction	9	0.31*	9.95*	0.04*
Error	30	0.04	7.257	0.02

*Significant at 5 % level

Appendix IX. Mean square values for seed per siliqua and 1000 seed weight of mustard, BARI sarisha 14

Source of variation	Degrees of freedom	Seed per siliqua	1000 seed weight
Replication	2	1.13	1.02
Sowing times	3	172.57*	6.97
Biostimulators	3	122.95*	2.34
Interaction	9	4.48*	3.35
Error	30	1.60	2.64

*Significant at 5 % level

Appendix X. Mean square values for seed yield per plot and seed yield (ton/ha) of mustard, BARI sarisha 14

Source of variation	Degrees of freedom	Seed per plot (g)	Yield (ton/ha)
Replication	2	225.20	0.004
Sowing times	3	24738.40*	0.489*
Biostimulators	3	8707.00*	0.172*
Interaction	9	254.50*	0.005*
Error	30	85.10	0.002

*Significant at 5 % level