

ISSN : 1812-5379 (Print)
ISSN : 1812-5417 (Online)
<http://ansijournals.com/ja>

J O U R N A L O F
AGRONOMY



ANSI*net*

Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Timing of GA₃ Application to Indian Mustard (*Brassica juncea* L.): Dry Matter Distribution, Growth Analysis and Nutrient Uptake

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Abstract: A field study was conducted to evaluate the effects of timing [single spray at pre-anthesis (T₁) and at anthesis (T₂) and double spray at pre- + at anthesis (T₃)] and concentration (0, 10⁻⁶, 10⁻⁵, 10⁻⁴ M) of exogenous application of GA₃ on growth, dry matter production, nutrient uptake and yield attributes of Indian mustard (*Brassica juncea* L. Czern and Coss) cv. Varuna. Depression of flowering and sink strength is associated with lower endogenous level of gibberellic acid. Therefore, it was hypothesized that foliar application of GA₃ will enhance the flower number and create a balance between source and sink. The impact of GA₃ application at T₁ was most conspicuous and resulted in a higher growth, efficient translocation and utilization of nutrients although; T₃ was equally effective but is not preferable as it requires the spray at two time intervals. Among different concentrations of GA₃, 10⁻⁵ M registered the maximum values for all the parameters studied. GA₃ increased partitioning of biomass to the leaves at the expense of appropriation to the stem at 20 DAF. In this way, an appreciation of the timing of foliar application of GA₃ can be used to manage the resources for maximum production of Indian mustard.

Key words: Gibberellic acid, *Brassica*, dry matter partitioning, nutrient uptake, source-sink relationship, growth ratio

INTRODUCTION

Remarkable accomplishments of Plant Growth Regulators (PGR) such as manipulating plant developments, enhancing yield and quality have been actualized in recent years using new emerging and efficient plant growth regulators. It has long been ascertained that plant hormones including auxins, gibberellic acid, cytokinin, abscisic acid and ethylene are involved in controlling developmental events such as cell division, cell elongation and protein synthesis. PGRs have been implicated in efficient utilization of nutrients and translocation of photo-assimilates in established sink-source system (Khan *et al.*, 1996; Patrick and Steains, 1987; Pereto and Beltran, 1987)

Several factors either endogenous or environmental contribute to sink strength but sink activity can mainly be enhanced by gibberellins (Kuiper, 1993). Extensive studies have demonstrated that Gibberellic Acid (GA₃) has potential to enhance growth, flowering, photosynthesis,

nutrient transport and yielding ability of mustard (Hayat *et al.*, 2001; Khan *et al.*, 1996, 2005). Precise timing of plant activities and the adaptive morphological modulations can only be realized if plants perceive signals of its direct environments. In comparison to the large number of studies on the foliar application of PGRs, much less effort has been applied to understanding how this exogenous application of PGR (GA₃) may elicit change in the allocation pattern with the leaf age. For the *Brassica juncea*, we suggest that ontogenetic differences in leaf age will differently mediate the reception of gibberellic acid signals (a mediator of sink-strength). To test this hypothesis, we applied single foliar spray of 0, 10⁻⁶, 10⁻⁵, 10⁻⁴M GA₃ at 40 (pre-anthesis) and 60 (at anthesis) DAS, or double spray in equal doses of 0, 10⁻¹², 10⁻¹⁰, 10⁻⁸M GA₃ both at 40 and 60 Days after Sowing (DAS) and studied the pattern of growth, dry matter partitioning, growth ratios, nutrient uptake and yield attributes of Indian mustard (*Brassica juncea* L.) cv. Varuna.

MATERIALS AND METHODS

Experiments were conducted at the Experimental Farm of Aligarh Muslim University; Aligarh, India (situated at 27°52' N latitude, 78°51' E longitude and 187.45 m altitude above sea level). It has a semiarid and subtropical weather with severest hot dry summers and intense cold winters. The mean annual rainfall is about 847.3 mm. More than 85% of the total downpour is delivered during a short span of four months from June to September.

Soil characteristics: Arbitrary samples of soil were collected from various chosen spots, upto depth of 15 cm, spread over the entire experimental field before sowing of the experimental crops and analyzed for physico-chemical characteristics of the soil. Data obtained on chemical characteristics and physical constant for soil are presented in Table 1.

Experimental layout, preparatory tillage and cultural operations: The field experiments were laid out in randomized block design with three replications for each treatment. The individual plot size was 10 m² (2×5 m). Prior to each trial, diligent ploughing of fields was done to turn the soil for maximum aeration and weed eradication. The plots were made with proper bunds along with necessary irrigation channels and then irrigated lightly before sowing to maintain proper moisture in the sub-surface of the soil. The seeds were sown by the usual behind the plough method at the rate of 10 kg ha⁻¹. Rows were separated by a distance of 30 cm while the plants in the row were 15 cm apart. After the establishment of the crop i.e., after 12 days of sowing seedlings were thinned to maintain the uniformity (approximately 22 plants m⁻²) of the plant population. Crop was irrigated prior to sowing

and subsequently, when ever found essential. There were two irrigations during the entire growth period of the crop.

Homogenous broadcasting of 80 kg N, 30 kg P and 30 kg K ha⁻¹ was applied to the soil at the time of leveling of the individual plot. The sources of nutrients were urea, Single Super Phosphate (SSP) and Muriate of Potash (MOP). Exogenous application of gibberellic acid was made at pre-anthesis (40 days after sowing) and at post-anthesis (60 days after sowing). The scheme of the treatments is given in Table 2. GA₃ was sprayed at the rate of 600 L ha⁻¹ together with 0.5% teepol (a surfactant). In control set, equal amount of de-ionized water with 0.5% teepol was sprayed simultaneously with the treatment.

Determination of growth variables and nutrient uptake: Plants were harvested by cutting at the ground level and were allowed for sun drying. After sun drying, threshing was done. Seeds were cleared and collected for seed yield. The observations were carried out at 20 days interval from 80 DAS till harvest (120 DAS). There were total three samplings, i.e., at 80, 100 and 120 DAS. Five plants from each plot were cut at the soil level at various sampling stages for analysis of different growth parameters using suitable covenant and nutrient concentrations and their accumulation in plants. At harvest (120 DAS), twenty five plants (equivalent to 1 m² land area) were removed to record the yield attributes.

Sampled plants were divided into different parts, like leaf, stem and pod corresponding to different sampling stages and were dried in hot air oven at 80°C for two days. The dried materials were weighed on physical balance and weight was recorded as dry weight. Leaf area was ascertained by gravimetric method. For analysis of growth ratio, crop growth rate (Watson, 1952), relative growth rate (Radford, 1967) and net assimilation rate (Milthorpe and Moorby, 1979) was calculated. Specific Leaf Area (SLA) and Specific Leaf Weight (SLW) was calculated according to Hunt (1978). Content of nitrogen (Lindner, 1944) and phosphorus (Fiske and Subba Row, 1925) was determined by the Kjeldahl method while content of potassium was estimated with the help of flame photometer. Uptake of nitrogen, phosphorus and potassium was calculated as the product of N, P and K content and their respective dry matter at these stages.

Table 1: Physico-chemical characteristics of the soil

Characteristics	Mean±Standard Error
Texture	Sandy loam
pH (1:2)	8.0±0.4
E.C. (1:2) (m mhos cm ⁻¹)	0.46±0.02
Available nitrogen (kg ha ⁻¹)	205±19
Available phosphorus (kg ha ⁻¹)	26.0±1.4
Available potassium (kg ha ⁻¹)	214±16
Available sulphur (kg ha ⁻¹)	19.3±1.25

Data are the means of 5 samples

Table 2: Scheme of treatments

Spray treatments	Spray stages (days after sowing)											
	40 (Pre-anthesis) [T ₁]				60 (At-anthesis) [T ₂]				40+60 (Pre+ at-) anthesis [T ₃]			
	0	10 ⁻⁶	10 ⁻⁵	10 ⁻⁴	0	10 ⁻⁶	10 ⁻⁵	10 ⁻⁴	0	10 ⁻¹²	10 ⁻¹⁰	10 ⁻⁸
Water (control)	+	-	-	-	+	-	-	-	+	-	-	-
GA ₃	+	+	+	+	+	+	+	+	+	+	+	+

+: Present, -: Absent

RESULTS AND DISCUSSION

Growth parameters: Table 3 shows that timing and concentration of GA₃ spray significantly affected all the growth parameters at 20 days after flowering. Dry weight of a plant represents a successful manifestation of a complex processes ranging from interception of solar radiation, availability of nutrients and water and active photosynthesizing area to hormonal status of the crop. Maximum above Ground Dry Matter (AGDM) was attained in 10⁻⁵ M GA₃ in pre-anthesis spray treatment (T₁) which was equal in effect of the double foliar spray (T₃) i.e., 40+60 DAS. The spray of this concentration at pre-anthesis (T₁) produced 22.5% more AGDM than the plants sprayed at anthesis (T₂). Significant differences were noted in the GA₃ concentration effect in T₁ and T₃ treatments, but in T₂ all the concentrations were equal in effect. The spray concentration of 10⁻⁵ M GA₃ recorded 34.3, 13.2 and 47.2% more AGDM than control in treatments T₁, T₂ and T₃, respectively. Exogenous application of GA₃ produced considerable increase in dry weight of rice (Singh, 1996) and mustard (Khan *et al.*, 1996). Plant height was significantly lower in treatment T₃ than T₁ and T₂ which were equal in effect. The concentration effect of GA₃ at each spray application showed a similar trend of increase over control. The increase in plant height was 19.3, 29.0 and 34.0% in T₁, 14.2, 29.6 and 26.5% in T₂ and 19.1, 26.1 and 33.8% in T₃ in 10⁻⁶ M, 10⁻⁵ M and 10⁻⁴ M GA₃ in comparison to control, respectively. Treatment with GA₃ causes microtubule reorientation favoring axial elongation (Shibaoka, 1994). The enzyme Xyloglucan Endotransglycosylase (XET) catalyzes the breaking and reforming of bonds between xyloglucan residues, thus

permitting transient increase in wall extensibility. Increase in XET activity is correlated with GA enhanced elongation in number of plant species (Potter and Fry, 1994). Possibly all these factors contributed for the increase in the plant height due to GA₃ treatment. Several investigators have reported GA₃ induce increase in plant height in rice varieties (Singh, 1996), *Steria anceps* (Carrer *et al.*, 1997) and flax (El- Shourbagy *et al.*, 1994).

Leaf area was significantly higher for T₁ (20.8%) and T₃ (25.8%) than T₂. Increase in the plant height, in the treatment T₁ and T₃, at early stage enhanced the opportunity for the formation of more leaf initials which may later developed into leaf increasing the total leaf area. Spray concentration of 10⁻⁴ M GA₃ at T₃ gave maximum leaf area which was equal in effect to that of 10⁻⁵ M and 10⁻⁴ M GA₃ at spray time T₂. The increase in the leaf area of GA₃ applied plants could be attributed to the increase in the phyllochron. In *Aegilops caudata* and *Aegilops tauschii*, addition of GA₃ increased the phyllochron as observed by Bultynck and Lambers (2004). The partitioning of dry matter to leaf area is an important determinant of plant growth rate during early phases of development (Nelson, 1988). GA₃ treatment at T₁ and T₃ had lower specific leaf area (leaf area/ leaf weight) than T₂. SLA was 11.2, 15.5 and 15.1% in T₁ and 11.9, 15.4 and 15.0% in T₃, less in 10⁻⁶ M, 10⁻⁵ M and 10⁻⁴ M GA₃ than water spray control. Dijkstra *et al.* (1990) recorded an increase in SLA of a slow-growing inbred line of *Plantago major* supplied with GA₃. Specific leaf weight (leaf weight/leaf area) was unaffected by the spray time T₃ in spite of large response in AGDM suggesting that leaf thickness was not altered. Allocation pattern of dry weight in the leaves (SLW) was similar for the plants received treatments at spray time T₁ and T₃.

Table 3: Growth parameters of Indian mustard (*Brassica juncea* L.) cv. Varuna as influenced by the timing and concentration of exogenous GA₃ application at 20 Days After Flowering (DAF)

Spray stages	GA ₃ Conc. (Molar)	Parameters				
		AGDM (g plant ⁻¹)	Plant height (cm)	Leaf area (cm ²)	SLA (cm ² g ⁻¹)	SLW (mg cm ⁻²)
T ₁	0	25.09	47.6	2020	258	3.9
	10 ⁻⁶	28.79	56.8	2288	229	4.38
	10 ⁻⁵	33.69	61.4	2618	218	4.59
	10 ⁻⁴	32.26	63.8	2414	219	4.58
T ₂	0	24.31	45.2	2008	254	3.93
	10 ⁻⁶	24.81	51.6	2001	258	3.89
	10 ⁻⁵	27.51	58.6	2168	245	4.08
	10 ⁻⁴	27.19	57.2	2149	247	4.05
T ₃	0	23.53	46.7	1992	253	3.96
	10 ⁻⁶	32.80	55.6	2636	223	4.52
	10 ⁻⁵	34.64	58.9	2727	214	4.72
	10 ⁻⁴	35.35	62.5	2823	215	4.67
LSD (0.05)						
Spray stages (T)		1.68	3.1	339	12.2	0.23
Concentration (C)		1.94	3.6	376	14.0	0.27
Interaction (T×C)		2.31	NS	NS	NS	NS

Table 4: Distribution of dry matter in to leaf, stem and pod (%) as influenced by the timing and concentration of exogenous GA₃ application at 20 (pod-initiation) and 60 (pod-fill) Days After Flowering (DAF) in Indian mustard (*Brassica juncea* L.) cv. Varuna

Spray stages	GA ₃ Conc. (Molar)	Sampling days					
		20 DAF			60 DAF		
		Dry matter (%)					
		Leaf	Stem	Pod	Leaf	Stem	Pod
T ₁	0	31.33	59.48	9.18	8.48	61.85	29.67
	10 ⁻⁶	34.06	54.90	11.04	9.45	58.67	31.89
	10 ⁻⁵	34.95	53.65	11.39	7.23	60.14	32.62
	10 ⁻⁴	34.51	54.26	11.23	8.34	59.10	32.56
T ₂	0	32.34	58.38	9.28	8.86	61.33	29.81
	10 ⁻⁶	31.33	58.54	10.16	9.18	61.87	28.95
	10 ⁻⁵	32.08	57.42	10.50	7.27	63.09	29.69
	10 ⁻⁴	31.99	57.68	10.34	8.34	62.06	29.6
T ₃	0	33.35	57.28	9.38	9.24	60.8	29.95
	10 ⁻⁶	36.06	52.62	11.32	11.74	57.25	31.01
	10 ⁻⁵	36.83	51.46	11.71	9.76	58.46	31.79
	10 ⁻⁴	37.07	51.42	11.51	11.01	57.23	31.77
LSD (0.05)							
Spray stages (T)		2.21	2.55	0.51	1.00	1.33	1.19
Concentration (C)		NS	2.94	0.58	1.15	1.53	1.40
Interaction (T×C)		NS	NS	NS	NS	NS	NS

Percent distribution of dry weight, number of flower and pod:

Table 4 shows the effects of GA₃ concentrations and timing of exogenous application on the pattern of biomass allocation at pod-initiation (20 DAF) and pod-maturity (60 DAF). The pattern of distribution of dry weight among different plant parts was strongly influenced by timing of GA₃ treatment while concentration was non significant. The contribution of leaf dry weight to the total plant dry weight was 33.7, 31.9 and 35.8% at spray time T₁, T₂ and T₃, respectively at pod-initiation phase. This was declined to 8.0-11.0% during pod-maturation phase of the crop. Interestingly, the period of decline in the specific leaf weight coincides with an increase in the pod dry weight. This was evident from the contribution of pod dry weight to the total dry weight which showed a steady increase from meager 9.0-10.0% at 20 DAF to 30.0-33.0% at maturity (60 DAF). It was found that GA₃ has changed the pattern of assimilate distribution and more assimilates were translocated to reproductive parts of the GA₃ treated plants from the source (leaves) organs. GA₃ directed mass movement of photosynthetic materials was also evident from the distribution of dry weight towards stem. In this case, per cent stem dry weight of water sprayed treatments were found to be much higher than GA₃ treated plants. This was due to the reduced translocation of photo-assimilates towards leaves and pods in water sprayed plants.

Number of flowers and pods were significantly responsive towards timing of spray application (Fig. 1 A and B). The setting of flower to pods were remarkably increased in the GA₃ treated plants at spray time T₁, but spray time T₂ recorded the lowest pod

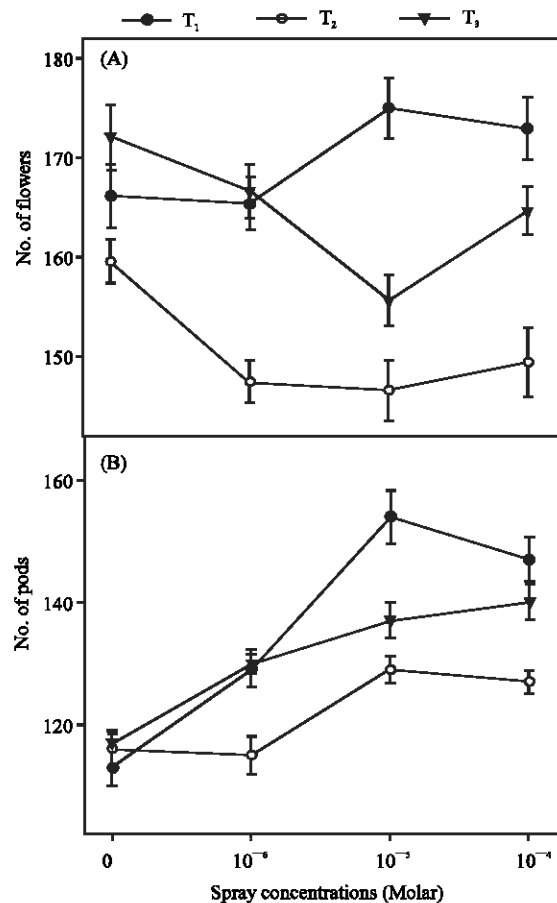


Fig. 1: Effect of timing and concentrations of GA₃ on number of flowers (A) at anthesis (20 DAF) and number of pods (B) at maturity (60 DAF) of Indian mustard (*Brassica juncea* L.) cv. Varuna. Vertical bars indicate ±SE (n = 5)

number even at higher concentration of GA₃. The spray at T₁ (pre-anthesis) and at T₃ (pre- + post anthesis) checked the abortion of flower which finally enhanced the chance for the plants to increase its yield potential by increasing the number of pods. Several investigators have positively correlated the initiation of flower with GA₃ treatment and endogenous level (Chandler and Dean, 1994; Takahashi and Kaufmann, 1992).

Growth analysis: According to Lambers (1987), growth analysis is an important first step in an analysis of morphological, physiological, or biochemical factors determining relative growth rate. The results show that all the growth variables were strongly affected by the timing of spray application (Fig. 2A-F). Between 0-20 DAF, crop growth rate (CGR) was found to be highest in T₁ while T₂ and T₃ recorded maximum between 20-40 DAF and 40-60

DAF, respectively. GA₃ spray gave significantly lower CGR than water sprayed control between 20-40 DAF this was a period which coincided with pod-filling stage and GA₃ sprayed plant has invested all its resources to pods (Fig. 2A and B). Relative Growth Rate (RGR), was significantly higher at T₁ while others i.e., T₂ and T₃ were equal in response between 0-20 DAF. Rate of increase in dry mass per unit starting mass and time (RGR) was enhanced under the influence GA₃ in comparison to the water sprayed control when applied at T₁ and T₃ between 0-20, 20-40 and 40-60 DAF. RGR in water sprayed control did not vary between any sampling stages but between 20-40 and 40-60 DAF it decreased significantly (Fig. 2C and D). In the present experiment a reduction in SLA was consistently associated with a decrease in RGR and the two parameters were positively correlated underlining the significance of leaf expansion for dry

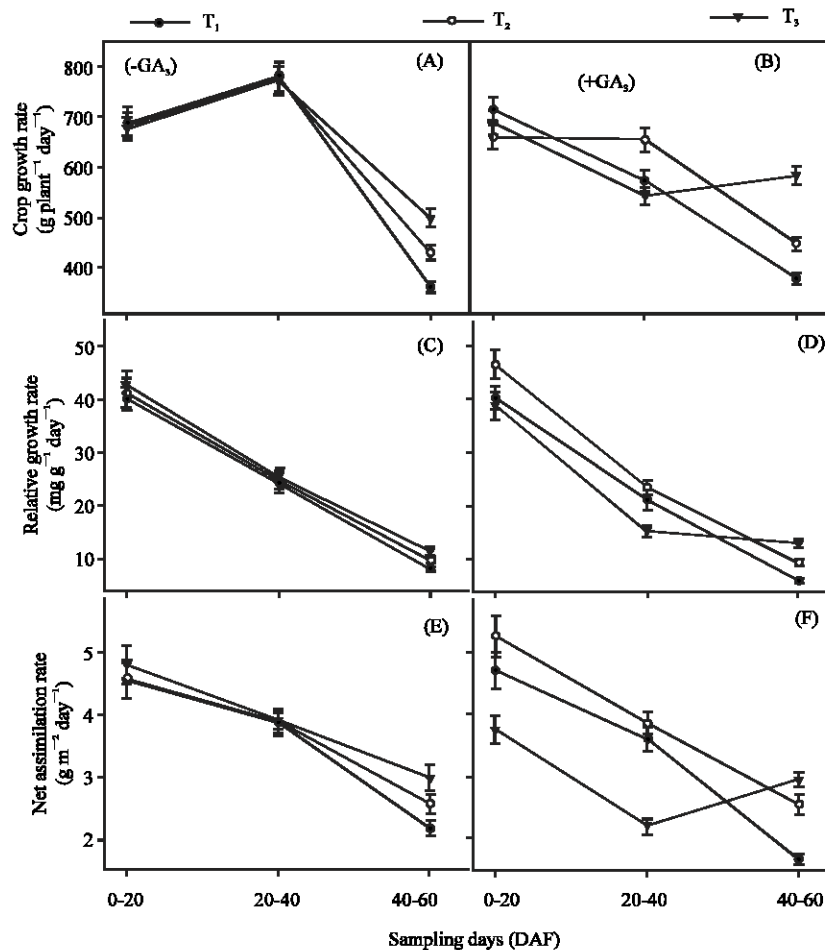


Fig. 2: Crop growth rate (A-B), relative growth rate (C-D) and net assimilation rate of Indian mustard (*Brassica juncea* L.) cv. Varuna, as influenced by timing of exogenous application of GA₃ at 20 days after flowering (20 DAF). Points are means of five replicates. Vertical bars indicate ±SE

matter accumulation. A positive relationship between SLA and RGR has also been reported by others (Lambers and Poorter, 1992; Poorter and Remkes, 1990; Reich *et al.*, 1998).

Net Assimilation Rate (NAR) is basically a function of photosynthesis particularly during the initial stage of growth. In GA₃ sprayed plants the rate of dry matter accumulation in a unit (NAR) was always higher in comparison to the water sprayed plants. This is consistent with the positive effect of GA on photosynthetic characteristics (Khan *et al.*, 1996, 2005). Significant differences were observed for the effect of timing of spray and T₃ was superior in effect between 0-20 DAF and 20-40 DAF while T₂ recorded maximum between 40-60 DAF (Fig. 2E and F). Contrary to our findings an increase in NAR was observed in low-GA mutant (Nagel *et al.*, 2001).

Nutrient uptake: Improving the use efficiency of applied nutrients to *Brassica* requires an understanding of the pattern of nutrient uptake by the crop during growth. The yield response of *Brassica* to applied nutrient will depend, in part, on the capacity of the crop to mobilize the nutrients from senescing vegetative organs and to translocate this to developing seeds. To achieve this, the crop should be manipulated in such a way as to utilize the maximum possible available resources. Present results on the pattern of uptake of nutrients showed that it was much more affected by the timing of spray at 20 DAF than at 60 DAF (Table 5). Pronounced effect of GA₃ was noted for the uptake of nitrogen, phosphorus and potassium in treatment T₁ and T₃ at 20 DAF. But the effect of spray stage T₃ on nutrient uptake was negligible. The difference in the nutrient uptake in response to spray timing remained as such until 60 DAF (pod-maturation) where T₃ recorded significantly lowest value. The observations in the

present experiment clearly manifest the importance of GA₃ application at pre-anthesis stage for enhanced uptake and better utilization of nutrients. Plant growth regulators control most of the characteristics of root systems, including primary root growth and the formation of lateral roots and root hairs. Many plant species respond to the exogenous application of auxins by producing large numbers of lateral roots and to auxins and ethylene by increasing the density and length of root hairs. In case of gibberellic acid, it is assumed that exogenous application of GA₃ at T₁ sends a signal to the roots for enhanced uptake of nutrients. Similar enhancement of nutrient uptake in response to GA₃ application was also noted in *Plantago major* (Kuiper and Saal, 1987) and *Brassica* (Khan *et al.*, 2005). However, application of GA₃ and IAA to flax (*Linum usitatissimum* L.) increased P, K and Ca accumulation in all plant organs (El-Shourbagy *et al.*, 1994).

Yield characteristics: Yield is the culmination of several comprehensive phases which starts at germination and end at harvest, encompassing through shoot growth, leaf development, photosynthesis, flowering, pollination and seed set. Better vegetative growth of a crop is largely responsible for higher seed yield because number of photosynthesizing sites i.e., number of vegetative branches is affected by initial growth stages. Two sequential steps are necessary for a mustard plant to produce pods; a sink of pollinated pods capable of further development must be created and this must be supplied with photosynthates over subsequent period of development. The consequences for yield attributes are clearly evident (Fig. 3A-C). Again there was consistent and significant reductions (17.8%) in the seed yield due to spray time T₂ than T₁. This reinforces our view that application at the phase that coincides with intense

Table 5: Effect of timing of foliar application of GA₃ on nitrogen, phosphorus and potassium uptake of Indian mustard (*Brassica juncea* L.) cv. Varuna at 20 Days After Flowering (DAF)

		Sampling days					
		20 DAF (Pod-initiation)			60 DAF (Pod-maturity)		
		Uptake (mg plant ⁻¹)					
Spray stages	GA ₃ (Molar)	N	P	K	N	P	K
Pre-anthesis	0	560bc ±23	60.93b ±3.5	537bc ±44	904c ±55	103b ±7	771c ±63
	10 ⁻⁵	803a ±39	85.13a ±4.4	751a ±64	1176a ±87	128a ±10	1016a ±77
At anthesis	0	552bc ±42	60.01b ±3.6	524bc ±35	936bc ±49	106b ±9	809bc ±58
	10 ⁻⁵	592b ±45	62.27b ±4.7	549b ±39	980b ±58	106b ±11	841b ±47
Pre- + at anthesis	0	542c ±39	58.91b ±3.1	509c ±32	969b ±64	108b ±9	848b ±54
	10 ⁻⁵	831a ±49	86.65a ±5.6	763a ±58	1259a ±105	133a ±11	1080a ±87

Values are means (±SE) of five plants. Different letters in the same column indicate significant differences (p<0.05)

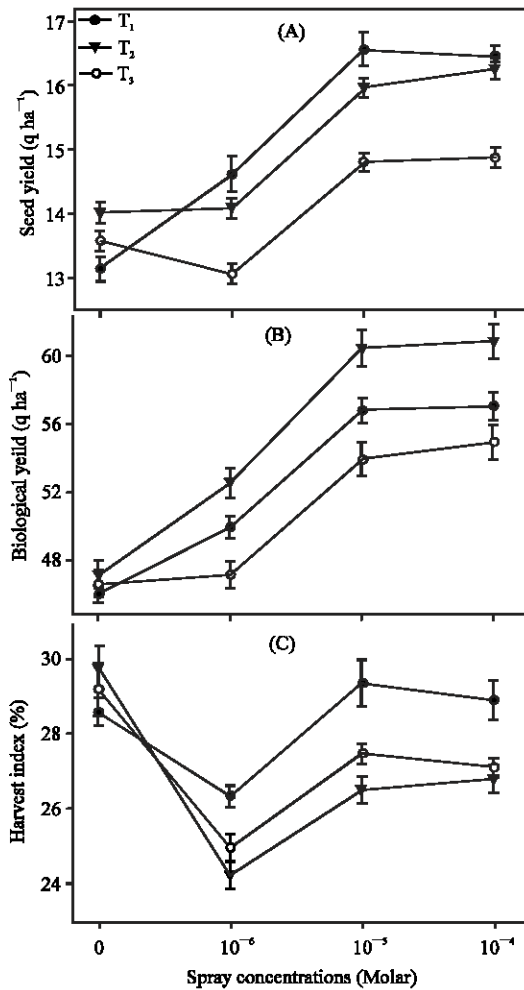


Fig. 3: Seed yield (A), biological yield (B) and harvest index (C) of Indian mustard (*Brassica juncea* L.) cv. Varuna, as influenced by timing and concentrations of GA₃ at harvest (60DAF). Vertical bars indicate \pm SE (n = 5)

vegetative growth had the tissues which was most responsive and created higher demands with efficient translocation of nutrients that finally culminated in to higher seed yield. As for concentration effect on the seed yield, the spray application of 10⁻⁵ M GA₃ produced 21.6% more seeds than water sprayed control. Similar increases in seed yield due to exogenous application of GA₃ have been observed by several workers (Harkess *et al.*, 1994; Metzger, 1987; Zeevart, 1983).

The increase in biological yield was 7.9 and 11.5% more in spray time T₁ and T₃ than T₂. The spray application of 10⁻⁵ M GA₃ increased the biological yield by 22.5% compared to water sprayed control. It was shown that photoassimilates are remobilized to

support both seed development and vegetative growth when plant source-sink relations were artificially modified (Griffith, 1992). Factors that control sink strength also control the partitioning in the crop. Harvest index of GA₃ applied plants were increased by 15.8% compared to water sprayed control. The increase in biological yield and harvest index owe much to the production of enough leaves due to GA₃ with improved radiation interception efficiency which ultimately resulted in higher translocation of photoassimilates towards seeds.

In conclusion, most suitable stage of growth for the exogenous application of GA₃ would appear to be at pre-anthesis for Indian mustard (*Brassica juncea* L.). The findings are relevant when considering the impact of nutrient utilization and related promotion of partitioning of photoassimilates under the spray influence of GA. Taking all together, GA₃ spray had a significant stimulatory effect on dry matter production and nutrient uptake. The findings reported here also have implications with respect to the use of plant growth regulators (GA) that lower the incidence of flower abortion and promote the formation of pods.

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