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Effect of Naphthalene Acetic Acid (NAA) on Oil Content and Quality of the Mustard Plant (*Brassica campestries* L.)

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ABSTRACT

An experiment was carried out to evaluate the biochemical response of mustard to naphthalene acetic acid (NAA). Six levels of NAA such as G0 (no NAA or control), G1 (30 ppm ha^{-1}), G2 (50 ppm ha^{-1}), G3 (70 ppm ha^{-1}), G4 (90 ppm ha^{-1}) and G5 (110 ppm ha^{-1}) were tested. The leaf chlorophyll content was recorded at 30, 40, 50 and 60 days after emergence (DAE). The NAA significantly influenced the biochemical property. The highest chlorophyll level was recorded in 70 ppm NAA ha^{-1} at 50 DAE. At 45 DAE, the highest nitrogen content was noted in 70 ppm NAA ha^{-1}. The 70 ppm NAA ha^{-1} also showed the maximum oil content. The minimum acid value, peroxide and saponification values were found in 70 ppm NAA ha^{-1}. The maximum iodine value was observed in 70 ppm NAA ha-1. Nonessential chemicals likes stearic, palmitic and erucic acid were augmented in the mustard with a decrease in the NAA level while necessary fatty acids were highest in 70 ppm NAA ha^{-1}. It is suggested that 70 ppm NAA ha^{-1} can be used to grow quality mustard plants.

Keywords: *Brassica campestries*, fatty acid, NAA, oil content, oil quality

INTRODUCTION

Mustard is a vital oilseed crop in Bangladesh and its oil is generally used as a cooking oil. Generally, *Brassica campestris* and *B. juncea* are cultivated in Bangladesh for producing edible oils (Kaul & Das, 1986). Edible oils are essential for meeting calorie requirements due to its high-energy

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component. Each gram of oil/fat provides 9 kcal of energy, whereas each gram of carbohydrate/protein supplies only about 4 kilocalories of energy (Stryer, 1980). Edible oils also carry fat-solubles vitamin such as A, D, E and K. In nutrition, fat or oil is necessary for the absorption of these vitamins. Cooking oil that contains necessary fatty acids such as linolenic and linoleic acid is assumed to be of better quality (Egesel, 2009). Moreover, better quality cooking oil possesses minor peroxide, acid and non-saponification values and greater iodine value (Ahsanullah, 1994).

Bangladesh has been suffering from severe scarcity of edible oils for the last several decades. Bangladesh receives about 30% oil from local production (3.8 g/day/head) while the remaining 70% is imported (Wahhab et al., 2002).

Growth-stimulating chemicals are used to modify the growth and development of the mustard plant to increase its yield and also to improve the quality of products. Naphthalene acetic acid (NAA) is an important synthetic auxin (Yamamoto & Yamamoto, 1998). It is important to study the performance of NAA on the growth, yield and biochemical attributes of mustard plant varieties especially the ones that have been approved for cultivation in Bangladesh. Setia et al. (1993) reported that the effect of growth regulators on photosynthesis assimilate partitioning and yield of *B. juncia* and *B. campestris* was positive. However, no data are available on the effect of naphthalene acetic acid (NAA) on the biochemical response of rapeseed. Therefore, there is scope for work on the biochemical aspects of rapeseed, especially growth regulators and sulphur-based fertilisers. The influence of naphthalene acetic acid on the oil content and quality of rapeseed has not been stated. So, the present study was undertaken with the following objectives: To study the influence of naphthalene acetic acid on chlorophyll and nitrogen content in the leaf, oil and protein content in the seed and the nutritive merits of the oil of the mustard plant.

MATERIALS AND METHOD

Experimental Site and Soil Characteristics

An experiment was conducted at the Oil Seed Research Centre, BARI, Gazipur from July 2010 to April 2012. The tested plot was situated in grey terrace soil. The geographical location was at about N-24° 23´ and E-90° 08´. The elevation was 8.4 m above sea level. The texture of the tested plot was silty clay and the plot was located on high ground. The chemical properties of the soil were pH 6.4, OM content 0.87%, total nitrogen 0.09% and exchangeable K 59 ppm. The sulphur level was critical (Table 1). The soil was slightly acidic (pH 6.0) in nature and low in total N (0.088%) and the available S was at 9 ppm. Available P and exchangeable K were above the critical level (Table 1).

Table 1
Initial soil properties of the experimental field

Properties	Initial Value	Critical Level
pH	6.0	-
Total –N (%)	0.088	- 0.12
P (ppm)	14.00	10.00
S (ppm)	9.00	10.00
K (ppm)	63.0	46.9

Extraction method:
Total-N: Kjeldahl method
Available P: Olsen method
Available S: Calcium dihydrogen phosphate
Exchangeable K: N NH_4OA_c extraction method

Cropping Period

Rabi, Kharif-I and Kharif-II are the main cropping periods in Bangladesh. The Rabi season lasts from 15 October to 15 March, while the Kharif-I season is from 15 March to the end of June and the Kharif-II season is from 1 July to 15 October. Rapeseed is sown in November. The weather data related to the planting seasons are presented in Table 2.

Table 2
Weather data related to the growing season

Month and Year	Temperature (°C)			Precipitation (mm)	Relative Humidity (%)		
	Max.	Min.	Avg.		Max.	Min.	Avg.
Nov. 2010	30.6	19.3	25.0		93.4	68.4	80.9
Dec. 2010	26.2	13.5	19.9	53	91.0	68.8	79.9
Jan. 2011	23.3	10.5	16.9		93.1	66.7	79.9
Feb. 2011	28.0	14.3	21.2		88.2	52.1	70.2
Nov. 2011	29.2	16.7	23.0	2	90.2	67.9	79.1
Dec. 2011	25.2	13.5	19.4		91.4	67.6	79.5
Jan. 2012	23.9	12.3	18.1	12	88.3	61.5	74.9
Feb. 2012	30.0	13.5	21.8		87.4	46.2	66.8

Source: Meteorological Centre, Ministry of Defense, BARI, Gazipur, BD

The Test Crop

Rapeseed (*Brassica campestries* L.) cv. BARI Sarisha-15 was used as the test crop. It was collected from ORC, BARI, Gazipur. BARI Sarisha-15 was a semi dwarf, early-growing plant.

Experimental Design and Treatments

The trial was set up in RCBD design and replicated three times. The six rates of NAA were: G0 (nil NAA or control), G1 (30 ppm ha^{-1}), G2 (50 ppm ha^{-1}), G3 (70 ppm ha^{-1}), G4 (90 ppm ha^{-1}) and G5 (110 ppm ha^{-1}). After certain periods (30, 40, 50 and 60 DAE), the leaf samples were collected for analysis of their biochemical properties. The experimental unit plot size was 4 m × 3 m.

Fertilizer Rate, Use and Other Actions

N, P, K, S, Zn and B were used from urea, triple superphosphate (TSP), muriate of potash (MoP) and gypsum, zinc oxide and

boric acid, respectively. N, P, K, S, Zn and B were used at the rate of 120, 34, 45, 60, 1.8 and 1.8 kg ha^{-1}, respectively. Half amounts of nitrogen and whole amounts of P, K, S, Zn, B and half of N were applied as the base during the final land preparation. The remaining N was top-dressed at the flower initiation time. During the whole growing season, irrigation was provided three times. At first, light irrigation was provided 5 days before planting. At the vegetative stage, irrigation was done for the second time. The last irrigation was provided at the siliqua filling stage. Mulching and other plant protection measures were taken as per requirement.

Seed Sowing and Harvesting

The rapeseeds were sown on 14 and 16 November of 2010 and 2011, respectively with 30 cm × 5 cm spacing. The rapeseed plants were harvested on February 25, 2011 and April 2, 2012, respectively.

Sampling Techniques

Chlorophyll content and nitrogen in the leaf samples were detected at 30, 45, 60 DAE and 30, 40, 50, 60 DAE for N and chlorophyll, respectively. Some properties such as oil content and protein content of the rapeseeds and the biochemical properties (acid, peroxide, iodine and saponification values and fatty acid content) were assessed after harvesting.

Analysis of Soil Sample

Analysis for soil acidity, nitrogen, phosphorus, potassium and sulphur was done following the standard laboratory method. A glass electrode pH meter was used to determine soil pH. Total nitrogen content was determined using the micro-Kjeldahl method (Page et al., 1989); available phosphorus was detected using the Olsen method (Jackson, 1973), exchangeable potassium was measured following the NH_4OAC extraction method (Black, 1965) and sulphur was measured using a spectrophotometer at the wave length of 420 nm (Page et al., 1989).

Biochemical Properties of Oil

The Cocks and Van Rede (1966) and Mehlenbacher (1960) methods were used to determine the oil content of rapeseed and fatty acid composition was determined using the gas-liquid chromatography method (Jellum & Worthington, 1966). A total of 12 mg oil was taken and transesterified at the same time with 5 ml ethylate reagent and shaken. A salt solution (80 g NaCl and 3 g sodium hydrogen sulphate in 1 L water) of 10 ml was added and shaken. As soon as the two layers were separated, the benzene phase was transferred to small test tubes and the samples were then ready for gas chromatography. Peak areas were measured with an electronic digital integrator Thermo Fisher Scientific, Trace GC Ultra- with Tri-Plus autosample (Nagraj, 2009). The

following operating parameters were used: split injection mode, the injection rate was 0.2 µml and the injector temperature was 250°C, where the capillary column (30 m × 0.25 µ × 0.25 µ) was used. The method for esterification of fatty acids was developed by the Swedish Seed Association, Svalov, Sweden; by this method, the oil was used for transesterification. The procedure to determine the acid value was from Devine and Williams (1961). A volume of 25 ml diethyl ether was mixed with 25 ml alcohol and 1 ml of phenolphthalein solution and carefully neutralised with 0.1 M sodium hydroxide. An amount of 2 g oil was dissolved in the mixed neutral solvent and titrated with aqueous 0.1 M sodium hydroxide. The mixture was constantly shaken until a pink colour was seen (Chapman, 1979; IUPAC, 1979). Iodine value was determined using the Hanus method (AOAC, 1960). An amount of 1 g oil sample was placed in a 500 mL volumetric flask. A volume of 15mL of carbon tetrachloride was added to the sample and the mixture was swirled to ensure that the sample was completely dissolved. A volume of 25 mL of Wijs solution was then dispensed into the flask. The flask was stoppered and swirled to ensure complete mixing. The sample was then placed in the dark for 30 min at room temperature. The flask was removed from storage and 20 mL of 10% potassium iodide (KI) solution was added, followed by 150 mL of distilled water. The mixture was titrated with 0.1 N thiosulphate ($Na_2S_2O_3$) solution that was added gradually and with constant and vigorous shaking until its yellow colour had almost disappeared. Then, 1.5 mL of starch indicator solution was added and the titration was continued until the blue colour disappeared. The Cocks and Van Rede (1966) method was used to determine the peroxide value. An amount of 1 g oil was placed in a clean dry boiling tube and 1 g powdered potassium iodide was added together with 20 ml of solvent mixture 2 vol. glacial acetic acid + 1 vol. chloroform. The tube was placed in boiling water so that the liquid boiled within 30 s. It was allowed to boil vigorously (<30 s). The contents were quickly poured into a flask containing 20 ml of potassium iodide solution 5%. The tube was washed out twice with 25 ml water and titrated with 0.002 M sodium thiosulphate solution using starch (Mehlenbacher, 1960; IUPAC, 1979). The saponification value was estimated using the Pearson technique (1970). A volume of 2 g oil sample was placed in a volumetric flask. Then, 25 mL of 1.0 N alcoholic KOH was pipetted and allowed to drain for about 1 min into the mixture. A condenser was connected to the flask and the mixture sample allowed to boil gently but steadily for 45 min for complete saponification. The flask and the condenser were then cooled but not sufficiently to form a gel. The inside of the condenser was washed down with about 1 ml of distilled water. The condenser was disconnected and 1 mL of phenolphthalein indicator was added. The solution was titrated with 0.5 N hydrochloric acid (HCl) until the pink colour of the mixture disappeared.

Chlorophyll Content Determination

The specific absorption co-efficient of Mckinney (1940) and the formula of Maclachalan and Zalik (1963) were used to determine chlorophyll a and b.

The formulae used were:

Chlo. a = {(12.3 × D663 – 0.86 × D645) × V} / {d X1000 × W} mg/g fresh leaf

Chlo. b = {(19.3 × D645 – 3.6 × D663) × V} / {d X1000 × W} mg/g fresh leaf

where, Chlo. a = Chlorophyll a

Chlo. b = Chlorophyll b

D = Visual density (OD) at wave length

V = Final volume

W = Weight (Fresh leaf pigment materials)

d = Light path length in cm

Statistical Analysis

The analysis of variance for different properties of oil were performed following the ANOVA technique and the mean values were adjudged by DMRT (p=0.05) method (Steel & Torrie, 1960). SAS software (version 9.1) was used to analyse the data. Microsoft EXCEL 2003 was used to compute and prepare the graphs.

RESULTS AND DISCUSSION

Different levels of napthalene acetic (NAA) significantly influenced the chlorophyll content and nitrogen in the leaf, oil and protein content in the rapeseed and its nutritive potential.

Influence of NAA on Chlorophyll 'a' Content in Rapeseed Leaf

The different NAA levels at different days after emergence (DAE) significantly influenced the chlorophyll 'a' content. From 2010-2011, 50 DAE showed the significantly (p=0.05) higher chlorophyll 'a' (1. 61 mg g-1) (Figure 1).

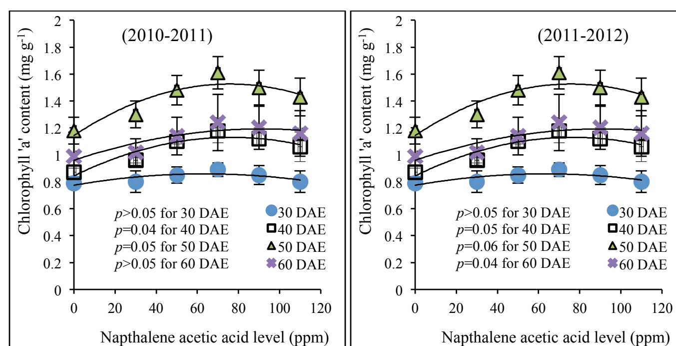

Figure 1. Chlorophyll 'a' content of rapeseed leaf as influenced by napthalene acetic acid at different sampling dates (DAE=days after emergence). The mean data (±SE) over replication are presented

The maximum chlorophyll ‘a’ content (1.61 mg g^{-1}) was found in G3 (70 ppm ha^{-1}) among the NAA levels. The response function showed the quadratic association among the chlorophyll ‘a’ content and NAA in regression analysis (Figure 1). For chlorophyll ‘a’ (1.51 mg g^{-1}), the calculated suitable dose of NAA was 72 ppm ha^{-1} at 50 DAE (Table 3). Therefore, applying 72 ppm ha^{-1}, the highest chlorophyll ‘a’ content (1.51 mg g^{-1}) of rapeseed leaf can be done. Chlorophyll ‘a’ content of rapeseed leaf was positively correlated (R^2=858^{NS}) with the applied NAA at 50 DAE. (Table 3).

Table 3
Response function of chlorophyll ‘a’ content (mg g^{-1}) in Rapeseed leaf to NAA

2010-2011	Regression Equation	R^2 Value	Optimum Dose (kg ha^{-1})	Maximum Chlorophyll ‘a’ content (mg g^{-1}) For Optimum Dose
30 DAE	y = -2E-05x^2 + 0.0027x + 0.7742	0.6458^{NS}	67	0.86
40 DAE	y = -5E-05x^2 + 0.0075x + 0.844	0.8802^{NS}	75	1.13
50 DAE	y = -7E-05x^2 + 0.0101x + 1.1442	0.8585	72	1.51
60 DAE	y = -3E-05x^2 + 0.0051x + 0.9604	0.8043^{NS}	85	1.18
2011-2012				
30 DAE	y = -2E-05x^2 + 0.0027x + 1.0836	0.4131^{NS}	74	1.17
40 DAE	y = -2E-05x^2 + 0.0025x + 1.272	0.8557^{NS}	63	1.35
50 DAE	y = -5E-05x^2 + 0.0074x + 1.2119	0.8531^{NS}	68	1.48
60 DAE	y = -2E-05x^2 + 0.0026x + 1.0511	0.975*	65	1.13

Note: NS, not significant; *, significant at 5% level

Apparently, chlorophyll ‘a’ at 40 and 60 DAE showed similar values but there is a significant difference in the values between the two dates. The control showed the minimum chlorophyll ‘a’ content in all the sampling dates (Figure 1). Chlorophyll ‘a’ content increased due to application of NAA in 2011-2012 that followed the same trend. At 30 and 60 DAE, the chlorophyll ‘a’ was significantly different in the rapeseed leaf samples (Figure 1b). At 30, 40, 50 and 60 DAE, the optimum doses of NAA were 74, 63, 68 and 65 ppm NAA ha^{-1} for maximising chlorophyll ‘a’ of 1.17, 1.35, 1.48 and 1.13 mg g^{-1}, respectively (Table 3). The chlorophyll ‘a’ content was increased with the increase in NAA level up to 70 ppm ha^{-1}; after that, chlorophyll ‘a’ decreased. Chlorophyll ‘a’ content also increased with the increase on different days after emergence but decreased after 50 DAE (Figure 1).

Chlorophyll ‘b’ Content in Rapeseed Leaf as Influenced by NAA

Different DAE significantly influenced the chlorophyll ‘b’ content. The chlorophyll ‘b’ content was increased with the increase in NAA level up to 70 ppm ha^{-1} in 2010-11

on different sampling dates. G3 (70 ppm NAA ha^{-1}) showed the highest chlorophyll ‘b’ content. A quadratic relationship was found between the chlorophyll ‘b’ and NAA level (Figure 2). At 40, 50 and 60 days after emergence the optimum doses were 71, 76 and 77 ppm NAA ha^{-1}, respectively (Table 4). The NAA level significantly influenced the chlorophyll ‘b’ of the mustard leaf at different sampling dates in 2011-12. The maximum chlorophyll ‘b’ (0.49 mg g^{-1}) was found at 50 DAE, which was significantly higher than at the other DAE. At 30 DAE, the lowest chlorophyll ‘b’ was recorded, while 70 ppm NAA ha^{-1} showed the maximum chlorophyll ‘b’ content. The response function showed a quadratic relationship between chlorophyll ‘b’ and the NAA level. At 50 DAE, the optimum dose of NAA was 72 ppm ha^{-1} (Table 4).

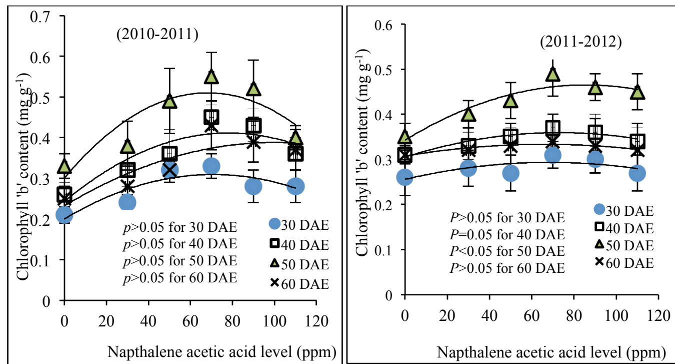

Figure 2. Chlorophyll ‘b’ of rapeseed leaf as influenced by NAA at different sampling dates (DAE=days after emergence). Data are presented as mean (±SE) over replications

Applying 72 ppm NAA ha^{-1}, the highest chlorophyll ‘b’ (0.50 mg g^{-1}) in rapeseed leaf can be predicted. Chlorophyll ‘b’ was positively associated with the applied NAA at 40 DAE (R^2=0.868), 50 DAE (R^2=0.892) and 60 DAE (R^2=0.835) (Table 4). At 40, 50 and 60 DAE, the optimum doses of NAA were 75, 72 and 66 NAA ppm ha^{-1} for maximising chlorophyll ‘b’ (0.38, 0.50 and 0.34 mg g^{-1} for 40, 50 and 60 DAE, respectively) (Table 4). The photosynthetic activities of green plants are directly influenced by the chlorophyll content of leaves. The NAA level significantly influenced the chlorophyll a and b content in leaf. This was attributed to the increased proportion of gana per plastid volume in the chloroplasts.

Table 4
Chlorophyll 'b' (mg g^{-1}) in Rapeseed leaf as influenced by NAA under response function

2010-2011	Regression Equation	R^2 Value	Optimum Dose (kg ha^{-1})	Maximum Chlorophyll 'b' Content (mg g^{-1}) for Optimum Dose
30 DAE	$y = -2E-05x^2 + 0.0031x + 0.2004$	0.7633^{NS}	77	0.32
40 DAE	$y = -3E-05x^2 + 0.0043x + 0.2429$	0.8123^{NS}	71	0.40
50 DAE	$y = -4E-05x^2 + 0.0061x + 0.3009$	0.7745^{NS}	76	0.50
60 DAE	$y = -2E-05x^2 + 0.0031x + 0.2322$	0.7634^{NS}	77	0.43
2011-2012				
30 DAE	$y = -8E-06x^2 + 0.0011x + 0.2556$	0.5109^{NS}	68	0.34
40 DAE	$y = -1E-05x^2 + 0.0015x + 0.3049$	0.868^{NS}	75	0.38
50 DAE	$y = -2E-05x^2 + 0.0029x + 0.3426$	0.8921^{NS}	72	0.50
60 DAE	$y = -6E-06x^2 + 0.0008x + 0.3074$	0.8353^{NS}	66	0.34

Note: NS, not significant

Lakshmamma and Rao (1996a) reported that spraying 5-20 ppm NAA at the flowering stage progressively increased the chlorophyll content in the leaves of black gram. The highest chlorophyll and RNA content were recorded with 20 ppm NAA in the presence of endogenous gibberellic acid (Brain & Hemming, 1958). Spraying NAA (0.04 % solution) at 35 and 75 DAE increased the total chlorophyll content in the soybean leaves by 2.08, 2.21 and 1.68% over the control (Kalarani & Jeyakumar, 1998). Sivakumar et al. (2002) reported that application of 20 ppm NAA increased the content of chlorophyll in the leaf of pearlmillet. Kumar et al. (2005) also found that application of NAA (20 ppm) increased the chlorophyll content of cotton.

Nitrogen Content in Rapeseed Leaf as Influenced by NAA

NAA levels significantly influenced the nitrogen content at different DAE (Figure 3). The highest nitrogen content (6.03 and 5.7% for 2010-11 and 2011-12, respectively) was recorded at 45 DAE, which was significantly higher than the other DAE. The maximum nitrogen content was found in 70 ppm NAA ha^{-1} at all sampling dates (Figure 3). A quadratic relationship was found between the nitrogen content and applied NAA (Figure 3). In 2010-11, the optimum doses of NAA were 75, 72 and 76 ppm NAA ha^{-1} for 30, 45 and 50 DAE, respectively (Figure 3). Applied NAA at 30, 45 and 50 DAE was positively correlated with the nitrogen content in rapeseed leaf (R^2=0.819 and 0.434 and 0.794 for 30, 45 and 50 DAE, respectively) (Table 5). At 30 and 45 DAE, the nitrogen content was apparently similar but there was a significant difference between 30 and 45 DAE. After application of NAA, the nitrogen content increased in the rapeseed leaf in 2011-12, which followed the same trend. In rapeseed leaf, the nitrogen content was positively

(p<0.01 and 0.18 for 30 DAE and 45 DAE, respectively) correlated (R^2=0.956* and 0.685 for 30 and 45 DAE, respectively) with NAA at 30 and 45 DAE. In 2011-2012, the optimum doses of NAA were 67, 79 and 77 ppm ha^{-1} for 30, 45 and 50 days after emergence, respectively (Table 5). It may be mentioned that the nitrogen content of the rapeseed leaf samples increased with the increase in NAA but after 70 ppm NAA ha^{-1} it was decreased. Shende et al. (1987) reported that foliar application of different growth regulators increased the nitrogen content in the leaves. Forty-five days after emergence, the nitrogen content was gradually decreased; this might have been due to ageing.

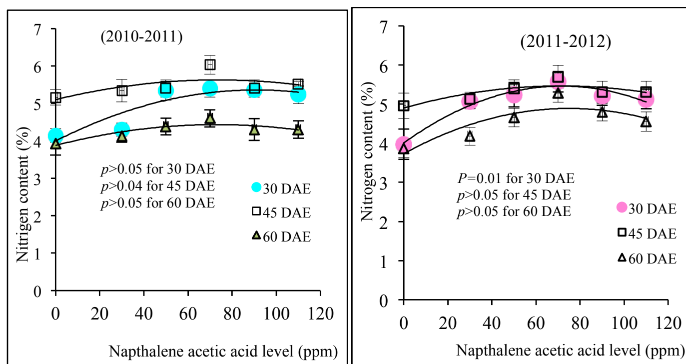

Figure 3. Nitrogen content (%) in rapeseed leaf as influenced by NAA at different DAE in 2010-11 and 2011-12
Note: DAE = days after emergence. Data are presented as mean (±SE) over replications

Table 5
Nitrogen content (%) in Rapeseed leaf as influenced by NAA under response function

2010-2011	Regression Equation	R^2 Value	Optimum Dose ($kg\ ha^{-1}$)	Maximum Nitrogen Content (%) for Optimum Dose
30 DAE	$y = -0.0002x^2 + 0.03x + 3.995$	0.819^{NS}	75	5.43
45 DAE	$y = -1E\text{-}04x^2 + 0.0144x + 5.1004$	0.434^{NS}	72	5.68
60 DAE	$y = -0.0001x^2 + 0.0153x + 3.8757$	0.794^{NS}	76	4.41
2011-2012				
30 DAE	$y = -0.0003x^2 + 0.0404x + 4.0083$	0.956*	67	5.45
45 DAE	$y = -0.0001x^2 + 0.0158x + 4.8988$	0.685^{NS}	79	5.61
60 DAE	$y = -0.0002x^2 + 0.0308x + 3.7334$	0.765^{NS}	77	4.70

Note: NS, not significant; *, significant at 5% level

Protein Content in Rapeseed as Influenced by NAA

The NAA significantly influenced the protein content in rapeseed. G2 (50 ppm NAA ha^{-1}) showed the maximum protein content (23.7%), which was significantly higher than in the other treatments (Figure 4A). G2 was also 20.3% higher than the control. Kalarani and Jeyakumar (1998) reported that spraying 0.04 % NAA solution at 35 and 75 DAE caused the soluble protein content of soybean leaves to increase by 9.84, 17.58 and 9.13% over the control. Karim (2005) also reported that spraying 20 ppm NAA on the foliage produced the highest amount of protein in chickpeas but the protein content in the seeds decreased with the increase in NAA concentration. Sivakumar et al. (2002) observed that application of 20 ppm NAA increased the protein content in the leaf of pearl millet. Ullah et al. (2007) also reported that the highest protein content was found with 50 ppm NAA.

Oil Content in Rapeseed as Influenced by NAA

The NAA significantly influenced the oil content in rapeseed. In rapeseed, oil content varied from 42.45 to 43.05, having the maximum (43.05%) in G3 (70 ppm NAA ha^{-1}) followed by G4 (42.85%) (Figure 4B). The control showed the minimum oil content (42.0%) (Figure 4B). The similar oil content was found in G4 and G5. With the increase in NAA level, the oil content of rapeseed increased up to 70 ppm NAA ha^{-1} (Figure 4B). The protein content was reduced in the same treatment, while the oil content was the highest (Figure 4A). The best rate of the NAA might have enriched enzyme activities; this implicates that oil synthesis resulted in increased amounts of oil in the seed.

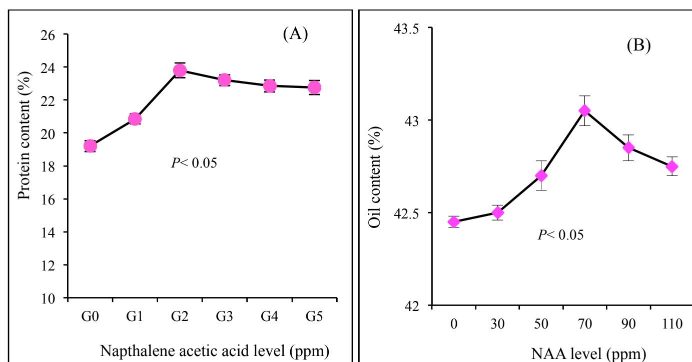

Figure 4. Effect of NAA on protein (%) (A) and oil content (B) in rapeseed. Data are presented as mean (±SE) over replications

Bhat et al. (2004) reported that application of 50 ppm NAA caused significant improvement in oil content of the seed of the mustard plant. Karim (2005) carried out an experiment with different concentrations of NAA on chickpea foliage and found that 20 ppm NAA produced the highest fat in the seeds. Oluwatosin (1997) reported positive correlation between protein and lipid content of cowpea.

Chemical Characteristics of Rapeseed Oil as Influenced by NAA

Acid, iodine, peroxide and saponification values determine the quality of oil. The NAA significantly influenced the acid value. The highest acid value (0.86) was found in G1 (30 ppm NAA ha^{-1}), which was statistically different form other treatments. G3 (70 ppm NAA ha^{-1}) showed the minimum acid value (0.37) (Figure 5A). Mondal (1999) reported that the growth regulators significantly influenced the acid value. G3 showed the maximum iodine value (86.7 and 95.6 for 2010-11 to 2011-12, respectively) (Figure 5B). In 2010-11, a quadratic relation was found between the NAA and iodine value. The optimum dose of NAA was found to be 70.7 ppm NAA ha^{-1}. A quadratic relation was also found between the iodine value and applied NAA in 2011-12. (Figure 5B). The optimum rate of NAA was 76.8 ppm NAA ha^{-1}. The control showed the lowest iodine value (75.0 and 85.2 for 2010-11 to 2011-12, respectively). After application of NAA, the iodine value increased in the synthesis process of fatty acid and this might have been due to the maximum amount of unsaturated fatty acids being converted to saturated fatty acids (Gangahara et al., 1990). The NAA also influenced the peroxide value. The maximum peroxide value (6.18 and 4.48 for 2010-11 to 2011-12, respectively) was found in G1 (30 ppm NAA $ha-^{1}$) followed by G0 (control) (Figure 5C). G5 and G3 showed the minimum peroxide value (5.11 and 3.87 for 2010-11 to 2011-12, respectively) in 2010-11 to 2011-12, respectively (Figure 5C). With the increase in the NAA level, the peroxide value was decreased. Better quality oil contains a low peroxide level.

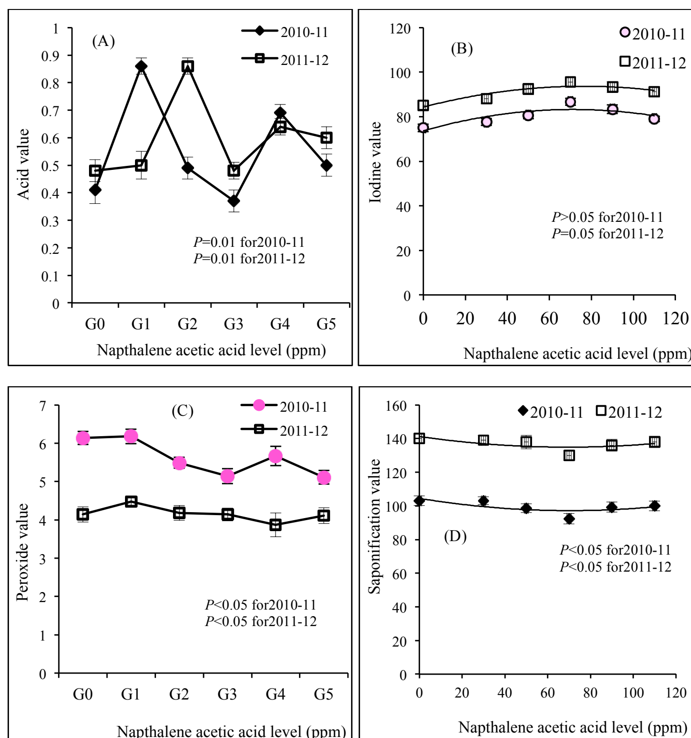

Figure 5. Acid value (A), iodine value (B), peroxide value (C) and saponification value (D) in rapeseed oil. Data are presented as mean (±SE) over replications

NAA also influenced the saponification value. The control showed the maximum saponification value (103 and 140 for 2010-11 and 2011-12, respectively), which was closely followed by G1 (Figure 5D & Table 6). The minimum saponification value (92.4 and 130 for 2010-11 and 2011-12, respectively) was found in G3 (70 ppm NAA ha^{-1}). The NAA was negatively correlated with the saponification value. With an increase in the NAA level, the saponification value decreased; this is beneficial to human health.

Fatty Acid Composition of Rapeseed Oil as Influenced by NAA

NAA significantly influenced the fatty acid composition. NAA positively influenced the palmitic acid content but the effects were not significant ($p>0.05$). The control showed the maximum palmitic acid content (1.97%) followed by G1 and G2 (Figure 6A).

Table 6
Response function of palmitic acid content, iodine value and saponification value of Rapeseed to NAA

	Regression Equation	R^2 Value	Optimum Dose (kg ha^{-1})
Iodine Value (2010-11)	$y = -0.0019x^2 + 0.2686x + 73.67$	0.7087^{NS}	70.7
Iodine Value (2011-12)	$y = -0.0016x^2 + 0.2459x + 84.297$	0.8622^{NS}	76.8
Saponification Value (2010-11)	$y = 0.0015x^2 - 0.2078x + 104.48$	0.4768^{NS}	69
Saponification Value (2011-12)	$y = 0.0013x^2 - 0.1825x + 141.23$	0.4181^{NS}	70

Note: NS, not significant

G5 (110 ppm NAA ha^{-1}) showed the minimum palmitic acid content (1.55%). The response showed a quadratic relationship in nature between the applied NAA and palmitic acid (%) value (Figure 6A). With the applied NAA level, the palmitic acid content in mustard oil was negatively correlated (R^2=955). The control showed the maximum stearic acid value (1.00 and 0.98% for 2010-11 and 2011-12, respectively) (Figure 6B). G5 and G3 showed the lowest stearic acid value (0.75 and 0.59 for 2010-11 and 2011-12, respectively) in 2010-11 and 2011-12, respectively (Figure 6B). With the applied NAA level, the stearic acid value was negatively and significantly ($p<0.05$) correlated (R^2=0.995* for 2010-11). The oil is not beneficial to health as it contains

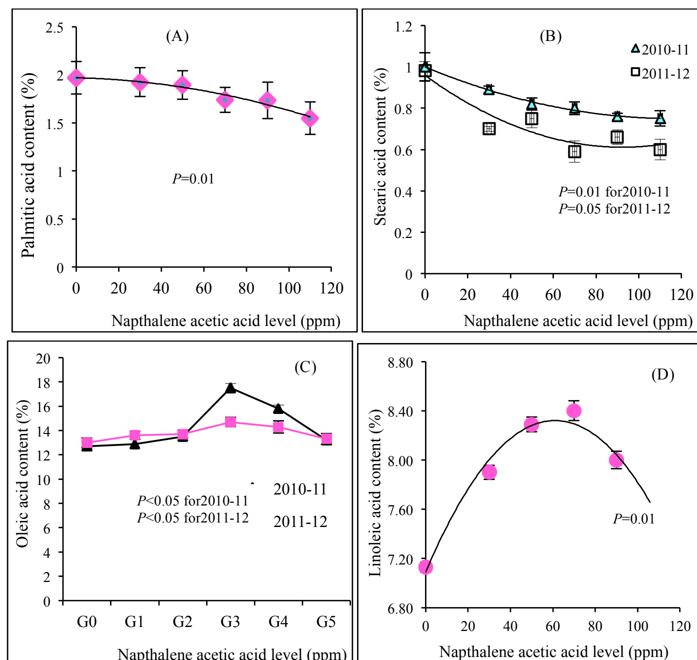

Figure 6. NAA influenced the palmitic acid (A), stearic acid (B), oleic acid (C) and linoleic acid (D) value (%) in rapeseed oil. Data are presented as mean (±SE) over replications

maximum levels of palmitic and stearic acid and it also recorded from 0-30 ppm NAA ha^{-1}. By increasing the NAA levels, these acid values were decreased; this is good for us. The NAA significantly influenced the oleic acid ($p<0.05$).

G3 showed the highest oleic acid content (17.5 and 14.7% for 2010-11 and 2011-12, respectively) followed by G4 (Figure 6C). The control showed the minimum oleic acid value (12.7 and 13.0% for 2010-11 and 2011-12, respectively). A quadratic relationship was found between the NAA level and linoleic acid content (Figure 6D). For maximising linoleic acid in rapeseed, the optimum dose of NAA would be 67 ppm NAA ha^{-1}. A positive and significant ($p<0.01$) correlation (R^2= 0.995**) was found between the linoleic acid and NAA level. According to regression analysis, a quadratic relationship was found between the linolenic acid content and NAA (Figure 7A). The positive correlation (R^2=0.741NS) was found between the linolenic acid value and the NAA level and the optimum dose of NAA was 68 ppm NAA ha^{-1}. The maximum ecosanoic acid content (1.2%) was found in G3. The control showed the lowest value (0.64%). A quadratic relation (R^2=0.623NS) was found between the ecosanoic acid value and NAA level but it was not significant (Figure 7A). To receive maximum ecosanoic acid value, the optimum dose of NAA was 68 ppm NAA ha^{-1}(Table 7). The maximum ecosenoic acid content (6 and 9.16 for 2010-11 and 2011-12, respectively) was recorded in G3 and the lowest was found in the control (Figure 7C). A quadratic relation was found between the NAA and ecosenic acid value but its effect was insignificant. To get the maximum ecosenic acid, the optimum doses of NAA were 76 and 68 ppm NAA ha^{-1} in 2010-11 and 2011-12, respectively (Table 7). By increasing the NAA level, oleic, linoleic and lenolenic acid content were increased up to a certain level, after which the content of these acids decreased. To lower plasma cholesterol and lipoprotein density, a high proportion of polyunsaturated fatty acids are required. This would reduce the risk of coronary heart disease and parthenogenesis (Skoric, 1988). Erucic acid is harmful to human health. Erucic acid was also influenced by the applied NAA levels (Figure 7D). The maximum erucic acid value (53.3%) was found in the control, followed by G1 (53.2%). The minimum erucic acid content (50.3%) was noted in G4. By increasing the NAA level, the erucic acid content was decreased. This is beneficial to human health. The composition of seed oil is largely controlled by genetic factors, but environmental factors may change the pattern of fatty acid in oil of mustard seed (Mondal, 1986).

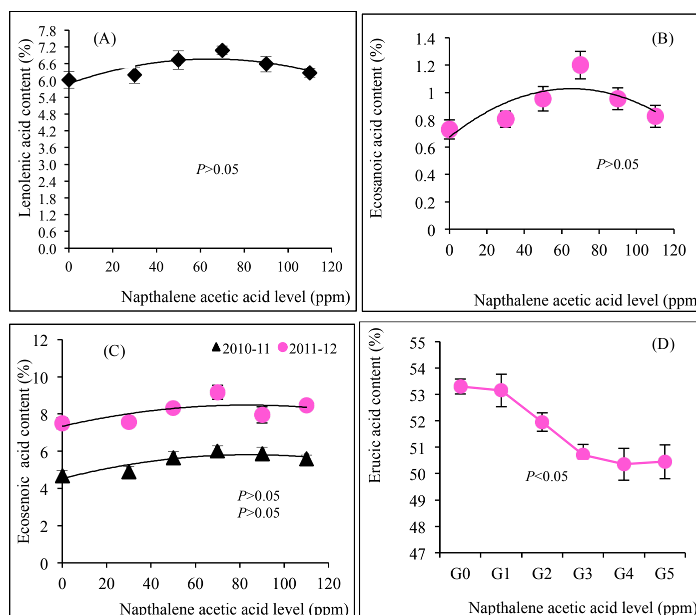

Figure 7. Linoleic acid (A), ecosanoic acid (B), ecosenoic acid (C) and erucic acid (D) content (%) in rapeseed as influenced by NAA. Data are presented as the mean (±SE) over replications

No previous report is available on the effect of NAA on fatty acid formation of oil. However, the nutritional and storage qualities of mustard depend on the relative proportion of saturated and unsaturated fatty acids in the oil.

Table 7
Influence of NAA on stearic acid, lenolic acid, lenolenic acid, ecosanoic acid, ecosenoic acid and erucic acid under different response functions

	Regression Equation	R^2 Value	Optimum Dose (ppm)
Palmitic Acid Content	$y = -3E\text{-}05x^2 - 0.0005x + 1.97$	0.9553^{NS}	-
Stearic Acid (2010-11)	$y = 2E\text{-}05x^2 - 0.0043x + 1.00$	0.9945 **	-
Stearic Acid (2011-12)	$y = 4E\text{-}05x^2 - 0.0075x + 0.96$	0.8621^{NS}	-
Lenolic Acid	$y = -0.0003x^2 + 0.0403x + 7.10$	0.9778 **	67
Lenolenic Acid	$y = -0.0002x^2 + 0.027x + 5.90$	0.7021^{NS}	68
Ecosanoic Acid	$y = -8E\text{-}05x^2 + 0.0109x + 0.67$	0.6225^{NS}	68
Ecosenoic Acid (2010-11)	$y = -0.0002x^2 + 0.0304x + 4.527$	0.83^{NS}	76
Ecosenoic Acid (2011-12)	$y = -0.0002x^2 + 0.0273x + 7.34$	0.4772^{NS}	68

Note: NS, not significant; *, significant at 5% level; **, significant at 1% level

CONCLUSION

The naphthalene acetic acid significantly influenced the biochemical character of rapeseed oil. G_3 (70 ppm NAA ha^{-1}) showed the highest chlorophyll content of rapeseed leaves at 50 days after emergence. The maximum nitrogen content in the leaves was also found in the same NAA level at 45 DAE. A value of 70 ppm NAA also showed the highest oil content. The minimum acid, peroxide and saponification values were observed in 70 ppm NAA. The same treatment also showed the highest iodine value. By decreasing the NAA level, nonessential fatty acids were increased in the mustard plant, while necessary fatty acids were highest in 70 ppm NAA ha^{-1}. So, 70 ppm NAA ha^{-1} can be used to grow quality mustard.

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