

Study of Some Agronomic Characteristics of *Nigella sativa* that validates its Yield Potential

Nazar Hussain^a, Syed Abdul Majid^a, M. Altaf Hussain^b

^aDepartment of Botany University of Azad Jammu & Kashmir Muzaffarabad-13100, Pakistan.

^bDepartment of Botany, Mirpur University of Science and Technology, Bhimber Campus Bhimber, Azad Kashmir.

Corresponding author : Nazar Hussain

Abstract

In the plant life-cycle, seed germination and seedling emergence are the two critical stages largely depend on temperature and soil moisture which are not always static. Two sets of seven *Nigella sativa* genotypes were plot in triplicate at 16 hours day length. One set was grown at 22 ± 2 °C in Glass-House under control conditions and other in Growth-Chamber at four temperature conditions viz., 10 °C, 15 °C, 20 °C and 25 °C. All the genotypes were recorded for percent germination and growth behavior which were observed with the similar time of emergence to flowering except an early response with N4 genotype, however its opposite following growth stages later. Phenological components were evaluated assessing yield at control and variable temperature conditions which showed many variations. Among seven genotypes N1 through N7, the N1, N2, N5 and N7 formed a group of similar phenological characteristics which can be optimized at 20 °C for better growth and yield. It was observed that the traits like plant height, number of branches and seeds capsule number can largely be correlated with total productivity. All the data obtained were statistically analyzed using computer base SPSS III program.

KEYWORDS: Germination; Temperature; *Nigella sativa*; Genotypes; Phenological components.

Introduction

The climate is the function of biotic and abiotic factors fluctuating around any plant species maternal environment. It is now common consensus backed through accumulative evidence that global warming mounting substantial crash on the agro-biological yields especially in tropic and sub-tropics that's links to the climate is obvious (Schlenker and Roberts, 2006). In the agro-climatic conditions, temperature predominantly impact on growth and productivity of any crop through 'cause and effect'. It is evident that the temperature is proportional to the relative hydraulic conductivity and inversely affects water surface tension and viscosity. In exposure to other biological factors this may be additively complex. So temperature is the single most cause have direct or indirect dominant growth regulating influence and is the function of yield. Plants grown prevailing unusual climatology vulnerable to stress which on continual exposure may change the phenotypic and genotypic characteristics. The optimization of maternal

plant space could facilitate in establishing better germination and growth, hence productivity at large. *Nigella sativa* is an annual herbaceous plant belongs to the family Ranunculaceae. Plant is now grown everywhere for its seeds which has huge medicinal potential against a number of diseases. It is widely cultivated in Pakistan, India, Syria, Egypt, Saudi Arabia, Iran, Turkey, Cyprus and Europe (Davis, 1965; Riaz *et al.* 1996). The seeds are frequently referred as kalonji or kalazira or onion seeds in the Sub-continent and black seeds or black cumin in Europe. Although *Nigella sativa* is native of Mediterranean region but it is well suited for arid and semi-arid regions (D' Antuno *et al.*, 2002). It is mainly grown for food additives, condiments and medicinal purposes. It is grown during February-April and harvested in June-August. Plant is hermaphrodite reproducing through self pollination. The height of plant ranges 35-80 cm, branched stem (4-12) each terminating with flower and dense pinnately dissected leaves. Plants grow erect with sparsely branched stiff stem. The leaves are linear, slender finely divided, pinnate-sect, 2-4 cm long cut into linear segment and segments are oblong. The flowers are delicate, showy (pale-blue and white) with perianth which is differentiated into an outer whorl of five sepals and an inner whorl of eight nectariferous petals. Androecium contains numerous stamens and gynoecium with five fused follicles terminating each by long indehiscent style. The fertilized flower developed into a capsule which on ripening dehisces black seeds. The seeds are trigonous, usually three-cornered, with two sides flat and one convex. They are rough textured and black in color with strong agreeable aromatic odors, like that of nutmegs, and a spicy, pungent taste. The seeds are mostly used as a spice. They can be used in a wide range of cooking's, baking products and impart a little bit heat effect to the dishes. The oil is mostly extracted mechanically from small, black, tough seeds of *Nigella sativa*. This dark yellow brown oil has long been a favorite in the Middle East and Asia and the main supply comes from Egypt, Syria and Turkey. They have been extensively investigated in recent years and used in folk medicine as a natural remedy for a number of diseases such as asthma, hypertension, diabetes, inflammation, cough, eczema, fever and gastrointestinal disturbances. Seed oil also has antipyretic, analgesic and antineoplastic activity (Ali and Blunden, 2003). Thymoquinone, an active constituent of *N. sativa* seeds, is a pharmacologically active quinone, which possesses several pharmacological properties including analgesic and anti-inflammatory actions (Abdel-Fattah *et al.*, 2000; Randhawa and Al-Ghamdi, 2002).

Materials and Methods

This study was carried out in Glass-House of Aberystwyth University, Wales UK during March to June, 2012. *N. sativa* seven seeds lines were collected from herbal stores of seven locations viz., Rawalpindi, Islamabad, Abbotabad, Muzaffarabad, Mirpur, Bhimber and Faisalabad of Pakistan. For each line, 10 seeds were sown at 2 cm distance in base soil filled pots (10"). All pots were placed on desk stands in triplicate at a constant temperature $22\text{ }^{\circ}\text{C} \pm 2$. Water was given when required and NPK (Scotts & Co.UK. Ltd.) supplied once in a whole life span. Time and Percentage germination was recorded at 2 mm plumule stage. Plant length measured after 7 days, 15 days, 30 days and 60 days.

These seven lines of *N. sativa* were also grown in Growth-Chamber at different Temperature (10, 15, 20 and 25 C°) to know their potential of growth at variable

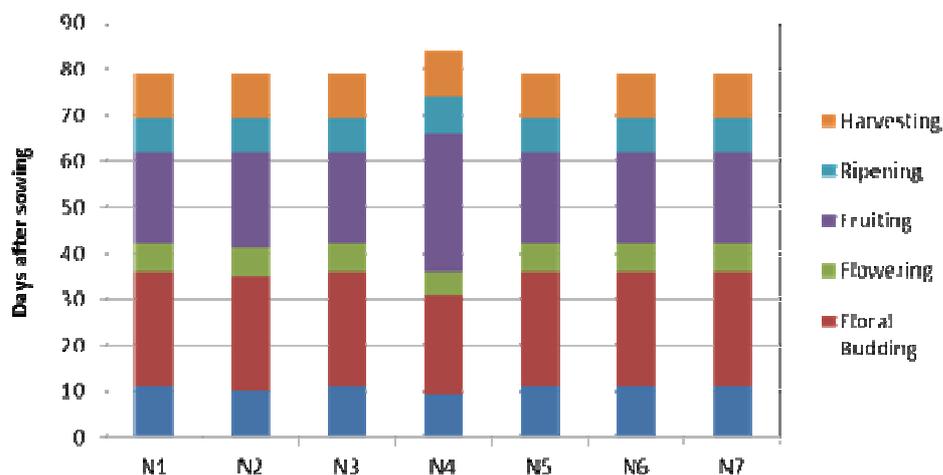
temperature conditions. Their days, percentage of germination and rate of growth was also measured to evaluate the potential variations among each line. The chlorophyll status was also recorded investigating any kind of change or difference due to underlying genotypic variations using SPADE-502 (MINOLTA, Japan), frequently applied as inexpensive and non-destructive (Uddling *et al.*, 2007; Hawkins *et al.*, 2009). The plant morphological and agronomic characteristics were closely observed, recorded and statistically analyzed as description given in the tables. Statistical analysis of the recorded data was performed through computer software SPSS III 13.0 for Windows.

Results and Discussion

The aim of the agronomical and phenological analysis of *N. sativa* was to characterize the variations within the lines that are the combinations of the genetic and biochemical functions. These variations in combinations of other morphological distinctiveness clarify and support the deviation within the *N. sativa* genotypes based on yield characteristics.

Agronomic Components and their Yields: The percentage germination of the seven genotypes of *N. sativa* was recorded by counting the days to emergence from the date of sowing to 80% of the emergence completed. All the genotypes were recorded with the similar time of emergence (11 days) except an early emergence of N2 (10 days) and N4 (9 days) genotypes. The similar pattern was recorded following the days of floral budding and days in flowering. However, in days of fruiting, ripening and harvesting the genotype N2 was recorded consistent with N1, N3, N5, N6 and N7 but N4 showed delayed considerably (Table 1). The growth pattern of the seven genotypes from germination to harvesting is presented in figure A.

Figure (A): Growth Patterns of *Nigella sativa* genotypes



Height of Plants: Therefore, noted significant ($p < 0.05$) differences among the height of all genotypes of *N. sativa* (Table 2; Fig. B). The tallest mean plant growth was recorded in N1 (62.27 cm) followed by N5 (55.07 cm), N2 (52.5 cm), N7 (49.94 cm), N6 (46.5 cm), N3 (44.54 cm) and N4 (34.89 cm) genotypes.

Dry Weight per Plants: The mean dry weight of the all lines calculated after seeds threshing and oven drying of the plants. The *N. sativa* line N3 observed with highest dry weight (9.43g) followed by N7 (8.14g), N1 (7.81g), N2 (6.86g), N5 (6.67g), N6 (5.57g) and N4 (4.13g). The dry weight is another potential yield component of any crop which influence all other components especially the seeds yield of the plant productivity (Table 2; Fig. C).

Figure B: *Nigella sativa* genotypes: Height

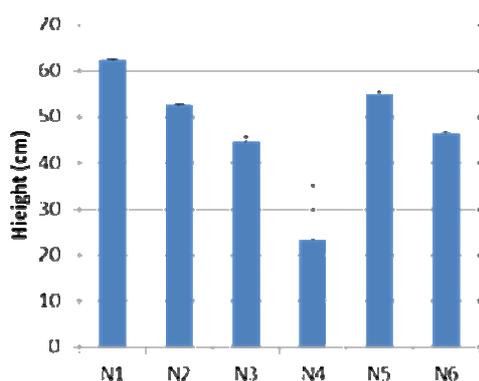
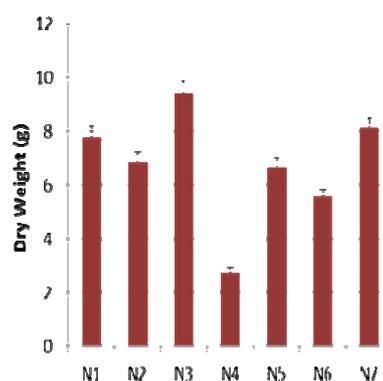


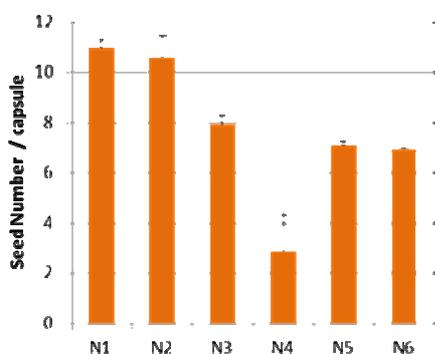
Figure C: *Nigella sativa* genotypes: Dry Weight



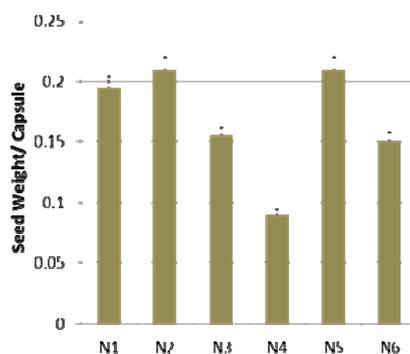
Number of Flowering Branches per Plants: The mean number of flowering branches/plant were higher in N2 (9.2) followed by N1 (8.17), N7 (7.2), N5 (7.07), N3 (5.5) and N6 (5.2) while N4 (4.2) was recorded with less number of branches (Table 2). All the genotypes showed a significant ($p < 0.05$) difference among each other in the distribution of flowering branches (Table 2).

Number of Capsule per Plant and Capsule Weight: The mean number of seed capsule/plant was found higher with N1 (10.97) followed by N2 (10.6), N7 (9.37), N3 (7.9), N5 (7.07), N6 (6.93) and N4 (4.3). Similarly in the mean total weight (Grams) of the capsules/plant, it was recorded high in case of N7 (0.25) followed by N5 (0.21), N2 (0.207), N1 (0.2), N3 (0.153), N6 (0.146) and N4 (0.17) genotypes (Table 2; Fig. A & B).

(A) *N. sativa* genotypes: Seeds Capsule No

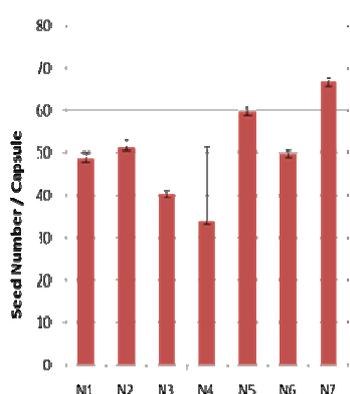


(B) *N. sativa* genotypes: Seed Capsule Wt

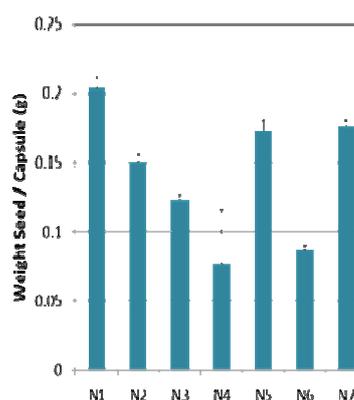


Number of Seeds and Seeds Weight per Capsule: In the mean total number of seeds the N7 count with maximum seeds number (66.7) and N3 with minimum (40.3), while the others N5 (59.7), N2 (51.3), N4 (51), N6 (49.7) and N1 (48.7) of the *N. sativa* genotypes (Table). Similarly the mean seeds weight/capsule (Grams) was recorded high with N1 (0.203) followed by N7 (0.18), N5 (0.17), N2 (0.15) and N4 (0.12) and N6 (0.08) of the *N. sativa* genotypes (Table 2; Fig. C & D).

(C) *N. sativa* genotypes: Seeds No / Capsule

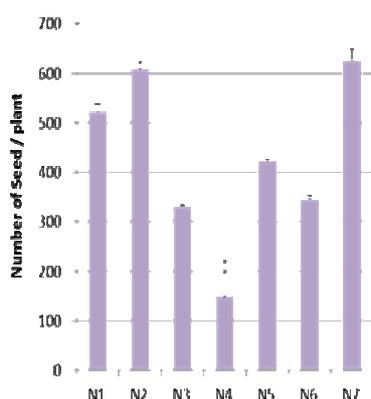


(D) *N. sativa* genotypes: Seeds Weight/ Capsule

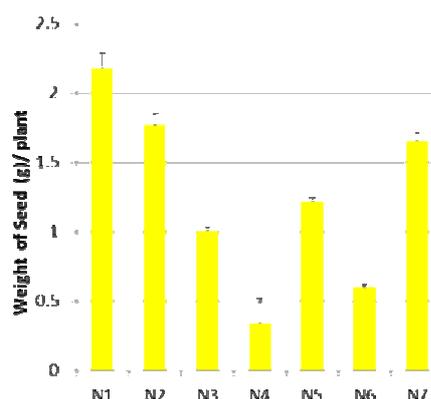


Number of Seeds and Seeds Weight per Plants: The mean number of seeds/plant was counted high with N7 (624.7) and lowest N4 (219.5) while N2 contains 607 followed by N1 (521.3), N5 (421.3), N6 (344.3) and N3 (329.3) of the *N. sativa* genotypes (Table 2). The mean total weight/plant (Grams) of the seeds was recorded highest with N1 (2.18) and lowest N4 (0.52) while the others N2 (1.8), N7 (1.7), N5 (1.22), N3 (1.00) and N6 (0.6) of the *N. sativa* genotypes (Table 2; Fig. E & F).

(E) *N. sativa* genotypes: Seeds No / Plant



(F) *N. sativa* genotypes: Seeds Weight/ Plant



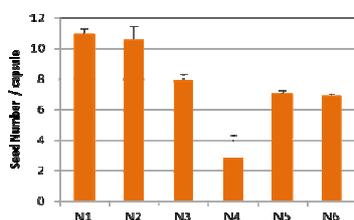
Phenology of Seeds Germination in Variable Temperature Conditions: The seven lines of *N. sativa* were also grown in Growth-Chamber at different temperature (10, 15, 20 and 25 °C) to estimate the potential of growth at variable temperature conditions. Their days, percentage of germination, chlorophyll status and rate of growth was also

measured to evaluate the potential variations among each line. All lines of *N. sativa* have taken about 10 to 11 days to germinate at 25-20 °C, however, there was noted long time to germinate all the seeds lines at 15 °C (~20-22 days) and 10 °C (~ 26-27 days). The germination time was noted to 50% germination (Time between first seed germination to the last seed germination). The mean germination time and percentage germination was recorded as described by Labouriau (1970 and 1983) under experimental conditions. The genotype N1 showed 100 percent germination at three temperature conditions except at 15 °C (95 percent) while all other genotypes were encounter with much variation. The genotypes N2, N3, N5, N6 and N7 exhibit about 80 to 100 percent except N4 recorded with 55 to 85 percent germination at four temperatures (25, 20, 15 and 10 °C). Among seven line of *N. sativa* N1 found to be the superior genotype in percentage germination while 20 °C is the best condition for germination followed by 10, 25 and 15 °C temperature (Table 3).

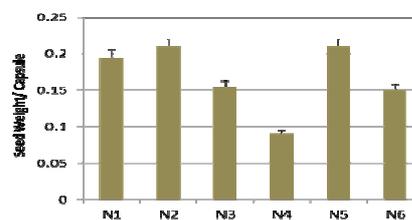
Chlorophyll Status: The chlorophyll meter give an empirical and non-destructive means of estimating foliar chlorophyll contents that promptly reports larger readings, thus instantaneous assessment of physiological variables (Hawkins *et al.*, 2009) like chlorophyll contents difference among species and relative plant health status. The greenness status or chlorophyll contents of all *N. sativa* genotypes were recorded to estimate the possible difference which on deficiency can also limit the yield. At optimal temperature conditions the chlorophyll contents improves plant vitality through increase in photosynthates productions. Higher the foliar green contents accounts for increased rate of photosynthesis which is the function of best temperature conditions. The results depicted a homogeneous chlorophyll contents values (~24.67) throughout the lines except minor variations however, N1 with high contents (26.03) and N4 lower (21.92). The results are also the indicative of positive correlation between the chlorophyll contents and yield functional components as in case of N1 with high contents and high yield similarly in case of N4 of lower chlorophyll contents and lower yield (Table 4).

Phenology of Growth in Variable Temperature Conditions: The growth recorded after two month of germination also showed great variations under different temperature conditions amongst all the seven *N. sativa* genotypes. At temperature 25 °C all the genotypes N1 through N7 about 20-22cm growth except N4 (16cm) measured from the plant soil surface to the proximal leafy-shoot end. Similarly, at temperature 20 °C growth recorded 24-29cm in all genotypes except N4 (18cm), at 15 °C from 12-13cm except N4 (9cm) and at 10 °C from 10-12cm except N4 (8cm). In terms of growth pattern behaviors, 20 °C exhibits best temperature condition for growth followed by 25 , 15 and 10 °C and N1 genotype showed superior growth behavior followed by N6, N7, N3, N5, N2 and N4 (Table 3; Fig. A, B, C, D & E).

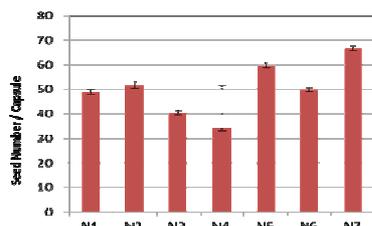
(A) *N. sativa* genotypes: Seeds Capsule No



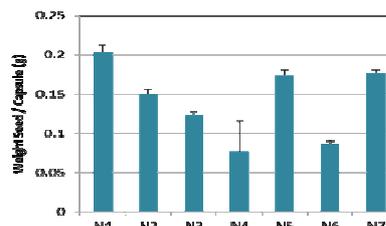
(B) *N. sativa* genotypes: Seed Capsule Wt



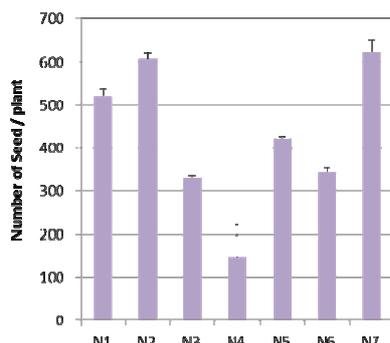
(C) *N. sativa* genotypes: Seeds No / Capsule



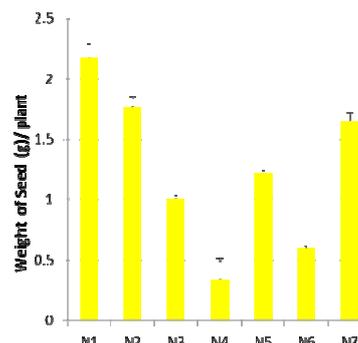
(D) *N. sativa* genotypes: Seeds Weight/ Capsule



(E) *N. sativa* genotypes: Seeds No / Plant



(F) *N. sativa* genotypes: Seeds Weight/ Plant



Although there has been seen apparently less difference among seven lines of *N. sativa* that might be is a result of continuous selfing of succeeding generation's lines. All lines were evaluated in height, number of branches, capsule number, weight, seeds numbers and weight for comparison to assess the possible variations within their lines. The data tabulated (Table 2) depicts many variations produced by these *N. sativa* lines in terms of the yield. These produced a statistically significant ($p < 0.05$) difference in average plants height, flowering branches/plant, seeds capsule/plant, seeds number/capsule, seeds weight/plant and dry weight/plant of all seven lines. Despite of some deviations there has been noted various positive interactions which correlates all lines of *N. sativa* except N4 (deviate to larger extent in all phenological components), however obscured the non-linear behavior that might be some genetic variability. The line N7 harvested with high number of seeds (624.7/plant) followed by N2 (607/plant), N1 (521/plant), N5 (421/plant), N6 (344/plant), N3 (329/plant and N4 (219/plant). But N1 line of *N. sativa* produced high yield as recorded with superior total weight of seeds/plant (2.18 g) than the other lines (Table 2).

The dry weight of N3, N7 and N1 were recorded high (9.43, 8.14 and 7.81g respectively) than other lines which showed that high dry weight is not necessarily responsible of high yield. Because N7 although with high dry weight and high seeds number per plant but less in total yield (1.653g/plant seeds weight). Similarly, N1 line with low dry weight and less seeds number per plant but produced high yield (2.18g/plant seeds weight). It shows that plants with high dry weight although produce seeds in larger number but smaller in size, hence lower weight and less yield. The results are in accordance Toncer and Kizil, (2004) who investigated that seeds number significantly influences plant height, branch per plant, capsule and seeds yield per plant. The number of capsule and number of seeds has also been found varied per plant which was proportional to the number of branches per plant (Table 2). These also support the results obtained by Tuncturk *et al.* (2005). It was investigated that seeds increases with the increase in size of capsule that is the effect of some genes which control the growth of capsule (Primack, 1987).

Planting space is also an important morphological feature that influences the agronomic characteristics. Although all lines showed better performance in height, number of branches, capsule number, weight, seeds numbers and weight as compare to other lines but N1 line proved at high rank in totality performance. An appropriate seeds sowing distances of 2 cm has positive effects on seed weight, capsule weight, stem weight, leaf weight and seed yield compared to other distances (Abdolrahimi *et al.*, 2011). Plants were grown at 16 hours day length at 22 °C ± 2 that also enhance its yield capacity. It is reported that longer day length favors better yield (Ahmad and Hague, 1986) as compared to shorter day length. In the phenological phenomenon temperature poses key factor in being phenotypic plasticity. It plays vital role in the process of germination through releasing seeds morpho-physiological dormancy. Under diverse conditions *N. sativa* have greater capability to grow even in salinity (Akram, 1999) and withstand drought conditions (Ghamarnia *et al.*, 2010) that's why best suited in arid regions of tropical and subtropical climates.

The present findings highlight the potential variations among all seven genotypes of *N. sativa* in average yield. Four genotypes (N1, N2, N5 and N7) with average mean values of plant height (49-62cm), number of branches (7-9), seeds capsule per plant (7-11), seeds weight per capsule (0.15-0.20g), seeds weight per plant (1.22-2g) and dry weight (7-8g) formed a single group while the other genotypes (N3, N4 and N6) assemble into separate due to lower values. This association into two groups might be the cause of genetic and biochemical similarities within the genotypes of each respective group. It was observed that the traits like plant height, number of branches and seeds capsule number can largely be correlated with total productivity. Plants of larger heights have a tendency to amass more than with low heights and increased seeds number have inverse effects on number of branches per plant that might be due to the interplant competition (Kizil, 2002).

Concluding Remarks

In view of the above findings it is concluded that N1 is an appropriate genotype in terms of yield and associated functions like percentage germination, growth patterns and

net productivity. N1 and allied genotypes (N2, N5 and N7) can successfully be cultivated under temperature conditions ranged 10-25 °C prevails in diverse agro-climatic areas of the region. However, incessant scientific researches still need to explore and develop genotypes which withstand under environmental stresses with high throughput (Riazuddin *et al.*, 2010). It is suggestive that more research should be carried out on the yield of different genotypic lines of *N. sativa* which clearly define the most potential cultivar-group.

Figures: Plant height at different temperature

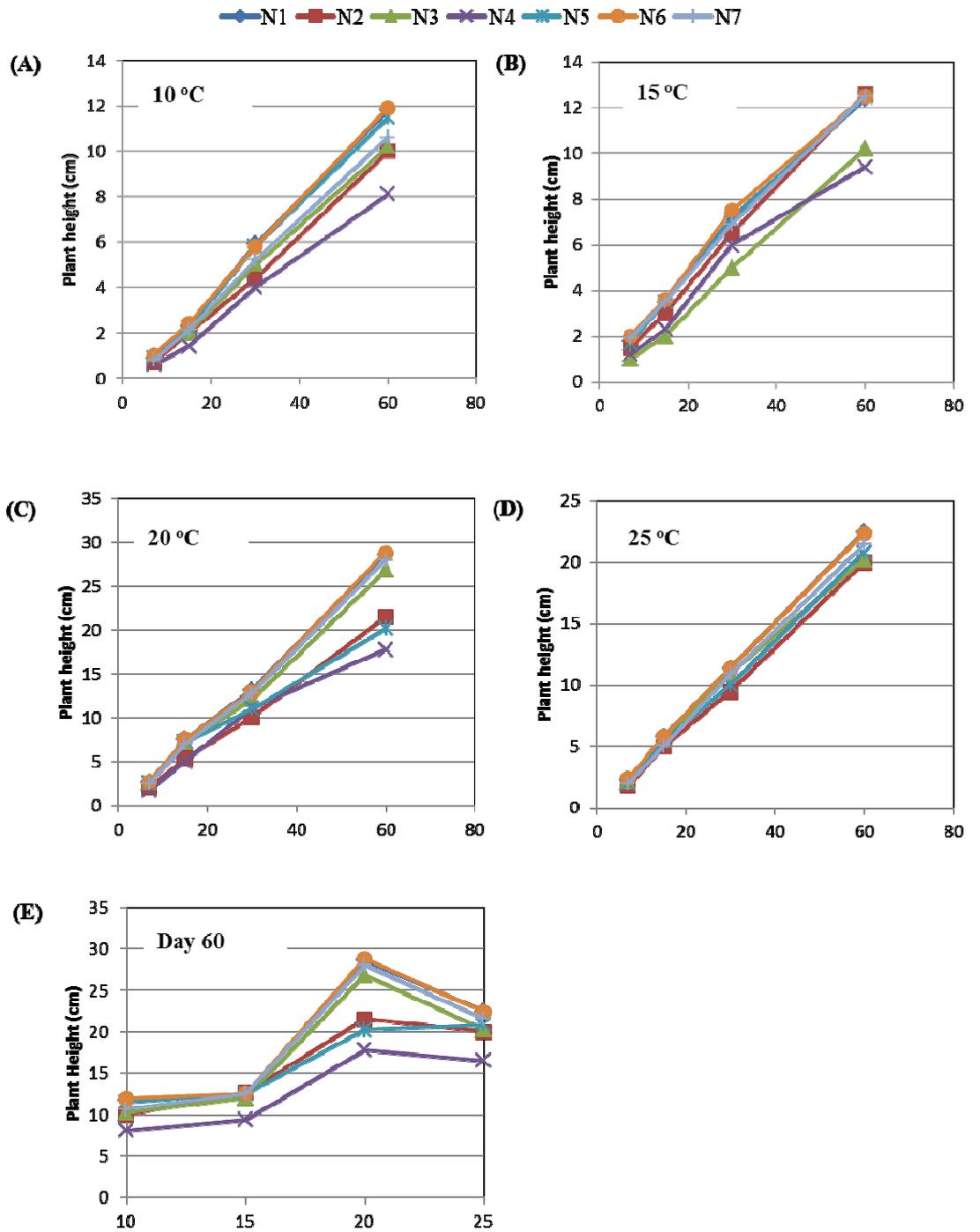


Table 1: Phenological stages of *N. sativa* seven lines grown in Glass House.

Nigella sativa 7 Lines	Date of Sown	Date of Germination	Date of Floral Budding	Date of Flowering	Date of Fruiting	Date of Ripening	Date of harvesting
N1	07.03.2012	18.03.12	12.04.12	18.04.12	08.05.12	15.05.12	25.06.12
N2	07.03.2012	17.03.12	11.04.12	17.04.12	08.05.12	15.05.12	25.06.12
N3	07.03.2012	18.03.12	12.04.12	18.04.12	08.05.12	15.05.12	25.06.12
N4	07.03.2012	16.03.12	07.04.12	12.04.12	12.05.12	20.05.12	30.06.12
N5	07.03.2012	18.03.12	12.04.12	18.04.12	08.05.12	15.05.12	25.06.12
N6	07.03.2012	18.03.12	12.04.12	18.04.12	08.05.12	15.05.12	25.06.12
N7	07.03.2012	18.03.12	12.04.12	18.04.12	08.05.12	15.05.12	25.06.12

Table 2: Agronomic Traits of *N. sativa* seven lines grown in Glass-House.

Nigella sativa 7 lines	Plant Height (cm)	Flower Branch/plant	Seeds Capsules / plant	Seeds Wt./ capsule	Seeds No./ capsule	Seeds Wt. / capsule	No. of Seeds / plant	Wt of Seeds /plant	Dry weight / plant
N1	62.27	8.17	10.97	0.20	48.7	0.20	521.3	2.18	7.81
N2	52.5	9.2	10.60	0.21	51.3	0.15	607.0	1.77	6.86
N3	44.54	5.5	7.93	0.15	40.3	0.12	329.3	1.00	9.43
N4	34.89	4.2	4.3	0.17	51.0	0.12	219.5	0.52	4.13
N5	55.07	7.07	7.07	0.21	59.7	0.17	421.3	1.22	6.67
N6	46.50	5.2	6.93	0.15	49.7	0.09	344.3	0.60	5.57
N7	49.94	7.2	9.37	0.25	66.7	0.18	624.7	1.65	8.14

Table 3: Days and percentage germination of *N. sativa* seven lines under different Temperature conditions.

Nigella sativa 7 Lines	Temperature	Days on Germination	% of Germination	Growth recorded 07 Days (cm)	Growth recorded 15 Days (cm)	Growth recorded 30 Days (cm)	Growth recorded 60 Days (cm)
N1	10C°	26	100	0.9	2.3	5.9	11.8
	15C°	20	95	1.8	3.5	7.2	12.4
	20C°	11	100	2.6	7.5	13.2	28.6
	25C°	10	100	2.3	5.8	11.4	22.5
N2	10C°	27	95	0.7	2.0	4.4	10.0
	15C°	22	90	1.5	3.0	6.5	12.6
	20C°	11	85	2.0	5.4	10.0	21.5
	25C°	10	100	1.8	5.0	9.5	20.0
N3	10C°	26	100	1.0	2.0	5.0	10.2
	15C°	22	85	1.5	3.0	7.0	12.0
	20C°	11	100	2.5	7.2	12.1	26.8
	25C°	10	80	2.1	5.5	11.0	20.2
N4	10C°	28	55	0.6	1.4	4.0	8.1
	15C°	22	70	1.2	2.3	6.0	9.4
	20C°	10	85	1.7	5.0	11.1	17.8
	25C°	9	60	1.3	4.0	8.0	16.5
N5	10C°	26	100	0.9	2.2	5.8	11.5
	15C°	20	85	1.7	3.5	7.1	12.5
	20C°	11	100	2.5	7.2	11.0	24.2
	25C°	10	90	2.0	5.4	11.0	21.8
N6	10C°	26	100	1.0	2.4	5.8	11.9
	15C°	20	80	2.0	3.6	7.5	12.5
	20C°	11	100	2.6	7.5	13.0	28.0
	25C°	10	80	2.4	5.8	11.4	22.4
N7	10C°	26	90	0.8	2.1	5.2	10.6
	15C°	20	90	1.9	3.5	6.9	12.5
	20C°	11	100	2.4	7.0	12.8	28.0
	25C°	10	100	2.0	5.0	10.9	21.5

Table 4: Chlorophyll Status of seven *N. sativa* lines Grown in Glass-House

Replicates	N1	N2	N3	N4	N5	N6	N7
R1	26.37	24.83	24.00	21.80	26.7	23.97	23.23
R2	26.20	22.93	23.30	22.95	23.43	27.87	25.20
R3	25.53	25.00	23.9	21.00	24.3	24.63	26.80
Means	26.03	24.25	23.73	21.92	24.81	25.49	25.08

All values mean of 10 plants fully expanded leaves from near the top (About 3 nodes down the apex)

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