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Determination of Effective Method of NPK Fertilization in Pea (*Pisum sativum* L.) Cultivars Grown in Pakistan

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ABSTRACT

Background: Nitrogen, phosphorus and potassium (NPK) are amongst the fundamental macronutrients, which are crucial for the growth and development plants. There is lack of information for the most effective method for NPK fertilization in pea cultivars grown in Pakistan. In most of the cases, NPK is applied as soil application rather than foliar spray.

Methods: Experiments were conducted to find out the most effective method of NPK fertilization i.e. soil application or foliar spray on three pea cultivars (Aleena, Climax and Meteor). Experiment was arranged in a completely randomized design with 4 replicates and data was recorded at different growth stages.

Result: Soil and foliar spray of NPK showed significant results for the enhancement of root and shoot development, foliage growth, relative growth rate (RGR), net assimilation rate (NAR), photosynthetic pigments, total carbohydrate contents and soluble proteins. Antioxidant activities i.e. catalases (CAT), peroxidase (POD) and yield attributes increased significantly with NPK applications. NPK foliar application showed better result only for the higher relative growth rate (RGR) and net assimilation rate (NAR). It was noted that NPK soil applications were more effective for the improvement of growth, quality and yield of pea as compared to foliar spray. Climax and Aleena cultivars were better in relation to growth, quality and yield than Meteor with soil NPK application. Changes in antioxidant activities by NPK significantly increased the defense mechanism in plants resulted improved in plant metabolism that increased the growth, yield and quality of pea. It was concluded that soil application of NPK was more effective for higher productivity of pea than foliar use.

Key words: Antioxidants, Growth, NPK, Pea, Yield.

INTRODUCTION

Pea (*Pisum sativum* L.) belongs to family Fabaceae producing high quantity of edible proteins (23-33%) along with vitamins and carbohydrates. Peas are also rich in carbohydrates with range of 21-25% in addition to supplementary nutrition (Gatti *et al.*, 2011). Pea ranks fourth in the world amongst legumes subsequent to soybean, French beans and groundnut on production basis (Jarosalva *et al.*, 2011).

Nitrogen, phosphorus and potash (NPK) fertilizers are important source of nutrients that is obtained by plant from the soil (Tanimu, 2013). Vegetative growth is considerably increased with the adequate supply of NPK and these nutrients are also helpful in photosynthesis because NPK is involved in chlorophyll synthesis (Futuless *et al.*, 2011). NPK are amongst the essential macronutrients, which plays crucial role for the enhancement of growth in the plants. Nitrogen enhance the synthesis of photosynthetic pigments, modifies the molecular structure of protein and some specific amino acids (Khalid, 2013). Potash is very important component that significantly improve the growth of the plants. P considerably increased the root growth and it also promoted the plant maturity (Akhtar *et al.*, 2003). K also enhances the root functioning such as uptake of water and permeability of soil and also works as a regulator of stomata for opening of guard cells and played an important role in improving the use of water proficiency (Tsegaye *et al.*, 2018).

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There are different opinions about the method of NPK application in plants. According to few researchers, various factors affect the effectiveness of foliar sprays, such as environmental factors that can effect on the absorption of foliar nutrient (Khalid, 2013). According to Fernandez and Eichert (2009), plants can uptake nutrients through rooting medium in a form of small molecules having no electric charge on it and translocation normally occurs by roots. However, fertilizers in the form of foliar spray can only be used when the availability of nutrients in the soil is limited. It was revealed from different studies that soil application produced higher NPK accumulation in underground storage

organs that was helpful for attaining better growth and yield in plants than foliar fertilization (Fernandez *et al.*, 2013).

In the view of above mentioned literature, this research was carried out to find the best method of NPK application for higher crop productivity in pea cultivars that are extensively grown in Pakistan.

MATERIALS AND METHODS

This experiment was carried out at botanical garden of University of Gujrat, Pakistan during 2018-19. Seeds of three pea cultivars (Aleena, Climax and Meteor) were obtained from Punjab Agriculture Department, Gujranwala, Pakistan. Seeds were sown during October, 2019 in sandy loam soil. After 21 days of sprouting treatments of nitrogen, phosphorus and potassium (NPK) were applied:

T_0 = Control.
T_1 = NPK foliar application (18:18:18% W/V).
T_2 = NPK soil application (18:18:18% W/W).

Experiment was arranged in a completely randomized design with 4 replicates. Four sets of plant samples were collected for recording observations at vegetative (21 days after treatment) and maturity (42 days after treatments) on like root and shoot lengths, fresh and dry weights, number of leaves, leaf area, leaf area ratio, activities of catalase and peroxidase, contents of chlorophyll and carotenoid, relative growth rate (RGR), net assimilation rate (NAR), carbohydrates and proteins contents in seeds. Number of pod, seeds per pod and weight of seeds were determined at maturity.

Leaf area was determined with portable Handheld Leaf Area Meter (CI-203 Laser scanner). Chlorophyll a, b, total chl and carotenoids were determined by the method of Arnon (1949). For determination of carbohydrate, 10 ml of 80% ethanol was used to grind fresh seeds (1g), extract was diluted with water and final volume was made up to 10 ml. Concentrated H_2SO_4 (96%) was added in test tubes (5ml) and gently shaked and incubated it at room temperature for 40 min. About 1 ml of 5% phenol was added to each tube and reading was taken from spectrophotometer at 490 nm. Fresh seeds (1g) were ground with 50 mM of potassium phosphate buffer having pH 7.8 in a cool environment. Centrifugation was done at 10,000 RPM at 4°C for about 15 min. Bradford reagent (2 ml) was mixed with 0.1ml of centrifuged extract and the reading was taken at 590 nm using spectrophotometer.

Estimation of catalases (CAT) and peroxidase (POD) activities were determined by the procedure of Chance and Maehly (1955). Fresh pea leaves (5g) were crushed with the volume of 5ml of 50mM cooled Phosphate buffer (pH 7.8) which was then placed in an ice bath for the accurate extraction of antioxidant enzymes. After that crushed samples were centrifuged at 15,000 RPM at 4°C for 20minutes. For the determination of enzymes activities supernatant was used. A solution of (3ml) was prepared to determine the antioxidant enzyme activity by adding 50 mM phosphate buffer (pH 7.0), 5.9 Mm H_2O_2 and 0.1ml extract. A change in the absorbance of CAT solution was determined at 240 nm. To characterize one unit activity of CAT absorbance change of 0.01 units per minute was utilized. Peroxidase dismutase (POD) solution of (3 ml) was made with 50 mM phosphate buffer (pH 5.0), 20 mM guaicol in addition with 40 mM H_2O_2 and with 0.1 ml enzymes extract to check the antioxidant activity. Changes in the absorbance of the solution was determined at 470 nm.

COSTAT computer software package was used to determine ANOVA (Analysis of Variance) and means were compared done by Duncan's New Multiple Range test at probability level of 5% (Steel and Torrie, 1986).

RESULTS AND DISCUSSION

Root development

Data obtained from the trial suggested that root development of pea at vegetative and maturity stages showed that the main effect of foliar spray and soil application of NPK on root length, its fresh and dry weights was significant (Table 1). Higher root length was observed in cultivar climax when NPK applied in soil medium at both growth stages (Fig 1A). Maximum increase in root fresh weight at both growth stages was recorded in Aleena cultivar when NPK was applied in soil (Fig 1B). It was noted that cultivars Aleena and Climax produced higher root dry biomass at vegetative and maturity stages with soil application of NPK (Fig 1C).

Table 1: Mean squares (MS) from analysis of variance (ANOVA) for root development in pea due to NPK application.

Source of Variance	df	Root length		Root fresh weight		Root dry weight	
		Vegetative stage	Maturity stage	Vegetative stage	Maturity stage	Vegetative stage	Maturity stage
Foliar spray NPK (FS)	1	148.202***	165.125***	0.2138**	0.2271***	0.0209**	0.0302***
Soil applicationNPK (SA)	1	54.765**	46.763*	0.1761*	0.2112*	0.0105*	0.054*
Variety (Var)	2	76.535***	60.166**	0.0019*	0.0138***	0.0117**	0.0067*
FS × SA	1	4.435ns	2.541ns	0.0367ns	0.0053*	0.0024ns	0.0022*
FS × SA × Var	2	12.4562**	121.652*	1.6532*	0.4832*	0.0572*	0.0572*
Error	28	5.235	4.2222	0.0426	0.0245	0.0018	0.0031
Total	35						

ns =non-significant levels; *,**,*** significant at 0.05, 0.01 and 0.001 levels, respectively.

Shoot development

Effect of foliar spray as well as soil application of NPK was highly significant on shoot development of pea (Table 2). Higher shoot length was observed in cultivar Aleena at vegetative stage and at maturity, cultivar Climax had higher shoot length with the soil application of NPK (Fig 2A). It was noted that Aleena had higher shoot fresh weight at vegetative stage while at maturity cultivar Climax produced higher shoot fresh weight with soil NPK application (Fig 2B). For shoot dry weight, cultivar Aleena showed the higher increase in dry weight with the soil application of NPK (Fig 2C).

Foliage growth

Analyzed data for number of leaves, leaf area and leaf area ratio of pea showed that soil and foliar application of NPK had highly significant effects on these attributes (Table 3). Higher number of leaves was counted in Aleena at both stages when NPK was applied in soil medium (Fig 3A). It was observed that cultivars Climax and Aleena had higher area with soil application of NPK (Fig 3B). In case of leaf area ratio, cultivar Meteor showed the higher values as compared to other cultivars with foliar application of NPK (Fig 3C).

Antioxidant activities

Soil application of NPK had highly significant results for catalases (CAT) and peroxidase (POD) activities, whereas, with foliar application results were significant at both stages (Table 4). CAT activity was considerably enhanced with the soil application of NPK in Climax at vegetative stage while

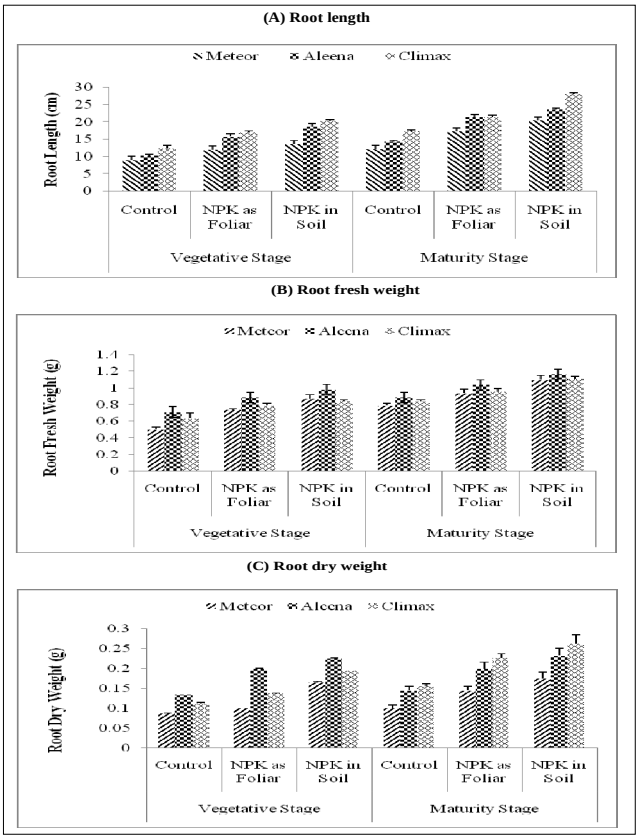


Fig 1: Development of root in pea due to foliar and soil application of NPK.

Table 2: Mean squares (MS) from analysis of variance (ANOVA) for shoot development in pea due to NPK application.

Source of Variance	df	Shoot length		Shoot fresh weight		Shoot dry weight	
		Vegetative stage	Maturity stage	Vegetative stage	Maturity stage	Vegetative stage	Maturity stage
Soil applicationNPK (SA)	1	376.880***	535.111***	133.291***	220.162***	3.054***	7.487***
Foliar spray NPK (FS)	1	321.238**	456.783**	76.894**	87.431*	2.875**	4.567*
Cultivar (C)	2	338.084***	428.131***	48.552***	96.951***	3.052***	1.858***
SA × FS	1	23.501*	28.986**	12.407**	24.572***	0.801***	0.745***
SA × FS × C	2	131.672**	432.675**	99.510**	65.252*	3.576'*	3.052**
Error	28	8.4942	5.0486	2.254	1.944	0.082	0.0983
Total	35						

ns =non-significant levels; *,**,*** significant at 0.05,0.01 and 0.001 levels, respectively.

Table 3: Mean squares (MS) from analysis of variance (ANOVA) for foliage growth in pea due to NPK application.

Source of Variance	df	Number of leaves		Leaf area		Leaf area ratio	
		Vegetative stage	Maturity stage	Vegetative stage	Maturity stage	Vegetative stage	Maturity stage
Soil applicationNPK (SA)	1	170.111***	188.861***	3968.69***	6033.44***	1234.08***	1964.20***
Foliar spray NPK (FS)	1	45.672*	65.687*	432.612**	1043.672**	3214.562**	1154.765*
Cultivar (C)	2	80.778***	72.5277***	537.52*	573.027**	8472.48***	2594.41***
SA × FS	1	3.111ns	3.6111ns	414.61*	213.944ns	194.29*	168.288*
SA × FS × C	2	76.439*	34.981*	763.841*	872.653ns	642.761*	652.532*
Error	28	5.592	4.6574	107.629	100.314	308.23	74.7156
Total	35						

ns =non-significant levels; *,**,*** significant at 0.05,0.01 and 0.001 levels, respectively.

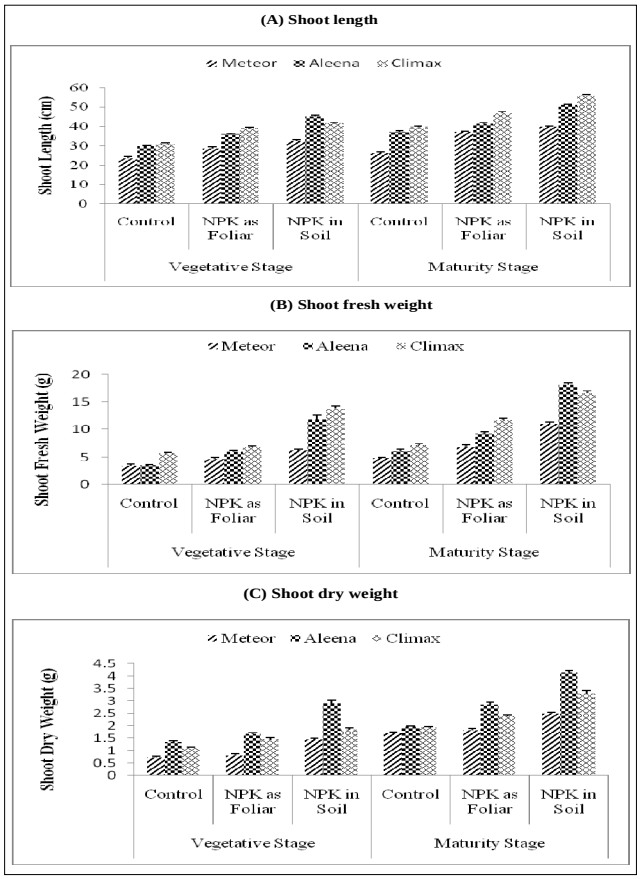


Fig 2: Development of shoot in pea due to foliar and soil application of NPK.

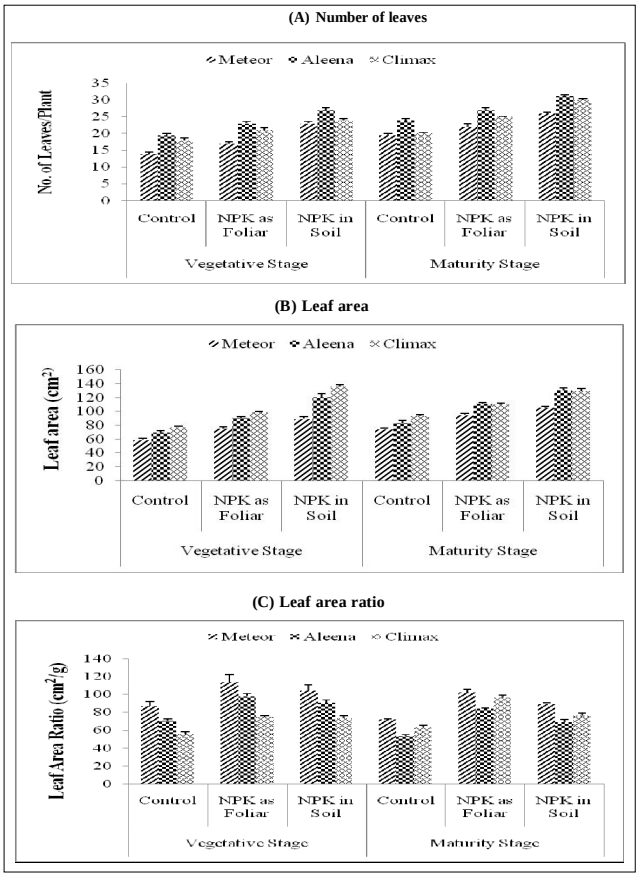


Fig 3: Foliage growth in pea due to foliar and soil application of NPK.

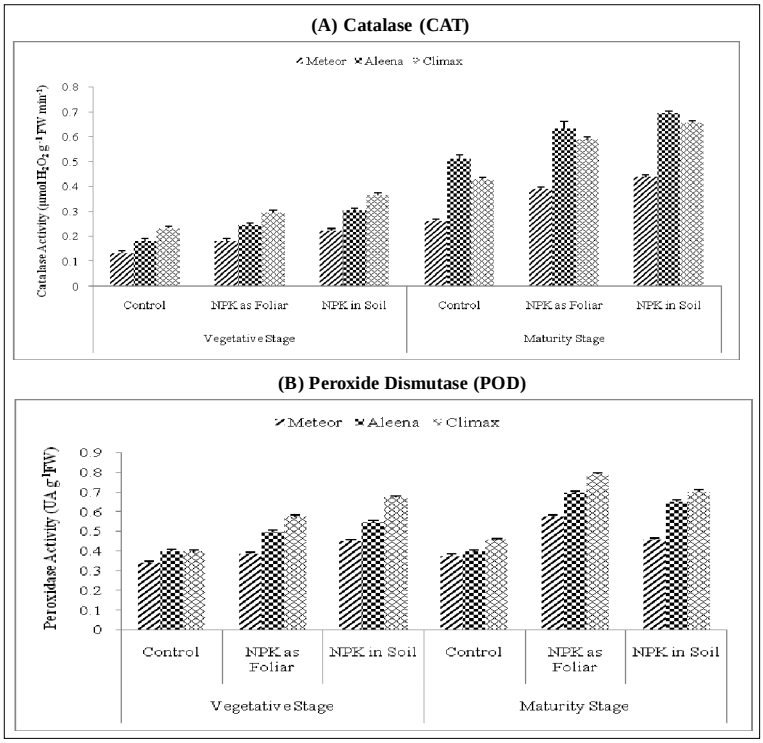


Fig 4: Antioxidant activities in pea due to foliar and soil application of NPK.

at maturity cultivar Aleena had higher CAT activity at both stages (Fig 4A). Significant increase in POD activity was observed in climax at vegetative stage with soil NPK and at maturity with foliar spray of NPK (Fig 4B).

Photosynthetic pigments

There was a significant result of NPK (soil and foliar spray) on chlorophyll a and b contents in pea, while it was highly significant in case of total chlorophyll and carotenoids (Table 5). Chlorophyll a and b contents significantly increased in Aleena and Climax at both stages when NPK was applied in soil medium. (Fig 5A-B). Higher increase in total chlorophyll content was observed in Climax at vegetative stage with soil application while at maturity, Aleena had higher total chlorophyll contents with the foliar spray (Fig 5C). Carotenoids content was significantly enhanced in climax at vegetative stage with soil NPK while at maturity, Aleena had higher carotenoids with foliar spray of NPK (Fig 5D).

Physiological activities

Effect of soil application of NPK had significant result on RGR (relative growth rate) and NAR (net assimilation rate) of pea (Table 6), whereas the interaction between foliar and soil application was non-significant. Higher RGR of pea was noted with the foliar application of NPK in Aleena (Fig 6A). Maximum increase in NAR of pea was observed in Aleena with foliar spray of NPK (Fig 6B).

Biochemical attributes

It was noted that NPK soil application had highly significant effects on carbohydrate contents in pea seeds. While, NPK applied as foliar spray had significant results on total carbohydrate contents (Table 4). Higher carbohydrate content was found in Aleena with foliar spray of NPK (Fig 7A). Soil application of NPK had highly significant effects on total soluble proteins, while there was significant results with foliar spray of NPK. Maximum increase in total soluble protein was recorded in Climax (Fig 7B).

Yield attributes

It was observed that different yield attributes i.e. number of pods per plant, number of seeds per pod and total seeds weight per plant were highly significant in the foliar and soil application of NPK (Table 6). Pea yield significantly increased

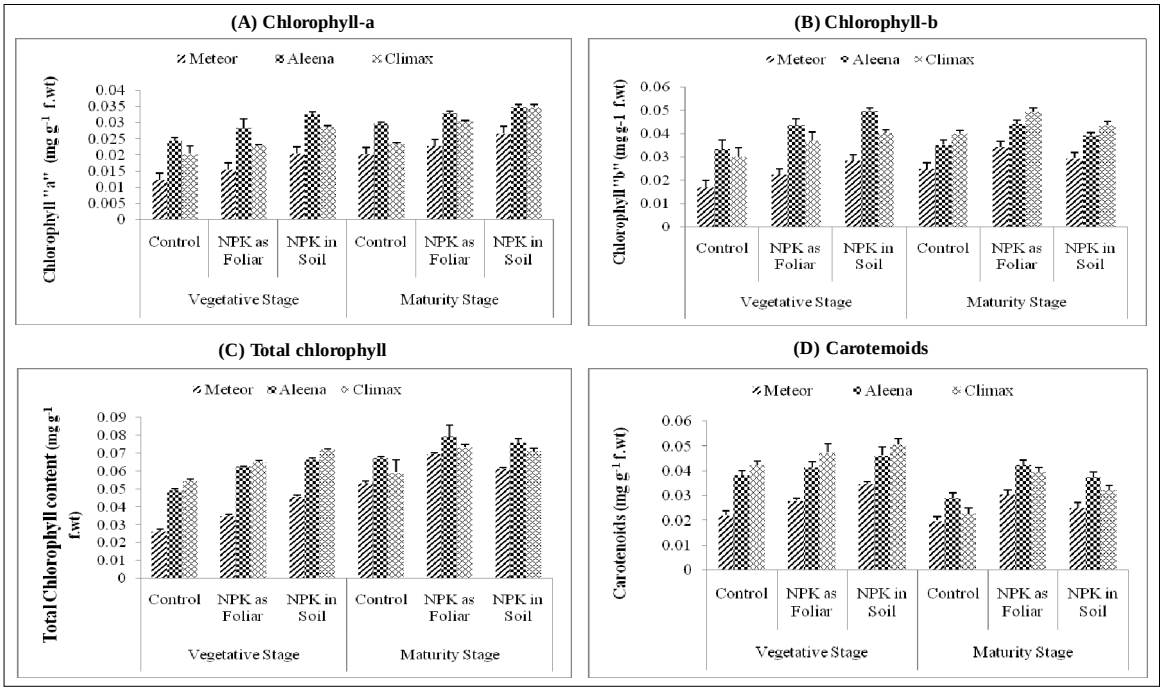


Fig 5: Concentrations of photosynthetic pigments in pea due to foliar and soil application of NPK.

Table 4: Mean squares (MS) from analysis of variance (ANOVA) for antioxidant activities in pea due to NPK application.

Source of Variance	df	Catalases		Peroxidase	
		Vegetative stage	Maturity stage	Vegetative stage	Maturity stage
Soil application NPK (SA)	1	0.0302***	0.1475***	0.0709***	0.2046***
Foliar spray NPK (FS)	1	0.0172**	0.1132*	0.0542*	0.2183**
Cultivar (C)	2	0.0074***	0.2262***	0.0488***	0.0936***
SA x FS	1	0.0013***	0.0083***	0.0057**	0.0064***
SA x FS x C	2	0.0091*	0.0052**	0.0531*	0.0072**
Error	28	8.5648	1.4522	9.3553	2.2270
Total	35				

ns =non-significant levels; *,**,*** significant at 0.05,0.01 and 0.001 levels, respectively.

Table 5: Mean squares (MS) from analysis of variance (ANOVA) for photosynthetic pigments in pea due to NPK application.

Source of Variance	df	Chlorophyll-a		Chlorophyll-b		Total chlorophyll		Carotenoids	
		Vegetative stage	Maturity stage	Vegetative stage	Maturity stage	Vegetative stage	Maturity stage	Vegetative stage	Maturity stage
Soil applicationNPK (SA)	1	4.670*	2.149*	3.711*	2.154*	0.0014***	7.761**	1.1202***	4.093***
Foliar spray NPK (FS)	1	2.482*	2.583*	0.1943*	3.762*	0.0034**	3.281**	0.652**	1.362**
Cultivar (C)	2	4.108***	2.371**	0.042***	6.433***	0.0035***	2.281**	0.0013***	1.291***
SA x FS	1	1.592ns	3.113ns	5.620ns	2.970ns	4.9193*	1.142ns	3.3045***	2.273***
SA x FS x C	2	6.095ns	3.641ns	7.612ns	3.921*	8.510**	2.532ns	0.541**	3.712*
Error	28	2.595	4.311	7.594	1.176	1.3979	1.161	3.756	1.044
Total	35								

ns = non-significant levels; *,**,*** significant at 0.05,0.01 and 0.001 levels, respectively.

Table 6: Means square (MS) from analysis of variance (ANOVA) for physiological activities, quality and yield of pea due to NPK application.

Source of Variance	df	Relative growth rate	Net assimilation rate	Total carbo-hydrates in seeds	Total soluble proteins in seeds	Number of pods/ plant	Number of seeds /pod	Seed yield /plant
Soil applicationNPK (SA)	1	0.0038***	3.1008**	0.3447***	0.1342**	31.083***	298.587***	73.562***
Foliar spray NPK (FS)	1	0.0024**	2.314*	0.2312*	0.1232*	22.452**	88.641*	31.419*
Cultivar (C)	2	1.1461**	1.3725*	0.0623***	0.1325**	11.083***	74.5424***	21.353***
SA x FS	1	1.9114ns	3.2083ns	0.0039**	0.0032***	2.7916**	26.733***	5.7982***
SA x FS x C	2	2.752ns	6.832ns	0.0643**	0.00341**	4.710**	231.762**	7.7219**
Error	28	2.9334	6.7518	6.6629	1.0435	0.537	3.0714	0.4826
Total	35							

ns = non-significant levels; *,**,*** significant at 0.05,0.01 and 0.001 levels, respectively.

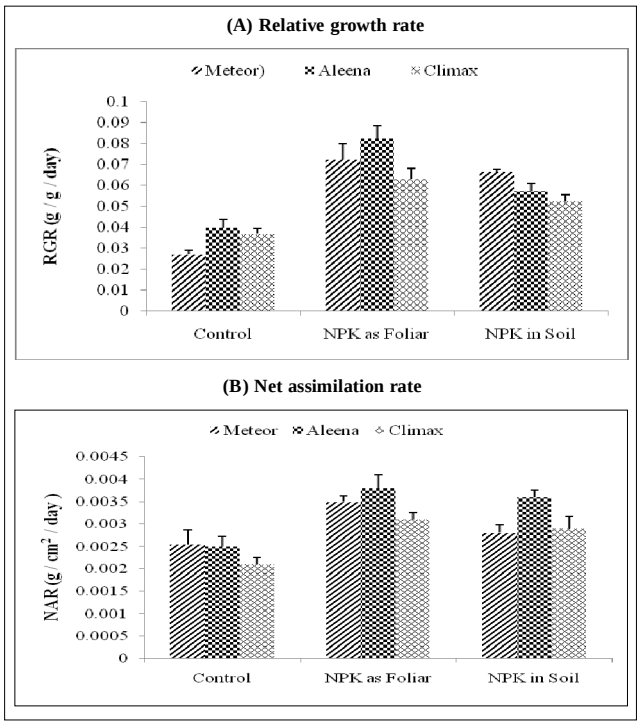


Fig 6: Physiological activities in pea due to foliar and soil application of NPK.

by NPK soil application. Higher increase in the pea pods production was observed with soil application of NPK in Climax (Fig 8A). Soil application of NPK also extensively increased the seeds number per pod in Aleena and Climax (Fig 8B). Higher seed weight was recorded in cultivar Aleena with soil application of NPK (Fig 8C).

It was observed that both methods of NPK application were effective for enhancement of pea productivity but soil application was more effective for higher growth and yield. It may be due to that roots system have the specialized structure that works efficiently in plant for the uptake of nutrients from soil rather than absorption of nutrient through foliage treatments (Fernandez and Eichert, 2009).

Various factors can effect on the effectiveness of foliar nutrient sprays, such as environmental factors have an effect on the absorption and assimilation of foliar nutrient sprays by influencing their responses on plant (Fernandez *et al.*, 2013). Plants can absorb and translocate nutrients through root via soil application in a form of molecules having no electric charge. Translocation of nutrient in plants normally occurs by roots, however, fertilizers in the form of foliar spray can only be used when the availability of nutrients in the soil is limited (Khalid, 2013). It was noted that soil NPK application was helpful for attaining better growth, yield and quality in plants than foliar fertilization (Fernandez *et al.*, 2013).

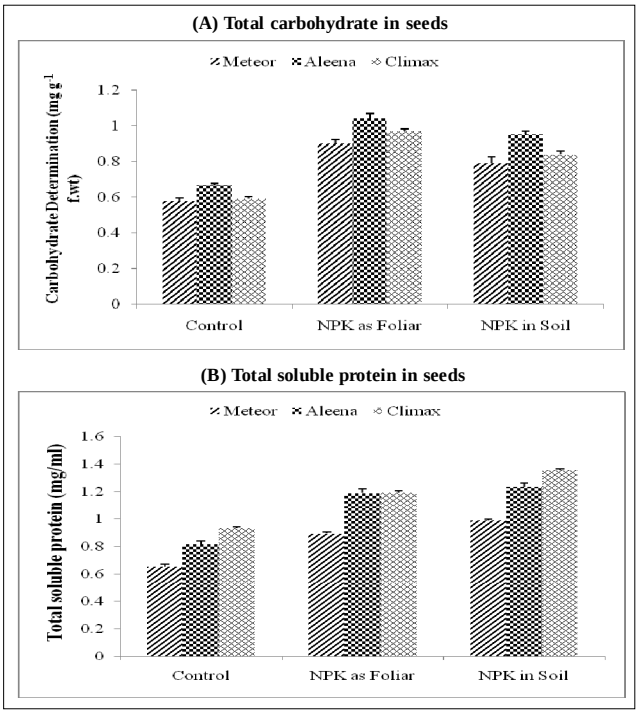


Fig 7: Total carbohydrate and protein contents in seeds of pea due to foliar and soil application of NPK.

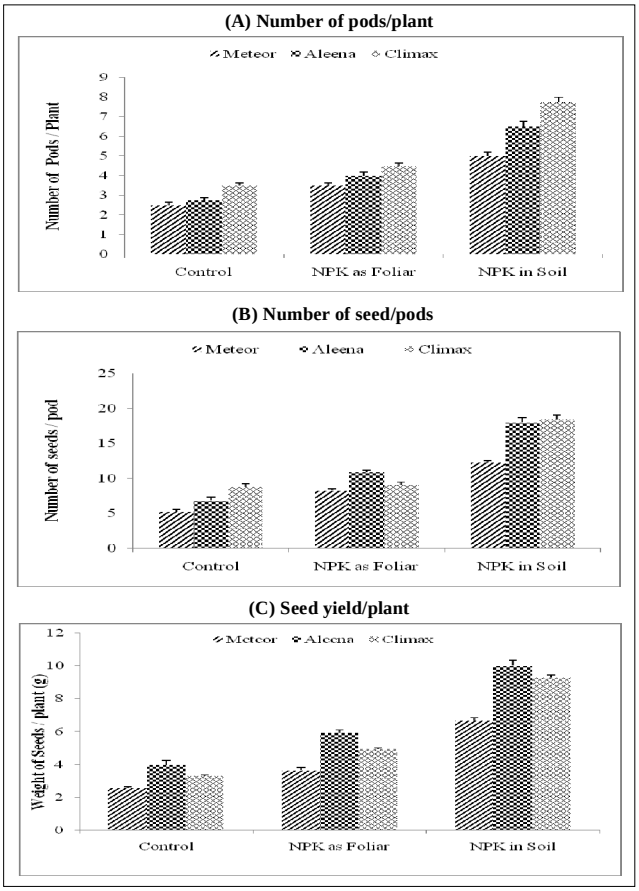


Fig 8: Yield attributes of pea due to foliar and soil application of NPK.

Similarly, Omotoso and Shittu (2007) confirmed that NPK applied in rooting medium significantly improved the growth attributes of okra including length of shoot and root, its fresh and dry weights and its yield. Okonwuh and Mensah (2012) described that NPK in soil application increased the leaf area, root, shoot fresh and dry weight and no. of leaves. RGR and NAR values were gradually increased with NPK fertilizer in pepper and eggplant (Balliu *et al*., 2007).

It was also noted that NPK had significant effects on photosynthetic pigments and antioxidant activities of pea. Peyvast *et al.* (2008) reported that significant increase in the production of chlorophyll a, b and total chl in plants was noted with the use of NPK. Abbas and Ali (2011) demonstrated that with the utilization of NPK through roots enhanced the total carbohydrates contents in roselle plant. NPK in soil application increased the total soluble protein contents in pea (Tisdale *et al.,* 1995). It was already described that NPK in soil considerably improved the antioxidant activities of (POD and CAT) enzymes (Gurgul and Herman, 1994). Due to the soil application of NPK, yield of pea increased because root system helped efficiently to uptake the nutrients from soil. According to Jaipaul *et al.* (2011) NPK in soil medium, considerably increased the number of pods and total seed yield of garden pea.

CONCLUSION

It was concluded that soil application of NPK was more effective for higher productivity of pea than foliar use. Climax and Aleena cultivars were best in relation to growth, quality and yield than Meteor with soil NPK application. NPK was useful for the enhancement of pea productivity.

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