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Research Article

Effect of FYM and NPK on growth, yield and oil production of mustard under the agro-climatic condition of Tandojam-Pakistan

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Abstract

In order to study the effect of FYM and NPK on growth, yield and oil production of mustard, Five treatments were formed including control T_1= Natural (Farmer practice), T_2= Recommended NPK 120-80-40 kg ha^{-1}, T_3= FYM 1 t ha^{-1} + NPK 120-80-40 kg ha^{-1} , T_4= FYM 3 t ha^{-1} + NPK 120-80-40 kg ha^{-1}, T_5= FYM 5 t ha^{-1} + NPK 120-80-40 kg ha^{-1} and T_6= FYM 7 t ha^{-1} + NPK 120-80-40 kg ha^{-1} results showed that significantly ($P<0.05$) of seed yield and mustard of oil content influenced through the FYM & NPK levels. Results further showed that the mustard crop given recommended NPK 120-80-40 kg ha^{-1} in crop performance with 134.33 cm plant height, 188.37 no of pods plant^{-1}, 11.31 no of seeds pod^{-1}, 3.42g in seed index, 1510.21 kg seed production ha^{-1} and 31.10% oil content. However the minimum lowest crop performance was observed in control, where no fertilizer ware applied, having 128.33 cm plant height, 145.41 number of pods plant^{-1}, 8.12 number of seeds pods^{-1}, 3.00g seed index, 1325.14kg seed yield production ha^{-1} and 26.35% content oil. It is concluded that mustard yield increase with NPK combined with FYM 120-80-40 + 7 ton FYM but 5 ton FYM combined with NPK120-80-40 was economic dose for mustard production. The oil production also increase at combined application of FYM and NPK fertilizers.

Keywords: FYM; Growth and yield; Mustard; NPK; Oil production

Introduction

Mustard (*Brassica juncia* L.) are important oilseed crops which rank third in vegetable oils after soybean and palm while second in oilseed proteins production after soybean in the world **[1]** . The global production of

rapeseed-mustard (*Brassica juncia* L.) was 62.45mt from an acreage of 33.64mha with a total productivity of 18.56 q/ha [2]. Pakistan economy mainly based on agricultural production, inappropriately 80% edible oil was imported for consumption in 2012-13, as local edible oil production of 20 % of the domestic demand. During 2012-13 the overall edible oil was 3.069 million tons. Native edible oil production remained figure is 0.606 million tons though 2.502 million tons were import. In 2012-13 import bill stood at Rs. 241.936 billion. During 2013-14 in July and March 1.719 million tons edible oil valued Rs. 148633 billion imported. In 2013-14 local oil production was 0.606 million tons and overall edible oil availability through overall source was estimated 2.325 million tons in 2013-14 [3]. It plays significant role in the development of seed. An oil seed crop requires Sulphur comparatively higher than other nutrient and it is now being recognized as the fourth major element of the plant. Primary nutrient of plants are nitrogen, phosphorus and potassium. Phosphorus plays a vital role in photosynthesis, respiration, cell conclusion cell enlargement and several other processes in living plants [4]. Application of potassium upto 60 kg ha^{-1} also significantly increased grain yields of Indian mustard. High potassium application of increase in grain yield reported by [5]. Seed yield and yield attributes of Indian mustard increased with increasing level of nitrogen and phosphorus upto 120 and 80 kg ha^{-1} respectively. It was also reported in maize-mustard sequence, FYM gave highest seed yield and quality of the oil [6]. Significant increase in protein and oil content in mustard seed due to application of 150 kg N ha^{-1} compared to 50 kg N ha^{-1} [7].

Thus, adequate nutrient supply increases the seed and oil yields by improving the setting pattern of siliquae on branches, number of siliquae per plant and other yield attributes. Farmers most commonly use di ammonium phosphate that supplies only nitrogen and phosphorus. So, for obtaining maximum efficiency of added fertilizer, balanced fertilization to crops including use of potassium and micronutrients, is essential. Micronutrient helps in photosynthetic activities and proper utilization of nitrogen and phosphorus [8]. Application of nutrients and its management is crucial agronomic practice that has a profound influence on Indian mustard's (*Brassica juncia* L.) yield. The soil physico-chemical properties can be improved by application of Farm yard manure (FYM) which results in improvement of necessary nutrients; eventually the crop yields increases [9, 10]. Indian mustard is more receptive to chemical fertilizers i.e. nitrogen and to some extent to sulfur, for better mustard production considering soil health. Day by day due to increasing prices of chemical fertilizers, there is urgent need to explore suitable substitute nutrients especially Nitrogen. The key to success is the assimilation of all possible nutrient sources for growing demand of oil seeds and by the more production of oil seed which lessen the load on the foreign exchange as oil import is costing highly. Addition of chemical fertilizers, Farm Yard Manure plus Biofertilizers could be a step forward, as they are low cost, highly nutritive for crops and inexhaustible. Some potential bio-fertilizers are Azospirillum and Azotobactor which are non-symbiotic bacteria, having the ability of adding Nitrogen to legumes by tapping nitrogen from air. The action of bio-fertilizers is subjective to be influenced with the addition of nutrients like Nitrogen. The present investigation was therefore investigated to know the effect of integrated nutrient management on mustard's (*Brassica juncia* L.) performance. The objectives of this study were to determine the effects of K with addition of different nutrients on

mustard's attributing traits, quality parameters and yield.

Materials and methods

The research experiment was conduct at Student's experimental farm, Sindh Agriculture University, Tandojam-Pakistan during Rabi, 2016-17. Three (3) year old FYM used in experimental plot. Recommended dose of NPK will applied in each treatment. Following details are as under:

Experiment research design = Randomized complete block design (RCBD)

Replication = Three

Name of variety = S-9

Plot size = 4m x 3m = ($12m^2$)

Following six treatments of FYM and NPK levels

T_1= Farmer practice (Natural)

T_2= Recommended ratio of NPK 120-80-40 kg/ha

T_3= FYM 1 t ha^{-1} + NPK 120-80-40 kg/ha

T_4 = FYM 3 t ha^{-1} + NPK 120-80-40 kg/ha

T_5 = FYM 5 t ha^{-1} + NPK 120-80-40 kg/ha

T_6 = FYM 7 t ha^{-1} + NPK 120-80-40 kg/ha

Observations to be recorded

1. Height of plant cm
2. Nos of pods per plant
3. Number of seeds per pod
4. Seed index in 1000 seed wt. (g)
5. Seed yield production kg/ha
6. Oil content (%)

Observations recording methodology

Height of plant cm: Measure at heading stage of crop through measuring tape from bottom to tip of plant in labeled plants in individual treatment.

Nos of Pods per plant

The pods counted per plant was at maturity stage of crop in labeled plants in individual treatment. The sum were then divided with the total number of plants.

No of seeds per pod

Selected labeled plant from the field at maturity of the crop plants than counted the seeds each pods from selected plant.

Seed index in 1000g seeds

Recorded manually 1000 seeds weight through including in individual treatment and weighted observed seed index grams.

Seed yield production kg/ha

After harvesting of crop, the total seed production of each treatment in all replications were weighted and divided by number of replications to achieve the mean seed yield (kg) $plot^{-1}$. Later, on the basis of seed yield $plot^{-1}$, the seed yield (kg ha^{-1}).

Oil content %

The oil content percentage was recorded under the laboratory of oil seed extracting from lab instruments first the seeds of mustard were taken and grinded by a grinding machine. The 3g of grinded seeds sample was weighed and put into the filter paper than the seed sample was put into the extractor tube of soxhlet machine with the help of the pair of the tong than the distillation flask was filled with the petroleum ethyl solution. The soxhlet apparatus was turned on and the temperature was raised gradually.

Cultural practices

Nitrogen (120 kg ha^{-1}) was split applied at sowing, 2nd and 3rd irrigations as per treatments in the form of Urea. All P (at varying levels) was applied in the form of single super phosphate at the time of sowing.

Statistical analysis

Analyzed the research data by used computer software Statistix 8.1 version **[11]**. The various differentiate along with treatments were compared through least significant various (LSD) test where compulsory.

Results and discussion

The experiment was conducted during 2016-17. Five treatments were formed including a T_1= Control (Natural), T_2 = Recommended dose of NPK 120-80-40 kg ha^{-1}, T_3= FYM 1 t ha^{-1} + NPK 120-80-40 kg ha^{-1}, T_4 = FYM

3 t ha^{-1} + NPK 120-80-40 kg ha^{-1}, T_5 = FYM 5 t ha^{-1} + NPK 120-80-40 kg ha^{-1} and T_6 = FYM 7 t ha^{-1} + NPK 120-80-40 kg ha^{-1} at Sindh Agriculture University, Tandojam Pakistan. The observations some characters of economy output value were following recorded: height of plant cm, no of pods/plant, no of seeds pod^{-1}, seed index 1000g seed wt., seed yield production kg ha^{-1} and oil content (%).This chapter contains the data (Table 1-6) along with the results interpretation in headings.

Height of plant cm: Data observed to height of mustard plant (Variety S-9) as prejudiced by different approaches of FYM & NPK levels applied shown in table-1. The variance analysis (Appendix-I) established significant (P<0.005) result FYM & NPK levels on mustard's plant height. Height of plant (145.38cm) observed fertilized in plots with FYM 7t ha^{-1} + NPK 120-80-40 kg ha^{-1}, followed by normal height of plant 144.00 cm, 141.00 cm and 139.22 cm achieved in plots given FYM 1t ha^{-1} + NPK 120-80-40 kg ha^{-1}, FYM 3t ha^{-1} +NPK 120-80-40 kg ha^{-1} and FYM 5t ha^{-1} +NPK 120-80-40 kg ha^{-1} respectively. Yet height of plant reduced considerably to 134.33cm when crop was supplied with recommended NPK 120-80-40 kg per ha respectively. However less height of plant (128.33cm) was observed in control plots, where fertilizer didn't applied. Way to scientific logic the plots receiving FYM 7t ha^{-1}+ NPK 120-80-40 kg ha^{-1} at sowing was moderate level so far the height of plant is concerned.

Table 1. Height of plant cm of mustard as resulted by FYM and NPK

Treatments	Mean
F_1 = Control (no fertilizer)	**128.33 D**
F_2 = Recommended NPK 120-80-40 kg ha^{-1}	**134.33 C**
F_3 = FYM 1t ha^{-1} + NPK 120-80-40 kg ha^{-1}	**139.22 B**
F_4 = FYM 3t ha^{-1} + NPK 120-80-40 kg ha^{-1}	**141.00 B**
F_5 = FYM 5t ha^{-1}+ NPK 120-80-40 kg ha^{-1}	**144.00 A**
F_6 = FYM 7t ha^{-1}+ NPK 120-80-40 kg ha^{-1}	**145.38 A**

S.E.± = 2.2420
LSD 0.05 = 4.9954

Number of pods per plant

The number of pods /plant has linear effect on seed yield per plant production under control soil, climatic & management conditions. Results are relative to no of plant per pods of mustard as increased through FYM and NPK doses are given away in table-2. Analysis of difference (Appendix-II) suggested that no of plant /pods in plant were significantly (P<0.05) influenced by FYM and NPK fertilizer levels. Maximum no of pods were (303.23) $plant^{-1}$ recorded in given plots FYM 7t ha^{-1} + NPK 120-80-40 kg ha^{-1}, followed by 293.34, 265.44 and 212.46 pods $plant^{-1}$ observed in plots given FYM 1t ha^{-1} + NPK 120-80-40 kg ha^{-1}, FYM 3t ha^{-1} + NPK 120-80-40 kg ha^{-1} and FYM 5t ha^{-1} NPK 120-80-40 kg/ha respectively. The no of pods $plant^{-1}$ declined noticeably to 188.37 $plant^{-1}$ in plots given recommended NPK 120-80-40 $kgha^{-1}$, respectively. However the less pods /plant (145.41) was recorded in (control) no fertilizer was applied. It was observed that plots receiving FYM 7t ha^{-1} + NPK 120-80-40 kg ha^{-1} at time of sowing was an optimum dose so far number of pods $plant^{-1}$ of mustard concerned.

Table 2. Number of pods per plant of mustard as resulted by FYM and NPK

Treatments	Mean
F_1 = Control (no fertilizer)	**145.41 D**
F_2 = Recommended NPK 120-80-40 kg ha^{-1}	**188.37 C**
F_3 = FYM 1t ha^{-1} + NPK 120-80-40 kg ha^{-1}	**212.46 B**
F_4 = FYM 3t ha^{-1}+ NPK 120-80-40 kg ha^{-1}	**265.44 B**
F_5 = FYM 5t ha^{-1}+ NPK 120-80-40 kg ha^{-1}	**293.34 A**
F_6 = FYM 7t ha^{-1}+ NPK 120-80-40 kg ha^{-1}	**303.23 A**

S.E.± = 41.539
LSD 0.05 = 92.555

Number of seeds pod^{-1}

The number of seeds pod^{-1} is generally influenced by the genetic make of different crop varieties, but the increased of crop management and proper input application is also of prime importance. The data in regards to number of seeds pod^{-1} of mustard as affected by various FYM and NPK levels showed in table-3. Analysis of the variance (Appendix-III) showed significantly ($P<0.005$) of seeds per pod by FYM & NPK levels. The highest no of seeds pod^{-1} (18.23) was observed in plots given soil applied FYM 7t ha^{-1} + NPK 120-80-40 kg ha^{-1}, followed by 15.33, 14.46 and 13.16 seeds pod^{-1} observed in plots supplied with FYM 1t ha^{-1} + NPK 120-80-40 kg ha^{-1}, FYM 3t ha^{-1} + NPK 120-80-40 kg ha^{-1} and FYM 5t ha^{-1} + NPK 120-80-40 kg/ha respectively. However, no of seed /pods decreased 11.31 in plot given recommended NPK 120-80-40 kg/ha, respectively. However, lowest no of seed pod^{-1} (8.12) observed in without fertilizer plots. It was observed that plots receiving FYM 7t ha^{-1} + NPK 120-80-40 kg/ha at time of sowing time was moderate level so afar the number of seeds pod^{-1} of mustard is concerned.

Table 3. Number of seed per pod of mustard as affected through FYM and NPK

Treatments	Mean
F_1 = Control (no fertilizer)	**8.12 D**
F_2 = Recommended NPK 120-80-40 kg ha^{-1}	**11.31 C**
F_3 = FYM 1t ha^{-1} + NPK 120-80-40 kg ha^{-1}	**13.16 B**
F_4 = FYM 3t ha^{-1}+ NPK 120-80-40 kg ha^{-1}	**14.46 B**
F_5 = FYM 5t ha^{-1}+ NPK 120-80-40 kg ha^{-1}	**15.33 A**
F_6 = FYM 7t ha^{-1}+ NPK 120-80-40 kg ha^{-1}	**18.23 A**

S.E.± = 0.8354
LSD 0.05 = 1.8613

Seed index 1000 weight in g

Seed index is seemingly a measureable trait, but it initially denotes the value of seed plant being evaluated. Seed index of mustard as affected FYM and NPK levels are showed in table no. 4. Analysis of different Appendix-IV, expressed seed index was significantly ($P<0.05$) varied of different levels of FYM and NPK. The highest seed index g (7.18) was applied fertilized with FYM 7t ha^{-1} + NPK 120-80-40 kg ha^{-1}, followed by seed index data 6.00 g, 4.47 g and 4.38 g observed in plots applied with FYM 1t /ha + NPK 120-80-40 kg /ha, FYM 3t ha^{-1} + NPK 120-80-40 kg ha^{-1} and FYM 5t ha^{-1} + NPK 120-80-40 kg ha^{-1} respectively. However, seed index decreased to 3.42 g in plots given recommended NPK 120-80-40 kg ha^{-1} respectively. Seed index (3.00 g) was recorded in control plots. It was observed that plots receiving FYM 7t ha^{-1} + NPK 120-80-40 kg ha^{-1} at sowing was an optimum fertilizers dose so far the mustard sex index is concerned.

Table 4. Seed index 1000g seed wt. of mustard as affected through FYM and NPK

Treatments	Mean
F_1 = Control (no fertilizer)	**3.00 D**
F_2 = Recommended NPK 120-80-40 kg ha^{-1}	**3.42 C**
F_3 = FYM 1t ha^{-1} + NPK 120-80-40 kg ha^{-1}	**4.38 B**
F_4 = FYM 3t ha^{-1}+ NPK 120-80-40 kg ha^{-1}	**4.47 B**
F_5 = FYM 5t ha^{-1}+ NPK 120-80-40 kg ha^{-1}	**6.00 A**
F_6 = FYM 7t ha^{-1}+ NPK 120-80-40 kg ha^{-1}	**7.18 A**

S.E.± = 0.5485
LSD 0.05 = 1.2220

Seed yield (kg ha^{-1})

The results in relation to seed yield ha^{-1} of mustard variety "S-9" as affected by different FYM and NPK levels are presented in Table-5 and its analysis of variance as Appendix-V. The analysis of variance demonstrated that seed yield ha^{-1} was significantly (P<0.05) influenced by FYM and NPK levels. The highest seed yield of 2090.47 kg ha^{-1} was obtained from the given plots FYM 7t ha^{-1} + NPK 120-80-40 kg ha^{-1}, followed by 1805.27, 1711.00 and 16.31 kg seed yield ha^{-1} achieved in plots fertilized with FYM 1t ha^{-1} + NPK 120-80-40 kg ha^{-1}, FYM 3t ha^{-1} + NPK 120-80-40 kg ha^{-1} and FYM 5t ha^{-1} + NPK 120-80-40 kg ha^{-1} respectively. However, seed yield production kg per ha much reduced 1510.21 kg ha^{-1} obtained in plots given recommended NPK 120-80-40 kg ha^{-1} respectively. The minimum seed yield production kg ha^{-1} (1325.14) was uptake in control plots, where is no fertilizers ware applied. It was concluded that plots receiving FYM 7t ha^{-1} + NPK 120-80-40 kg ha^{-1} at sowing was an optimum level so far seed yield per hector of mustard is concerned.

Table 5. Seed yield (kg ha^{-1}) of mustard as affected by FYM and NPK

Treatments	Mean
F_1 = Control (no fertilizer)	**1325 D**
F_2 = Recommended NPK 120-80-40 kg ha^{-1}	**1510 C**
F_3 = FYM 1t ha^{-1} + NPK 120-80-40 kg ha^{-1}	**1631 B**
F_4 = FYM 3t ha^{-1}+ NPK 120-80-40 kg ha^{-1}	**1711 B**
F_5 = FYM 5t ha^{-1}+ NPK 120-80-40 kg ha^{-1}	**1805 A**
F_6 = FYM 7t ha^{-1}+ NPK 120-80-40 kg ha^{-1}	**2090 A**

S.E.± = 95.089
LSD 0.05 = 211.87

Oil content (%)

The oil content percentage in (*Brassica junacea* L.) as affected by levels of FYM and NPK is recorded in Table-6 and its analysis of variance as Appendix-XI. The analysis of variance suggested that FYM and NPK levels in different split doses showed value significantly (P<0.05) on percentage of oil content. Data showed in table 6. That higher amount of oil content (40.36%) was observed in crop receiving FYM 7t ha^{-1} + NPK 120-80-40 kg ha^{-1} followed by oil contents of 38.15%, 36.15% and 35.00% recorded from the crop given FYM 1t ha^{-1} + NPK 120-80-40 kg ha^{-1}, FYM 3t ha^{-1}+ NPK 120-80-40 kg ha^{-1} and FYM 5t ha^{-1} + NPK 120-80-40 kg ha^{-1}, respectively. The oil content decreased to 31.10% when recommended NPK 120-80-40 kg ha^{-1} were applied, respectively. However, minimum content oil (26.35%) obtained in control, where is no fertilizer ware applied. It was noted that plots receiving FYM 7t ha^{-1} + NPK 120-80-40 kg ha^{-1} at sowing was

optimum fertilizer dose far the oil content percentage ha^{-1} of mustard is concerned.

Table 6. Oil content (%) of mustard as affected by FYM and NPK

Treatments	Mean
F_1 = Control (no fertilizer)	**26.35 D**
F_2 = Recommended NPK 120-80-40 kg ha^{-1}	**31.10 C**
F_3 = FYM 1t ha^{-1} + NPK 120-80-40 kg ha^{-1}	**35.00 B**
F_4 = FYM 3t ha^{-1}+ NPK 120-80-40 kg ha^{-1}	**36.15 B**
F_5 = FYM 5t ha^{-1}+ NPK 120-80-40 kg ha^{-1}	**38.15 A**
F_6 = FYM 7t ha^{-1}+ NPK 120-80-40 kg ha^{-1}	**40.36 A**

S.E.± = 1.0193
LSD 0.05 = 2.2711

The existing oilseed production in the country is far less than the domestic requirement and it is essential to develop advanced crop management practices and by balancing nutrients [12]. The results showed that growth, seed yield and oil content significantly (P<0.05) mustard crop effected by FYM & NPK levels. Fertilized mustard with FYM 7t ha^{-1} + NPK 120-80-40 kg ha^{-1} ranked 1st with, 145.38 cm plant height, 303.23 number of pods plant^{-1}, 18.23 number of seeds pod^{-1}, 7.18 g seed index, 2090 kg seed yield ha^{-1} and 40.36% oil content. The mustard crop getting FYM 5t ha^{-1} + NPK 120-80-40 kg ha^{-1} with 144.00 cm plant height, 193.34 number of pods plant^{-1}, 15.33 number of seeds pod^{-1}, 6.00 g seed index, 1805 kg seed yield ha^{-1} and 38.15% oil content. The crop provided with FYM 3t ha^{-1} + NPK 120-80-40 kg ha^{-1} with 141.00 cm plant height, 265.44 numbers of pods per plant, 14.46 numbers of seeds per pod, 4.47 g seed index, 1711 kg seed yield ha^{-1} and 36.15% content oil. Mustard crop applied FYM 1t ha^{-1} + NPK 120-80-40 kg ha^{-1} with 139.22 cm plant height, 212.46 numbers of pods plant^{-1}, 13.16 number of seeds pod^{-1}, 4.38 g seed index, 1631 kg seed yield ha^{-1} and 35.00% oil content. The effect further showed that the mustard crop received recommended NPK 120-80-40 kg ha^{-1} in crop performance with 134.33 cm plant height, 188.37 number of pods plant^{-1}, 11.31 number of seeds pod^{-1}, 3.42 g seed index, 1510 kg seed yield ha^{-1} and 31.10% oil content. However, the minimum lowest crop performance was observed in control plots, wherever no manure was applied, having 128.33 cm plant height, 145.41 number of pods plant^{-1}, 8.12 number of seeds pod^{-1}, 3.00 g seed index, 1325 kg seed yield ha^{-1} and 26.35% oil content. It is concluded that mustard yield increase with NPK combined with FYM 120-80-40 + 7 ton FYM but 5 ton FYM combined with NPK 120-80-40 was economic dose for mustard production. The oil production also increased at combined application of FYM and NPK fertilizers. These results are further supported by [13] under water stress environment the foliar appliance of fertilizers is most efficient to maintain the crop growth. [14] accounted that increasing doses of potassium fertilizers improved the seed yield and other agronomic parameters. In the same way, [15] for attaining higher seed mustard yields 80 kg K_2O ha^{-1} is recommended the results were potentially better with the application of both soil and foliar Potassium as contrast to K application through soil or foliar appliance only. [16] reported with enhancing k levels that mustard seed yield increased also enhances. The findings of [17] presented highest seed production of 2085 kg ha^{-1} in mustard is gained by the appliance of 60 kg K_2O ha^{-1}, 3.48% was higher than the control yield. [18] tested K in different levels with examined agronomical parameters i.e., seed yield, growth and oil contents of canola with

attained seed production of 3473 kg ha^{-1} in dose of K 150 kg ha^{-1}. But, mustard responded considerably through foliar application of K for growth and seed yield. **[19]** has obtained 3473 kg ha^{-1} production of mustard seed yield with K 150 ha^{-1}, while **[20]** reported enhancement split appliance of Potassium and additional essential elements by foliar application. **[21]** specified 60kg K_2O ha^{-1} through soil showed most favorable with enhance seed yield. **[22]** accounted that K application demonstrated optimistic influence on yield of mustard and cost advantage ratio were wonderful when K fertilizer applied over rest of fertilizer mixture. Research regained from the past investigations are fully harmony with conclusions of current finding. It is recommended that foliar application of K is very advantageous somewhat to enhance quantity of potassium application applied through soil. Therefore, to fulfill the crop necessities foliar application of K is more recommended as compared to application of Potassium through soil.

Conclusions

It is concluded that Mustard yield increased with NPK combined with FYM 120-80-40 + 7 ton FYM but 5 ton FYM combined with NPK 120-80-40 was economic dose for mustard production .The oil production also increased at combined application of FYM and NPK fertilizers..

Authors' contributions

Conceived and designed the experiments: JA Abro, M Habib & MI Jakhro, Performed the experiments: JA Abro, A Abro, AQ Gola & GB Bugti, Analyzed the data: S Ahmed, A Latif, SA Shahwani & M Anwar, Contributed reagents/ materials/ analysis tools: U Nawaz, F Maham & M Habib, Wrote the paper: JA Abro, M Habib & MI Jakhro.

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