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NEW AGRO-TECHNOLOGY TO INCREASE THE PRODUCTIVITY OF WILD MARIGOLD (*TAGETES MINUTA* L.)

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Abstract: *Tagetes minuta* is an industrially important medicinal & aromatic plant. A field well planned filed experiment was conducted to standardize an economic and efficient dose of fertilizer for wild marigold (*Tagetes minuta* L.) cultivation during rabi season in the year 2019-20. For this purpose, fourdoses of Nitrogen fertilizer were evaluated for their response with respect to plant height, essential oil content, freshherb and essential oil yield of wild marigold. Essential oil quality analysis was done by GC/MS. The experiment results revealed that application of fertilizer as Nitrogen (N), Phosphorous (P), and Potash (K) @ 120:60:40 kg ha^{-1} in (T_4) recorded significantly highest yield attributes; plant height (137.62 cm), fresh herb yield (94.84 quintal ha^{-1}), essential oil content (0.33%), and essential oil yield (31.14 kg per ha) with comparison to T_3, and T_2. Control showed the least plant height (98cm), fresh whole herb yield per ha (42.52 quintal), essential oil content on fresh weight basis (0.30%), and essential oil yield per ha (12.76 kg). On analysis, major constituent dihydrotagetones (56.87%) of essential oil sharply increases with increase in the nitrogen dose.

Keywords: Wild marigold, nutrient management, agro-technology, essential oil

INTRODUCTION

Wild marigold (*Tagetes minuta* L.) are annual and industrially important aromatic herbs belonging to family asteraceae. According to Sadia [1], asteraceae family is one of major and predominat plant taxonomy group, with 1,000 genera with 23000 species. T*agetes genus* itself contain 56 species, including 29 (perennial) and 27 (annual) [2]. This plant is native to South America and introduce in several countries of the world as weed in crop field. In India, *T. minuta* was found as a wild species in western Himalaya which contain rich amount of essential oil, increases its commercial production. Due to its high demand of essential oil, it is now cultivated in other countries also Asia, Africa and Europe. The essential oil contain major chemical composition monoterpene ketones (Z)-β-ocimene, dihydrotagetone, (Z)-and (E)- tagetone, ocimenones (E, Z) and limonene(hydrocarbons) have interesting aromatic properties[3]. *T. minuta* essential oil is used in the cosmetic, flavouring agent in beverage and food, as medicine and some perfumery product [4]. Previous study reported that *T. minuta* has strong nematocidal, anti-inflammatory and antimicrobial activity too [5-8]. Studies also confirm the presence of insect repellent properties, especially against ants and fly [9]. Environmental and different management factors influence the herb yield and oil content and quality of some medicinal plant. Agronomic practices, planting methods and time of planting plays very important role in the production of crop by increasing its herb yield and improving the quality of essential oil [10-,13]. Therefore, present study has made to investigate the influence of Nitrogen applied in increasing order on herb growth and analyze its effect on

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quality essential oil production of Tagetes minuta under sub-tropical northern Indian plains.

2. EXPERIMENTAL MATERIALS & METHODS

2.1. Experiment location and site

The planned experiment on marigold executed at CSIR-CIMAP, Pantnagar (29^{o} N latitude and 79.38^{o} E longitude) during 2019-20. The location of the experiment is warm and temperate with a range between 35 to 45 ^{o}C maximum and upto 12 ^{o}C minimum.

2.2. Experimental design and Field preparation

Good quality seeds of *T. minuta* were sown in small pot during mid- October, 2019 at 1 cm depth in row. Seeds covered by fine soil and irrigate the field just after sowing. After 45 days of sowing, healthy plants of wild marigold were transplanted at 50 cm row to row and 50 cm plant to plant spacing during November, 2019 in the plot having bed size 4×3 meter and transplanted 48 numbers of plants each bed in the Randomized Block Design (R.B.D.) experiment with four treatments and in three replications. Weeding was done in at 25 days of after transplanting.

2.3. Details of treatment

Good quality farmyard manures was applied in the field 30 days before transplanting of wild marigold crop. Soil was fertilized with phosphorus (P_2O_5), and potassium (K_2O) at 60:40 kg ha^{-1}, respectively, with Nitrogen treatment vary different level as; T_1- Control (no NPK), T_2- nitrogen 80 kg/ha, phosphorous 60 kg/ha, and potash 40 kg/ha, out of these fertilizer dosed fist part of that applied basal application i.e. soil incorporation as 1/3rd part of nitrogen and complete amount of phosphorous (P_2O_5) and Potash (K_2O) applied in field before last ploughing, and remaining 2/3rd part of nitrogen (N) applied in two equal splits at 25 to 30 and 40 to 45 days after transplanting, T_3- nitrogen 100 kg/ha, phosphorous 60 kg/ha, and potash 40 kg/ha, out of these fertilizer dosed fist part of that applied basal application i.e. soil incorporation as 1/3rd part of nitrogen and complete amount of phosphorous (P_2O_5) and potash (K_2O) applied in field before last ploughing, and remaining 2/3rd part of nitrogen (N) applied in two equal splits at 25 to 30 and 40 to 45 days after transplanting,, and T_4- nitrogen 120 kg/ha, phosphorous 60 kg/ha, and potash 40 kg/ha, out of these fertilizer dosed fist part of that applied basal application i.e. soil incorporation as 1/3rd part of nitrogen and complete amount of phosphorous (P_2O_5) and Potash (K_2O) applied in field before last ploughing, and remaining 2/3rd part of nitrogen (N) applied in two equal splits at 25 to 30 and 40 to 45 days after transplanting.

2.4. Plant growth and essential oil yield parameters

The desired parameters related to growth and yield such as height of plant (cm), fresh herb biomass/yield (quintal per ha), essential oil content (%), and essential oil production/yield per ha (kg) were recorded during harvesting time.

2.5. Essential oil extraction

Aerial parts of *Tagetes minuta* were harvested from each treatment plot and were hydro distilled in a Clevenger type apparatus for 6 hours (Clevenger, 1928). The volume/weight basis of essential oil was measured in mL per one hundred gram weight of fresh herb.

2.6. Gas chromatography

The essential oil of Tagetes analyzed by Nucon gas chromatograph with model no. 5765 equipped by flame ionization detector (FID) and possess two different numbers of different polarity stationary phases, namely, BP-20 (length, internal diameter, and film thickness of 30 meter × 0.25 mm × 0.25 µm, respectively) and DB-5 (length, internal diameter, and film thickness of 30 meter length × 0.32 mm internal diameter × 0.25 µm respectively) fused silica capillary columns. The gas namely hydrogen used as haulier at the rate of 1.0 millilitre per minute. The programmme regarding temperature ranging 70 °C to 230 °C and at 4 °C per minute as well as time regarding first and last hold is 2 minute (BP-20) and from 70 °C to 250 °C at 3 °C per minute

(DB-5). The constant temperatures of 210 °C and 230 °C were maintained at injector & detector, respectively. Split ratio was 1:30.

2.7. Statistical analysis

The experimental data were analyzed statistically by applying ANOVA using a randomized block design. Critical difference was tested (considering significant when P≤ 0.05) compared at 0.05 level of probability [14,15].

3. RESULTS

3.1. Plant height

Growth parameter (plant height) of *T. minuta* (Table 1), recorded before harvesting clearly showed significant effects on wild marigold crop. Height of *T. minuta* measured in treatment (T_4) where applied fertilizers doses was 120:60:40 kg NPK ha^{-1} was significantly higher as 137.62 cm in the crop as compared to remaining treatments viz., T_2 (80:60:40 kg NPK ha^{-1}), T3 (100:60:40 kg NPK ha^{-1}), and in T_1 (Control) possess plant height 134 cm, 125.22 cm, 98 cm, respectively.

3.2. Fresh herb yield

T. minuta fresh herb yield responded to N fertilization in very well (Table 1). Fresh herb yield of *Tagetes minuta* was significantly higher with recorded higher yield in T_4 (94.84 q ha^{-1}), followed by T_3 (81.42 q ha^{-1}), T_2 (80.04 q ha^{-1}), and lowest fresh herb yield was found in T_1 (42.52 q ha^{-1}). The response of *T. minuta* to high level of N application i.e. in T_4 (120:60:40 kg NPK ha^{-1}) is significantly enhanced the plant growth and yield both. It is cleared from results that fresh herb yield increases with increase in the nitrogen dose.

3.3. Essential oil content

The data presented in Table 1 showed essential oil content (%) of wild marigold. *T. minuta* essential oil of treatment (T_4) nitrogen 120 kg/ha, phosphorous 60 kg/ha, and potash 40 kg/ha, out of these fertilizer dosed fist part of that applied basal application i.e. soil incorporation as 1/3rd part of nitrogen and complete amount of phosphorous (P_2O_5) and Potash (K_2O) applied in field before last ploughing, and remaining 2/3rd part of nitrogen (N) applied in two equal splits at 25 to 30 and 40 to 45 days after transplanting, recorded maximum essential oil content on fresh weight basis (0.33%) with comparison to treatments of T_3 (100:60:40 kg NPK ha^{-1}) and T_2 (80:60:40, kg NPK ha^{-1}) which contain oil content 0.30% essential oils. The lowest essential oil content (0.3%) was recorded in T1 (control).

3.4. Essential oil yield

Table 1 showed the essential oil yield calculated. Fertilizer application as 120 kg. nitrogen, 60 kg. phosphorous, and 40 kg potash per ha (T_4) provided significantly maximum essential oil yield (31.14 kg per ha) as compared to remaining treatments as well as trends of essential oil yield noted in decreasing order in T_3 (100:60:40 kg NPK ha^{-1}), T_2 (80:60:40, kg NPK ha^{-1}), T_1 (control) with the oil yield of 26.07 kg ha^{-1}, 25.06 kg ha^{-1}, 12.76 kg ha^{-1}, respectively.

3.5. Essential oil quality

Essential oil of *Tagetes minuta* chemically analysed by GC to as certain the quality of the produced oil (Table 2). For better quality essential oil with enriched (Z)–β-Ocimene was recorded highest 33.57 % in T_3 (100:60:40 kg NPK ha^{-1}) as compared to T_2 (17.06 %). The lowest amount 10.01 % of (Z) –β- Ocimene found in essential oil of *T. minuta*. The major constituents of essential oil dihydrotagetone, sharply increases (56.87%) with increase in N level T_4 (120:60:40 kg NPK ha^{-1}), followed by 44.55% in T_2 and the lowest composition of dihydrotagetone 26.94% present in T_3 while (Z)-tagetone per cent decreased with increase in the nitrogen level viz; T_1 (control) 31.87% >T_2 (23.93%) >T_3 (20.94%) >T_4(15.72).

4. DISCUSSION

From the above results, application of nitrogen fertilizer at the rate 120:60:40 kg NPK ha^{-1} (T_4) can be used for higher plant height, fresh herb yield, oil content, and oil yield of *T. Minuta.* Applied phosphorous and potassium basally and increase dose of nitrogen in 3 split at planting stage, vegetative stage and budding stage promote the higher production of the

crop. All the parameters measured were directly proportional to the fertilizer applied that is increase in nitrogen dose, increases the herb yield and oil yield and oil. This increase was probably due to efficient utilization of natural as well as sufficient applied resources of nitrogen. Table 1 clearly showed the total oil content increases due to increase in fertilizer. This may also due to favourable condition or nutrient uptake capacity of the T. minuta. There is a reported study of Buresh and Pampolino who stated that the site specific nutrient management enhanced the rice crop production [16,17]. This study confirm the presence of dihydrotagetone (56.87%) increases with increase in N level @ 120 kg ha-1, while (Z)-tagetone % declined sharply with increase N level as shown in Table 2. In recent studies found that essential oils from United States America (USA), have been dominated and enriched by β-ocimene (36.4%), and reflected uncharacteristically high level or content of limonene content is (26.9%), high (Z)-tagetone (16.9%). Essential oil obtained

Table 1: Effect of nutrient management on yield attributes and yield of *Tagetes minuta*

Treatments	*Plant height (cm)*	*No. Brach (plant^{-1})*	*Fresh herb yield (q ha^{-1})*	*Oil content (%)*	*Oil yield (kg ha^{-1})*
T_1	98	3	42.52	0.30	12.76
T_2	134	3	80.04	0.31	25.06
T_3	125.22	4	81.42	0.32	26.07
T_4	137.62	4	94.84	0.33	31.14
SEM_+	4.37	1.51	2.79	0.008	1.33
$LSD_{(0.05)}$	15.14	NS	9.67	NS	4.60

T_1- control,**T_2**-NPK @ 80:60:40 kg/ha, **T_3**- NPK @ 100:60:40 kg/ha, **T_4**- NPK @ 120:60:40 kg/ha

Table 2: Quality analysis and composition of essential oil of *Tagetes minuta*

S. No	*Compounds*	*RI[a]*	*RI[b]*	*Content (%)*			
				T_1	*T_2*	*T_3*	*T_4*
1	Sabinene	968	969	0.51	0.43	0.52	0.40
2	Myrcene	994	988	0.10	0.10	0.12	t
3	α-Phellandrene	1002	1002	0.10	0.10	0.19	0.56
4	Limonene	1024	1024	4.82	3.81	4.40	4.35
5	(Z) – β- Ocimene	1032	1032	**14.54**	**17.06**	**33.57**	**10.01**
6	(E) – β- Ocimene	1032	1044	0.17	0.20	0.42	t
7	Dihydrotagetone	1052	1046	**35.58**	**44.55**	**26.94**	**56.87**
8	(E) –Tagetone	1136	1139	1.99	1.45	1.09	0.75
9	(Z) – Tagetone	1148	1148	**31.87**	**23.93**	**20.94**	**15.72**
10	(Z) – Ocimenone	1226	1226	0.58	0.48	1.05	t
11	(E) – Ocimenone	1240	1235	2.48	1.63	5.35	0.10
12	β- Elemene	1385	1389	0.39	0.19	0.17	0.20
13	β- Caryophyllene	1418	1417	0.95	0.58	0.70	1.11
14	α - Humulene	1448	1452	0.33	0.26	0.29	1.20
Class Composition							
Monoterpene hydrocarbons				20.24	21.7	39.22	15.32
Oxygenated monoterpenes				72.5	72.04	55.37	73.44
Sesquiterpene hydrocarbons				1.67	1.03	1.16	2.51
Total identified				94.41	94.77	95.75	91.27

[a]Retention index (experimental); [b]Retention index (Adams, 2007); t: trace (<0.05%)

T_1- control,**T_2**-NPK @ 80:60:40 kg/ha, **T_3**- NPK @ 100:60:40 kg/ha, **T_4**- NPK @ 120:60:40 kg/ha

from Brazilian plant material[18] having the following primarly and major constituents: dihydrotagetone (54.2%), (Z)-β-ocimene (5.1%), limonene (7.0%), and (E)-tagetone (6.7%). Wild marigold is harvested manually during full flowering stage. As chemical constituent of *T. minuta* essential oil in T_1 (control) was found to be rich in (Z)-tagetone 31.87% [19]. In his study in 2007 reported that best time for harvesting is budding stage for higher essential oil yield of *Tagetes minuta* (wild marigold), but for higher secondary metabolites/chemical content as (Z)- ocimene, (E)-ocimenone and (E)-tagetones, dihydro-tagetones, (Z)- ocimenone, etc the full bloom stage is suitable for harvest.

5. CONCLUSIONS

Tagetes minuta crop cultivated under the application of nitrogen 120 kg/ha, phosphorous 60 kg/ha, and potash 40 kg/ha, out of these fertilizer dosed fist part (1/3rd) of that applied basal application i.e. soil incorporation as 1/3rd part of nitrogen and complete amount of phosphorous (P_2O_5) and Potash (K_2O) applied in field before last ploughing, and remaining 2/3rd part of nitrogen (N) applied in two equal splits at 25 to 30 and 40 to 45 days after transplanting; (T_4) recorded the effective growth and development of wild marigold as compared to the other treatments. Thus it is recommended that *T. minuta* planted at 50x50 cm row to row and plant to plant spacing and fertilized with 120:60:40 kg nitrogen, phosphorous, and potash per ha^1 (T_4) 1/3rd Nitrogen and complete dose of P_2O_5 and K_2O as soil incorporation before the last ploughing, and remaining 2/3rd part of nitrogen (N) applied in two equal splits at 25 to 30 and 40 to 45 DAT to obtain higher essential oil yield. The results of the experimental trial explored new way for *T. minuta* cultivation in economical and more profitable way. The farmers of sub-tropical areas northern India can produce good quality essential with higher yield through newly developed agro technology for quality cultivation of *Tagetes minuta*.

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