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Long term effect of different plant based nitrification inhibitors and calcium carbide on total soluble solids (TSS), ascorbic acid and protein content of cauliflower (*Brassica oleracea* var. Botrytis L)

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Abstract
The study was devoted to assess the effect of different nitrification plant based nitrification inhibitors and chemical inhibitor on total soluble solids (TSS), ascorbic acid and proteins content of cauliflower. The study was conducted as a field experimental with eight treatment combinations replicated thrice in completely randomized block design during *rabi* season 2018-19 and 2019-20. Different treatments combinations *viz*. melia fruit powder, pomegranate rind powder, commercial neem cake, calcium carbide (CaC_2) and absolute control were incorporated in the study. The results revealed that during both the years of study maximum TSS values of 6.70 and 6.83 ^{o} brix were recorded in MF@ 20 g kg^{-1} soil and NC @ 20 g kg^{-1} soil, respectively. While the least TSS value recorded in control treatment. Maximum ascorbic acid was attributed to NC@ 20 g kg^{-1} soil i.e. 74.43 and 76.19 mg 100 g^{-1} during 2018-19 and 2019-20, respectively. Moreover, protein content in cauliflower varied from 2.44 g $100g^{-1}$ in control to 2.76 g $100g^{-1}$ in NC @20 g kg^{-1} soil during 2018-19 and 2.49 g $100g^{-1}$ in control to 2.76 g $100g^{-1}$ during 2019-20 in the same treatment. Application of plant based nitrification inhibitor neem cake@ 20 g kg^{-1} soil with recommended dose of NPK and farmyard manure was found effective over the chemical inhibitor and therefore improved the quality of cauliflower.

Keywords: nitrification inhibitors, nitrogen economy, nutrient management, sustainable agriculture

Introduction

Application of nitrogenous fertilizers has been considered as the major factor for increased agricultural productivity world-wide since last few decades. Nitrogen is regarded as a most important essential nutrient which controls the diversity and functioning of many terrestrial, freshwater and marine ecosystems. However, huge application of nitrogenous fertilizers are being practiced in the developing countries causing hazards like land degradation. Being a highly mobile and dynamic element nitrogen losses occurs through different ways i.e. NO_3^- leaching, denitrification, runoff, NH_3 volatilization, gaseous emissions of N_2O and NO to the atmosphere (Zaman *et al*., 2009) [23]. The losses from the fertilizers have long term adverse effects on the ecology and environment by means of eutrophication, losses in aquatic biodiversity and increased N_2O emissions (Warneke *et al*., 2011) [22]. Nitrogen metabolism is primarily a microbially mediated process. Some microorganisms play an important role in improving the fertility of soil by metabolizing the N which is not taken up by the plants.

At present, heavy inputs of N fertilizer are being practiced to sustain the productivity, as the naturally fixed N is rarely enough for high production systems (Dinnes *et al*., 2002; Subbarao *et al*., 2006; Abbasi *et al*., 2012) [5, 20, 1]. Therefore, for lowering the pollution hazards to environment, efficiency of nitrogenous fertilizers needs to be improved. Adoption of nitrification inhibitors could prove to be a boon for improving N use efficiency. Nitrification inhibitors can reduce N losses by reducing the rate of nitrification through their action in inhibiting the nitrifying bacteria and thus improving the uptake rate of plant for ammonium (NH_4^+). The nitrification inhibitors play an important role in increasing the yields and lower down the N_2O emissions (Zhang *et al*., 2015) [24].

Nitrification is two steps natural process occurring in the soil in which ammonium is converted into nitrite and further to nitrate form of nitrogen. The *Nitrosomonas* spp. of soil bacteria extract the energy from ammonium while converting it to nitrite form. Furthermore, another group of bacteria i.e. *Nitrobacter* spp. convert the nitrite form into nitrate. Both types of bacteria are commonly found in soil and determine the overall rate of nitrate production. Nitrification inhibitors are generally the compounds which depress the activity of *Nitrosomonas* bacteria and ultimately delay the nitrate production from ammonium. These nitrification inhibitors fasten their activity when provided with adequate soil moisture and soil type. Nitrification inhibitors work more effectively in sandy soils or soils which are low in organic matter and exposed to low temperatures. Retarding the nitrification process results in maintaining appreciable higher amounts of inorganic N in the ammonium form in soil and hence less losses and movement of nitrogen into the soil system. There are several compounds which are commercially recognized as nitrification inhibitors, the most commonly used are 2-chloro-6-(trichloromethyl)-pyridine (Nitrapyrin), dicycandiamide (DCD), 3,4-dimethylpyrazole phosphate (DMPP), neem cake and *Melia azedarach (*McCarty 1999; Abbasi *et al*., 2003; Fangueiro *et al*., 2009; Khalil *et al*., 2009; Zaman *et al*., 2009; Souri 2010; Pereira *et al*., 2010) [15, 3, 7, 10, 23, 19, 17]. Besides these chemical origin compounds, nitrification inhibitory properties of some inexpensive compounds like calcium carbide (CaC_2) and several plants materials wastes like Karanji (*Pongamia glabra*), Neem (*Azadirachta indica*), Dharek (*Melia azedarach*. L) and tea (*Camellia sinensis*) have been reported by many researchers (Freney *et al*., 2000, Kiran and Patra 2003; Majumdar 2002; Moreno *et al*., 2010 ; Abbasi *et al*., 2011) [8, 11, 12, 14, 2].

The ease of using the nitrification inhibitors depends on its easy availability and lower input cost. Dharek (*Melia azedarach*. L) is a deciduous tree belonging to the family Meliaceae. Taxonomically, this plant resembles neem which mostly grows in the tropical to sub temperate regions of Europe and Northern America. Leaves and fruits of melia possess the nitrification inhibitor properties and hence could be used as an amendement. Incorporation of melia derivatives into the soil has been reported to increase the soil fertility (Toselli *et al*., 2010) [21]. In India around 80,000 tons of oil and 3,30,000 tons of neem cake is being prepared from 14 million growing trees as reported in a study (Nicoletti *et al*., 2012). Similarly, wild pomegranate (daaru) is a very common plant in the mid- Himalayan region and its rind is also supposed to have properties which can help in the inhibition of nitrification and their non-economical portions can be effectively used as nitrification inhibitors.

Material and methods

The study was conducted as field experiments during 2018-19 and 2019-20 at the experimental farm of Department of Soil Science and Water Management Dr. YSP UHF Nauni, Solan (H.P.). The experiment was laid out in a randomized block design with eight treatments replicated thrice. The following treatments were incorporated in the study to compel on cauliflower.

Treatments were laid in randomized block design

Serial No.	Treatments
T_1	Powdered pomegranate rind @10 g kg^{-1} of soil
T_2	Powdered pomegranate rind @20 g kg^{-1} of soil
T_3	Powdered melia fruits @10 g kg^{-1} of soil
T_4	Powdered melia fruits @20 g kg^{-1} of soil
T_5	Commercial neem cake @10 g Kg^{-1} of soil
T_6	Commercial neem cake @20 g kg^{-1} of soil
T_7	Calcium carbide (CaC_2) @ 15 g kg^{-1} of soil
T_8	Absolute control

Treatments were laid in randomized block design with plot size of 6 m^2 (3m×2m) and the seedlings were transplanted at a planting distance of 60cm×45 cm. All the treatments were given recommended dose of NPK (125:76:72) except absolute control. FYM was also incorporated @ 250 q ha^{-1} in all treatments except control. Plant derivatives rates were calculated and applied as per the treatments.

Result and discussion

Total soluble solids (TSS)

The data presented in table (1) showed that the maximum TSS (6.70 ^{o} brix) during 2018-19 was recorded in the treatment T_4 and T_6 which at par with 6.58 ^{o} brix in T_7 i.e. CaC_2 @ 15 g kg^{-1} soil. The minimum TSS of 6.33 ^{o} brix was recorded in control (T_8). During the year 2019-20 the maximum TSS of 6.83 ^{o} brix followed by 6.65 ^{o} brix were recorded in T_4 and T_6, respectively, while the minimum was again in absolute control. The pooled data analysis revealed a maximum TSS of 6.76 ^{o} brix in T_4 but was not statistically significant to all other treatments. The interaction T×Y showed that a maximum TSS of 6.83 ^{o} brix was in T_4 during 2019-20 which was at par with T_4, T_6 in 2018 and T_6 and T_7 in the year 2019-20. Thus, the curd obtained under the soil treatment with melia fruit powder @ 20 g kg^{-1} soil (T_4) had the maximum TSS values. Singh *et al,* 2018 also reported that better nutrition (nitrogen management) results in improved levels of carbohydrates and other quality parameters of cauliflower curd through enzymatic activity.

Table 1: Effect of nitrification inhibitors on TSS and ascorbic acid in cauliflower

Treatment *	TSS (º brix)			Ascorbic acid (mg 100 g^{-1})		
	2018-19	2019-20	Pooled	2018-19	2019-20	Pooled
T_1: PR @ 10 g kg^{-1} soil	6.40	6.44	6.42	66.82	67.51	67.17
T_2: PR @ 20 g kg^{-1} soil	6.50	6.35	6.43	68.73	69.98	69.35
T_3: MF @ 10 g kg^{-1} soil	6.42	6.42	6.42	66.77	69.13	67.95
T_4: MF @ 20 g kg^{-1} soil	6.70	6.83	6.76	72.26	73.41	72.83
T_5: NC @ 10 g kg^{-1} soil	6.45	6.42	6.44	69.14	70.86	70.00
T_6: NC @ 20 g kg^{-1} soil	6.70	6.65	6.68	74.43	76.19	75.31
T_7: CaC_2 @15 g kg^{-1} soil	6.58	6.62	6.60	71.26	72.64	71.95
T_8: Absolute control	6.33	6.24	6.29	58.41	59.83	59.12
Mean	6.47	6.54		69.26	69.15	
CD (0.05)	0.14	0.34		2.05	3.78	
T	NS			1.99		
Y	NS			NS		
T × Y	0.24			2.81		

* RD of NPK were applied uniformly to all the treatments except T_8 (control)
PR = Pomegranate Rind MF = Melia Fruits NC = Neem Cake

Ascorbic acid

The data presented in table (1) revealed that during 2018-19 the maximum ascorbic acid content (74.43 mg 100 g^{-1}) was recorded in the treatment T_6 comprising of the application of neem cake @ 20 g kg^{-1} soil was followed by 72.26 mg 100 g^{-1} in T_4 i.e. MF @ 20 g kg^{-1} soil.

The minimum ascorbic acid of 58.41 mg 100 g^{-1} was in T_8 (control). During the year 2019-20 the maximum ascorbic acid of 76.19 mg 100 g^{-1} was again recorded in T_6 which was at par with T_4 and T_7, while the minimum remained in absolute control.

The pooled data analysis revealed a maximum ascorbic acid of 75.31 mg 100 g^{-1} in T_6 which was statistically superior to all other treatments and minimum was in T_8 (control). The interaction T×Y showed that maximum ascorbic acid of 76.19 mg 100 g^{-1} was in T_6 during 2019-20 which was at par with 74.43 mg 100 g^{-1} in the same treatment during 2018-19, and 73.41 mg 100g^{-1} in T_4 during 2019-20. Amongst pomegranate and melia powders, the application of melia fruit powder @ 20 g kg^{-1} soil (T_4) was superior, but statistically lower than the maximum values. These results are also supported by the findings of Oyinlola *et al.*, (2017) [16] who ascribed it to the changed soil conditions and plant nutrition.

Proteins

During 2018-19 the maximum protein content (2.76 g 100g^{-1}) was recorded in the treatment T_6 comprising of the application of neem cake @ 20 g kg^{-1} soil which was at par with T_4, T_5 and T_7 (Table 2). The minimum protein content of 2.44 g 100g^{-1} was, however, recorded in control (T_8). Similarly in the year 2019-20 the maximum protein content of 2.79 g 100g^{-1} was again recorded in T_6 being at par with T_4, T_5 and T_7, while the minimum was again in absolute control. The pooled data analysis revealed a maximum protein content of 2.78 g 100g^{-1} in T_6 which was statistically at par with T_7 but superior over all the other treatments. The average protein during 2019-20 (2.63 g 100g^{-1}) was higher as compared to that in 2018-19 (2.61 g 100g^{-1}) but statistically non-significant. The interaction T×Y showed a maximum protein content of 2.79 g 100g^{-1} in T_6 during 2019-20 which was at par with T_4, T_5, T_6 and T_7 in 2018-19 and T_4, T_5 and T_7 in 2019-20.

Table 2: Effect of plant derivatives on protein content in cauliflower

Treatment *	Protein (g 100g^{-1})		
	2018-19	2019-20	Pooled
T_1: PR @ 10 g kg^{-1} soil	2.55	2.56	2.56
T_2: PR @ 20 g kg^{-1} soil	2.58	2.58	2.58
T_3: MF @ 10 g kg^{-1} soil	2.51	2.53	2.52
T_4: MF @ 20 g kg^{-1} soil	2.67	2.68	2.68
T_5: NC @ 10 g kg^{-1} soil	2.66	2.67	2.67
T_6: NC @ 20 g kg^{-1} soil	2.76	2.79	2.78
T_7: CaC_2 @15 g kg^{-1} soil	2.71	2.75	2.73
T_8: Absolute control	2.44	2.49	2.47
Mean	2.61	2.63	
CD (0.05)	0.12	0.14	
T	0.09		
Y	NS		
T × Y	0.13		

* RD of NPK were applied uniformly to all the treatments except T_8 (control)
PR = Pomegranate Rind MF = Melia Fruits NC = Neem Cake

Combined application of plant derived N-inhibitors with N fertilizers and organic sources resulted in better quality indices over the control treatment. Similar findings were supported by Bairwa *et al.* (2009) [4] who reported that application of neem cake along with NPK fertilizer increased the protein content of fruit and total chlorophyll content of the leaves at 30 and 60 DAS in okra. Application of biological inhibitors was also found to improve the chlorophyll content, photosynthetic rate in peanut (Hou *et al.*, 2014). Application of nitrification inhibitors improved the ascorbic acid in the crop. The results in the present expeiment were in agreement with Martinez *et al.* (2015) [13] and Dong *et al.* (2016) [6].

Moreover, application of calcium carbide improved the ascorbic acid and proteins in the crop. Similar findings were reported by Shakar *et al.*, (2017) [18]. Lower ascorbic acid in control treatment could be due to reduced carbohydrates production, since vitamin C is composed of carbohydrates and hence the synthesis of vitamin C might have been reduced.

Conclusion

The results of the study revealed that of plant based nitrification inhibitors showed promising results on quality parameters of cauliflower. Treatment of neem cake @20 g kg^{-1} soil improved the TSS, ascorbic acid and protein content of the cauliflower during both the years of study and therefore adoption of neem cake as nitrification inhibitor could be a good practice for the farmers.

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Conflict of interest: The authors declare no conflict of interest.

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