

Original Research Paper

Elicitors induced changes in essential oil constituents of turmeric (*Curcuma longa* L.) rhizome

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ABSTRACT

An experiment was conducted at IISR, Kozhikode to study the effect of foliar application of chemical elicitors, namely, chitosan (100, 200 and 500 ppm), phenylalanine (0.1, 1 and 10 mM) and salicylic acid (0.01, 0.1 and 1 mM) on volatile constituents of turmeric rhizome essential oil (EO). Three genotypes (Pragati, Rajapuri and Acc.849) which vary in growth duration and volatile profile were taken for the study in randomized block design with three replications. The highest EO content in Pragati (6%) and Acc. 849 (5.3%) was found in Phenylalanine (1 mM) treatment. No significant changes in EO content were observed in the genotype Rajapuri. Phenylalanine and salicylic acid were found to have positive influence on ar-turmerone, the major sesquiterpenoid in Pragati. Acc.849 and Rajapuri did not produce any significant changes to ar-turmerone content in elicitor treated samples. Moreover, the treatment related variation in the total monoterpenes and total sesquiterpene content was found significant among the genotypes. Multivariate analysis using partial least square discriminant analysis supported the variation observed among the treatments and variable importance in projection score identified the metabolites responsible for variation among treatments.

Keywords: Chitosan, essential oil, phenylalanine and salicylic acid

INTRODUCTION

Turmeric (*Curcuma longa* L.), revered as “Golden Spice”, is a rhizomatous crop belonging to Zingiberaceae family. The crop is native to tropical Southeast Asian region (Ferreira *et al.*, 2013). India is the largest producer, consumer and exporter of this crop. The economic produce of the crop is the processed dried rhizome which varies in color from lemon yellow to dark orange. The earthy flavor of turmeric is contributed by its essential oil (EO) constituents. The turmeric EO is comprised of monoterpenes and sesquiterpenes compounds, namely, ar-turmerone, curlone, β -sesquiphellandrene, α -phellendrene, ar-curcumene, α -terpinolene, β -caryophyllene, etc. Leela *et al.* (2002) reported that EO of turmeric rhizome grown in Kerala, India contained ar-turmerone (31.1 %), curlone (10.6 %) and ar-curcumene (6.3 %) as the main components. Many factors namely genotypes/varieties, soil type, climate, altitudinal variation, etc. decides the differential accumulation of these terpenoids resulting

in non-uniform flavor profile of turmeric (Anandaraj *et al.*, 2014). The turmeric rhizome EO is reported to have numerous biological activities. It is reported to have anti-oxidant, anti-hyperlipidemic, hypoglycemic, anti-diabetic, cytotoxic, anti-inflammatory, anti-arthritic, hepatoprotective, neuroprotective, anti-bacterial and anti-fungal activities. (Dosoky and Setzer, 2018).

Many studies have proven the effectiveness of elicitors like chitosan, carrageenan, sodium alginate, salicylic acid and others to improve the essential oil components in medicinal and aromatic plants (Ahmed *et al.*, 2020; Shabbir *et al.*, 2017). Due to its numerous beneficial bio-activities, the need arises to increase the bioactive essential oil constituents in turmeric rhizome. Based on the above facts, the study was conducted to test the hypothesis that the foliar application of elicitors like chitosan, salicylic acid and phenylalanine in turmeric would increase the essential oil constituents in its rhizome.



MATERIALS AND METHODS

Plant material and treatments

The experiment was conducted at ICAR - Indian Institute of Spices Research (IISR), Kozhikode, Kerala at rainfed condition in randomized block design with three replications. The soil parameters of the experimental plot were as follows: pH (4.3-4.6); organic carbon content (2.0-2.1 %) and N, P and K content in the range of 235-272 kg/ha, 10-23 kg/ha and 344-503 kg/ha, respectively. Average minimum and maximum temperatures were 23.8 and 31.9 °C with mean annual rainfall of 2313 mm. Three different varieties/genotypes namely Pragati (a short-duration dwarf variety released from ICAR – IISR), Rajapuri (traded variety from Central Indian region) and Acc. 849 (germplasm collection from Sangli region of Maharashtra) which have inherent variation in the content of essential oil constituents were selected for the study. The experiment included the treatments viz., 1. Control, 2. C₁ - Chitosan at 100 ppm, 3. C₂ - Chitosan at 200 ppm, 4. C₃ - Chitosan at 500 ppm, 5. P₁ - Phenylalanine at 0.1 mM, 6. P₂ - Phenylalanine at 1 mM, 7. P₃ - Phenylalanine at 10 mM, 8. S₁ - Salicylic acid at 0.01 mM, 9. S₂ - Salicylic acid at 0.1 mM, 10. S₃ - Salicylic acid at 1 mM. The stock solutions of elicitors, chitosan (CHT) at 2000 ppm, salicylic acid (SA) and phenylalanine (PHE) solution at 100 mM concentration each were prepared and different dilutions were made freshly with 0.02 % Tween 20 on the day of spray. The elicitors were sprayed at rhizome development stage, i.e. 120-150 DAP depending upon the growth duration of the genotypes. Plants sprayed with 0.02 % Tween 20 served as the control. Once the above ground vegetative parts are dried, rhizomes are harvested, cleaned, cured by boiling them in hot water and dried in the sun for two weeks until the moisture content of the samples were brought down to 10-12 %.

Hydro-distillation and GC-MS analysis of volatile constituents

Hydro-distillation of essential oil from the dried and powdered rhizomes were done as per the method prescribed in AOAC, 2005. The separation and identification of EO constituents were done in Shimadzu GC/MS fitted with RTX-5 (5 % Phenyl and 95 % di-methyl polysiloxane) column with the dimension of 30 m x 0.25 mm x 0.25 µm. The temperature programming of the column was set as

follows: 60° C for 5 min, then gradient increase to 110° C at the rate of 5° C min⁻¹, to 200° C at the rate of 3° C min⁻¹ and finally to 240° C at the rate of 5° C min⁻¹ with hold time of 3 minutes. Ion source and interface temperature was set as 220° C and 240° C, respectively. Other operational parameters include column oven temperature at 60° C, injection temperature at 250° C and helium flow rate at 1.0 mL/min. The EO was injected in split mode (split ratio – 1:160) and ion fragments in the range of 40 – 650 m/z were scanned with a scan speed of 1428. The mass spectra of the components were compared with the standard mass spectral library of NIST/WILEY and identified by similarity search (Adams, 2007). The identification was confirmed based on their retention indices calculated using the formula suggested by Vandendool and Kratz (1963) by injecting homologous series of n-alkanes standard (C8-C40).

Statistical analysis

The data were analysed in SAS software and the treatment means (± S.E.) were compared by Duncan's multiple range test (DMRT) (p < 0.01 and p < 0.05). Multivariate analysis namely partial least square discriminant analysis (PLS-DA) was conducted on the identified metabolites using Metaboanalyst 5.0. Metabolites with significant differences among treatments were identified based on the variable importance in projection (VIP) scores (Xia and Wishart, 2011).

RESULTS AND DISCUSSION

The essential oil content of turmeric genotypes showed significant variation in response to elicitor treatment (Fig 1). In the genotype Pragati, the treatments P₃, C₂ and S₂ showed 13, 11 and 5 % increase in EO content, respectively over control. Whereas, Rajapuri genotype did not produce any statistically significant increase in the treated plants. In the genotype Acc.849, the treatments P₃ (9 %) and S₁ (9 %) has given significant increase in EO content as compared to control. By comparing the results, variation towards elicitors influence were found among the genotypes studied. Phenylalanine and salicylic acid treatments were effective in the genotype Pragati and Acc. 849 whereas, chitosan increased the EO content in Pragati. Our results were in consonance with earlier reported results of various crops (Pirbalouti *et al.*, 2019; Poogadhir *et al.*, 2020). Researchers all over the world tried to influence the terpenoid pathway to

enhance the volatile profiles of industrially relevant crops. The augmented production of terpenoids without transgenic approaches could be achieved in a limited extent using the application of elicitors (Hussain *et al.*, 2012; Ahmed *et al.*, 2020). The elicitors increased the content of essential oil by increasing photosynthetic carbon assimilation products as well as increasing the expression of key enzymes involved in terpenoid biosynthetic pathway (Srivastava *et al.*, 1990; Ahmed *et al.*, 2020; Vosoughi *et al.*, 2020). Few studies were available on the effect of chitosan, salicylic acid and phenylalanine on growth, physiology and curcumin content of turmeric, but our study is a first report on elicitor's effect on turmeric's volatile constituents.

The EO constituents analyzed using GC-MS threw more light on the effect of these elicitors on major volatile aroma compounds of turmeric rhizome. The statistically analyzed full data set is available in Tables S1-S3. Major sesquiterpenoid compounds identified in the EOs of genotypes used in the study were ar-turmerone (principal aromatic sesquiterpenoid), curlone (also known as β -turmerone), β -sesquiphellandrene, ar-curcumene, germacrone and zingiberene. Among monoterpene compounds, α -phellandrene, α -terpinolene, 1,8 cineole and cymene-8-ol occupied significant share in the turmeric EO.

In the genotype Pragati, relative peak area percentage of α -terpinolene showed significant increase in C₂ (3.57 %) and C₃ (3.54 %) as compared to control (3.29 %). All other treatments showed significant reduction of this compound. Elicitor treatments increased the content of ar-turmerone with the highest content detected in S₃ (51.68 %) followed by P₁ (51.49 %). Phenylalanine and salicylic acid were found to have positive influence on ar-turmerone content. The sesquiterpenoid compounds curlone and sesquiphellandrene has showed mutual exclusivity in chitosan treatments where former showed significant reduction whereas later showed significant increase in the content (C₁ - 7.56%; C₂ - 7.13 % and C₃ - 7.24 %) as compared to control (6.93 %). Chitosan treatments also produced significant increase in zingiberene content (C₁ - 7.22 %; C₂ - 6.58 % and C₃ - 6.75 %). These two sesquiterpenoids, sesquiphellandrene and zingiberene was responsible for the modest increase in its content in chitosan treated plants (Fig. 2).

In Rajapuri genotype, major monoterpene compounds, α -terpinolene and 1,8-cineole did not produce significant variation among treatments. The content of ar-turmerone was also not significant among treatments. On the contrary, the content of curlone was increased in P₃ as compared to control. Salicylic acid

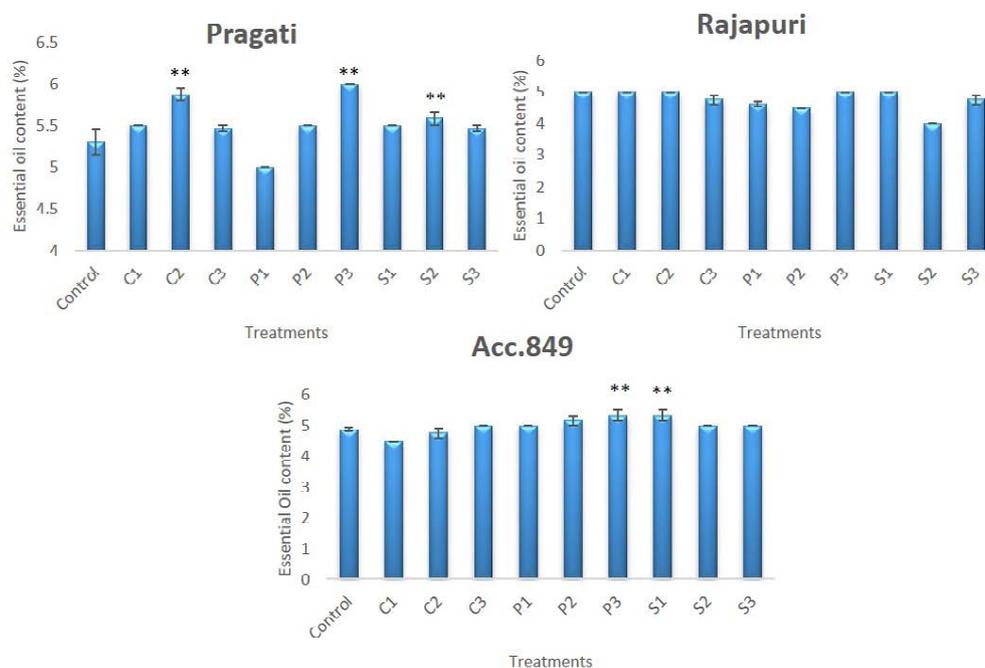


Fig. 1. Essential oil content of elicitor treated turmeric rhizomes (** indicates significant ($p < 0.01$) differences among treatments)

treatments produced some noticeable changes in the content of β -sesquiphellandrene and germacrone content as other treatments are either on par or registered lower content as compared to control. The influence of elicitors on monoterpene and sesquiterpene groups was also found negligible in this genotype (Fig. 2). Overall, influence of elicitors on volatile profile of this genotype is minimum.

In the genotype Acc.849, the main monoterpene compound α -terpinolene showed significant reduction in its content in elicitor treated plants as compared to control. The content of 1,8-cineole was the highest in salicylic acid treatment. Treatment related significant increase or decrease was not noted down in the content of ar-turmerone. Likewise, except in C₁ (6.24 %), all other treatments did not exhibit changes in the content of curlone. Another major sesquiterpene compound, β -sesquiphellandrene showed significant increase in P₁ (16.57 %) and P₂ (15.95 %) treatments over control (14.93 %). Likewise, P₂ (24.57 %) showed significant increase of zingiberene content over control (22.88 %). By comparing the results, the phenylalanine treatments had good influence on the volatile content of the genotype Acc.849. The phenylalanine treatment produced significant decrease in monoterpene content in this genotype. On the other hand, salicylic acid

produced increase in total monoterpene compounds with subsequent reduction in sesquiterpenoid compounds (Fig. 2) in this genotype.

The 2D plot of PLS-DA showed more pronounced treatment related variation in the genotype Pragati and Acc.849 (Fig. 3). In the genotype Pragati, P₁ treatment group is found distinct and distant from all other group. Likewise, C₁ treatment also showed distinct grouping as compared to other groups. When this was compared with VIP score, we found that compounds like ar-curcumene, zingiberene, β -sesquiphellandrene, ar-turmerone, α -bergamotane, α -bisabolene, curlone, α -himachalane and nerolidol with score >1 are the source of variation among the treatment groups. The previous results of absence of major influence of elicitors on the volatile constituents of the genotype Rajapuri was confirmed in the PLS-DA also. The 2D score plot of this genotype showed no distinct grouping of any treatments compared to control. If sesquiterpene compounds dominated the variation caused in the genotype Pragati, the equal influence of some monoterpene and sesquiterpene compounds are observed in Acc.849. Compounds with >1 VIP score are isoborneol, α -phellandrene, 4-terpineol, β -farnasene, α -humulene, curlone, α -terpinolene, camphor, 1, 8 cineole, nerolidol, ar-curcumene and cymene. In the 2D score plot, the treatments C₁ and

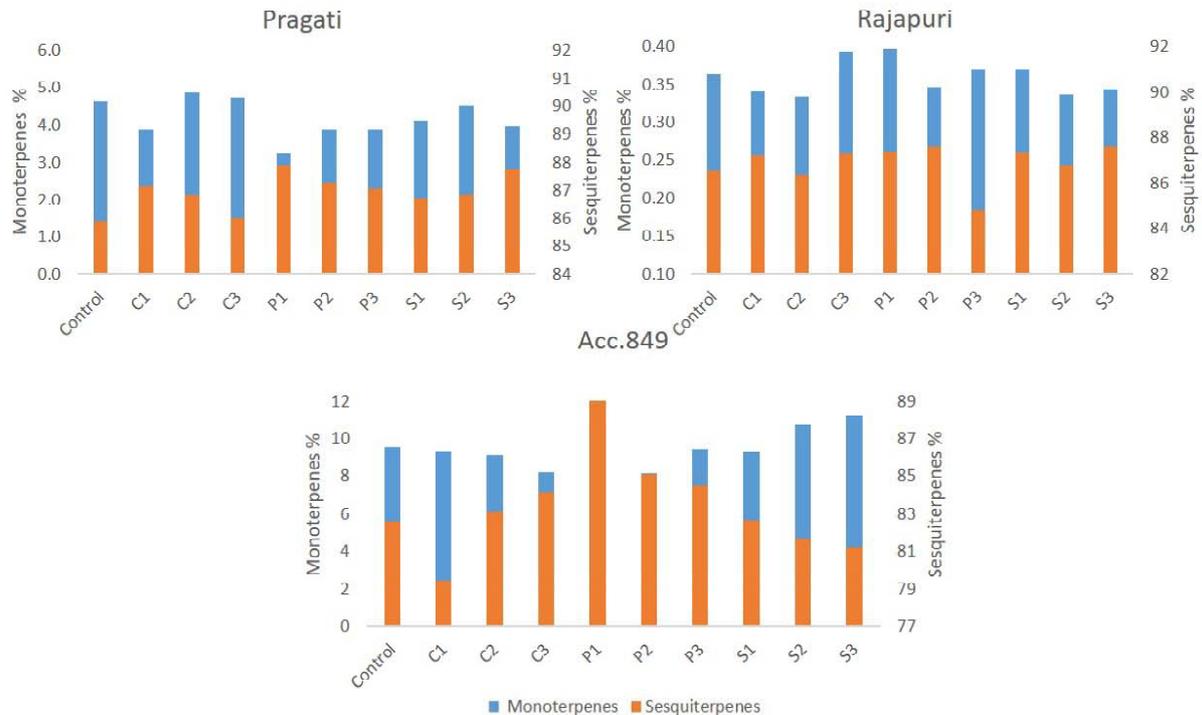


Fig. 2. Monoterpenes and sesquiterpenes content of elicitor treated turmeric rhizomes

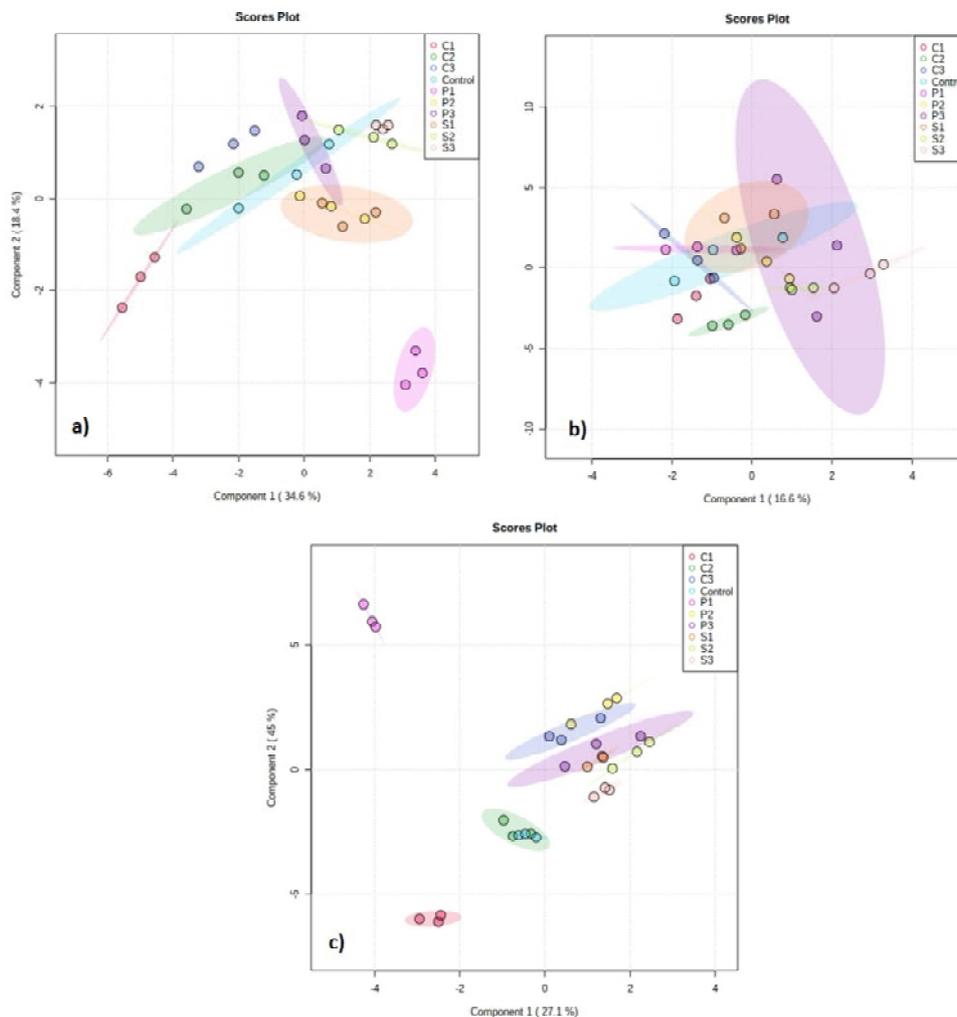


Fig. 3. 2D score plot of PLS-DA analysis of elicitor treated turmeric rhizomes a) Pragati b) Rajapuri c) Acc.849

P₁ showed distinct grouping as compared to control and other treatment groups. The results of multivariate analysis confirmed the differential influence of elicitors on volatile constituents for the three genotypes studied.

Our research finding of increased EO content in elicitor treated plants were supported by previous studies which showed that foliar application of elicitors like chitosan, salicylic acid and phenylalanine increased the quantity and quality of essential oil in different crops (Reham *et al.*, 2016; Ahmed *et al.*, 2019; Garde-Cerdán *et al.*, 2018; Alizadeh *et al.*, 2020; Goudarzian *et al.*, 2020; Momeni *et al.*, 2020). Foliar application of chitosan not only enhanced EO content but also increased the concentrations of monoterpene compounds namely limonene, 1,8-cineole, β -thujone and α -humulene in sage plant (Vosoughi *et al.*, 2018). Similar results were observed in our study in the genotype Pragati.

The foliar spray of phenylalanine as growth regulator and elicitor to improve the volatile profiling of few crops were available. In grapes, foliar spray of phenylalanine increased the relative content of volatile compounds such as benzyl alcohol, total benzenoids (aromatic compounds) and total positive compounds whereas total terpenoids and hexen-1-ol were decreased as compared to control (Garde-Cerdán *et al.*, 2018). In our study also, we found that phenylalanine treatment increased the content of β -sesquiphellandrene and zingiberene in the genotype Acc.849 and increased the content of ar-turmerone in the genotype Pragati. Phenylalanine application increased not only the growth and metabolism of crops, but also the biosynthesis of secondary metabolites including terpenoids (Gonda *et al.*, 2018; Poorghadir *et al.*, 2020). Elsayed *et al.* (2022) reported that foliar spray of phenylalanine increased

the monoterpene hydrocarbons in bitter fennel, which was not observed in our study. Alternately, we found increase in sesquiterpenoid hydrocarbon content in phenylalanine treatment especially in Pragati and Acc.849 genotypes.

Likewise, foliar spray of salicylic acid was reported to improve the EO yield and constituents by increasing the growth, nutrient uptake and induction of enzymes involved in terpenoid biosynthesis (Pirbalouti *et al.*, 2014; Mohammadi *et al.*, 2019). Our study also found the positive influence of salicylic acid on sesquiterpenoid in general and ar-turmerone content in particular in the genotype Pragati. Momeni *et al.* (2020) studied the effect of foliar spray of chitosan and salicylic acid on EO content and constituents of Mediterranean thyme (*Thymbra spicata* L.). They reported that the content of carvacrol, the predominant essential oil constituent is also increased in the plants sprayed with salicylic acid and chitosan.

The increase in volatile constituents like ar-turmerone, curlone and β -sesquiphellandrane observed in our study is in congruence with above mentioned literatures. We also observed increase in photosynthetic pigments and photosynthetic rate with the elicitor application (Sivaranjani *et al.*, 2022) in turmeric which could have increased the supply of base carbon compounds to terpenoid biosynthesis. Being a vegetatively propagated crop, genetic improvement to increase beneficial volatile constituents in turmeric rhizome is a limiting factor which could be alleviated by elicitor application to considerable extent. Our study was first of its kind in this direction by including varied turmeric genotypes in the field experiment. Since this is an open field experiment under natural growing conditions, the concentration-dependent decrease or increase in volatile constituents were not observed in our study.

CONCLUSION

The influence of different elicitors was not uniform among different genotypes. The study concluded that the short duration turmeric genotype, Pragati has responded well with respect to EO content by elicitors application. Phenylalanine treatments increased the percentage of sesquiterpenoids in Pragati and Acc.849 genotypes. Chitosan at 200 ppm, phenylalanine at 1 mM and salicylic acid at 0.1 mM could be sprayed to increase the ar-turmerone content in these genotypes.

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