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Impact of organic and inorganic fertigation on the preliminary phytochemical constituents of petals of *Rosa bourboniana*

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Abstract
The primary objective of the study is to find the potentiality of organic (combination of vermicomposts prepared from cow dung. press mud, tea dust and vegetable wastes in the ratio of (1:1:1:1) and inorganic fertilizer (NPK, 1:2:1 ratio) amendment on the preliminary phytochemical constituents of petals of *Rosa bourboniana*. The plot with no amendment served as the control. The experiment was arranged in a complete randomized block design (CRBD). An extrapolated value of 6 tons ha $^{-1}$ of amendments were used during the overall 330 days of cultivation period. At the end of the cultivation period the petal extracts of *Rosa bourboniana* with various solvents viz., Hexane, chloroform, ethyl acetate and ethanolic fractions were evaluated for preliminary phytochemical analysis. In the present study petals of *Rosa bourboniana* showed positive results for the presence of carbohydrates, glycosides, cardiac glycosides, flavonoids, alkaloids, tannins, steroids, phenols and aminoacids within hexane, chloroform, ethyl acetate and ethanolic fractions. The preliminary phytochemical screening of petals of *Rosa bourboniana* showed significantly higher rates for the presence of various phytochemicals. Since phytochemical screening gives the exact profile of the phytochemical quality of plants the results obtained with the higher content of phytochemical constituents in the flowers from the vermicompost amended plot (T1) confirms the positive role of vermicompost in modifying the phytochemical content of *Rosa bourboniana* comparing to that of the chemical fertilizer. Levels of phytochemicals are found to be lower in the flowers from the chemical fertilizer amended plot (T2) and slightly higher than the control plot (T3).

Keywords: *Rosa bourboniana* vermicompost, Inorganic fertilizer, phytochemicals

Introduction
Rose is a perennial ornamental plant belonging to the family Rosaceae which includes 200 species and 18,000 cultivars **[1].** The plants form a group of erect shrubs and climbing plants with hispid stems armed with sharp prickles. The flowers are large, attractive, showy and of multi colours. The wild species are mostly shrubs, distributed in the temperate zones of the northern hemisphere [2]. The cultivation practise of roses dates back to about two thousand years. In the era of the Han dynasty (141- 87 BC) roses were used to decorate the gardens of the royal palace in China [3]**.** Similarly, roses go far back in history in west asia and europe, particularly in eastern countries where people used to cultivate roses for oil extraction as well as for the purpose of beautification. Further promotion of roses extended to Egypt, Greece and the Roman Empire from the eastern countries. Blooms are mainly used for showy purposes and for oil extraction. From ages, mankind have been involved to make new scent by using the foliage and blooms to stimulate the surroundings. The utilization of Damask rose dates back to 1500 years ago. Rose petals are widely used in perfumes, creams, soaps, lotions, rose concentrate, rose absolute and other cosmetic products [4]. Petals are also used as flavoring ingredients in various food products like cakes, cookies, confectionaries, sweets, ice creams, jams, syrups, gulkand and in making jams.

Plants and Phytochemicals
Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients [5]. Phytochemicals are accumulated in different parts of the plants, such as in the roots, stems, leaves, flowers, fruits or seeds in varying compositions. They protect plants from disease and contribute to the plant's color, aroma and flavor. In general, the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals [6]. The use of plants as medicines goes back to early man. Certainly the great civilizations of the

ancient Chinese, Indians and North Africans provided written evidence of man's ingenuity in utilizing plants for the treatment of a wide variety of diseases.

A group of phytochemicals known as secondary plant metabolites have biological properties such as antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, modulation of hormone metabolism and anticancer property over living systems. Phytomedicine almost went into extinction during the first half of the 21st century due to the use of the 'more powerful and potent synthetic drug'. However, because of the numerous side effects of these drugs, the value of medicinal plants is being rediscovered as some of them have proved to be as effective as synthetic medicines with fewer or no side effects and contraindications. It has been proved that although the effects of natural remedies may seem slower, the results are sometimes better on the long run especially in chronic diseases. Recent research studies have investigated and reported that plant phytochemicals can be used for the treatment of a number of ailments like diabetes, dysentery, diarrhoea, cough, asthma, bleeding disorders, bronchitis, fever, AIDS, inflammation, ulcers, malaria, prostate cancer, hypertension, atherosclerosis, hyper lipidemia, male infertility, infant brain ischemia and obesity [7].

Roses apart from commercial perfumery and commercial cut flower crops, flowers with its rich content of phenols, tannins, flavonoids, steroids and anthraquinones were also used for medicinal purpose [8]. In ancient medical books several therapeutic effects of roses are stated such as for the treatment of abdominal and chest pain, strengthening of heart, treatment of menstrual bleeding, digestive problems and anti-inflammation are reported. North American Indian tribes use a decoction of the root of *Rosa damascena* plant as a cough remedy to ease children's cough and as a gentle laxative. Essential oil from *Rosa damascena* is reported to have analgesic, hypnotic, antispasmodic and anti-inflammatory effects [9]. Since only very few varieties of roses have been evaluated for medicinal value and lack of much medicinal properties of *Rosa bourboniana*, the present study was designed to analyze some of its phytochemical constituents from its petal extracts.

Cultivation of *Rosa bourboniana* under field condition

Cultivation of *Rosa bourboniana* was done based on the methods described by Nagaraja [10]. Cultivation study was done from January 2012 to February 2014. The study area is located in the Gummidipoondi (National Highway NH5) Panchayat union of Thiruvallur District located geographically at 13.41° North latitude, 80.12° East longitude and 17 meters elevation above the Mean Sea Level (MSL) located at a distance of about 42 Km from Chennai, Tamilnadu, India. The temperature in the study area ranged between 35 ºC - 40 ºC in summer and 27 ºC – 30 ºC in winter. The study area received rainfall in both seasons namely South West Monsoon prevailing from June to September (451.6 mm) and North East Monsoon (589.3 mm) prevailing from October to December every year.

Land preparation for cultivation of *Rosa bourboniana*

The land selected for cultivation was well prepared and leveled prior to cultivation. The field selected for cultivation was such that the plants can get proper sunlight for at least 6 hours in a day in every season avoiding shade beneath trees and root competition with those of rose plants for available nutrients and moisture. Double-dig bed raise method was adopted for the cultivation of *Rosa bourboniana*. The top soil was removed, mixed and tiled to a depth of about 2- 4 meters facilitating aeration to roots, good drain and easy penetration of roots deeper into the soil. Double-digging provided a reservoir of steady nutrients and sufficient water which can be accessed by rose plants. A tractor was employed for leveling the cultivation bed. Both clockwise and anti-clockwise mode of leveling was done during the preparation of cultivation bed. Pebbles and rocks were handpicked manually. Weeds, grasses and other herbs were removed that colonized the field. The cultivation bed was leveled in such a way to ensure proper drain during rainy season.

Plot preparation, experimental design and treatment details

The experimental plot was a completely randomized block design (CRBD). A plot was designed in the prepared cultivation bed. The size of the study plot was in an area of 1,000 sq. ft with equal length and width. Three rows were partitioned so as to plant rose seedlings in single line pattern. Each row was planted with ten siblings of *Rosa bourboniana.* Plants were planted at a spacing of 60 cm × 30 cm. The reason for maintaining space is to avoid collision of plants and to ensure proper aeration when it attains a bushy nature. The plot was designed in such a way to prevent nutritional mixing with the adjacent rows. Each row is designated for the application of its respective amendments. T1 is the Organic plot (Mixture of vermicomposts prepared from cow dung. press mud, tea dust and vegetable wastes in the ratio of (1:1:1:1), T2 (Chemical fertilizer plot, NPK in 1:2:1 ratio) and T3 (Control plot). An extrapolated value of 6 tons ha $^{-1}$ of amendments were used during the overall 330 days of cultivation period. The experiments were done in triplicates. The plants were irrigated daily during peak summer and as per requirement during rainy season. Irrigation was done by canals constructed from bore wells to the study plot.

Collection of floral material

For phytochemical analysis fully opened floral blooms were plucked in the early hours of dawn and was collected in separate labeled wet cloth bags and carried to the PG & Research Department of Botany, Pachaiyappa's college, Chennai for further studies.

Processing of floral material for solvent extraction

The plucked flowers were rinsed in distilled water to get rid of contaminants adhering to the petals. The petals were drawn and air-dried under shade for a week at room temperature. The dried petals were segregated and pulverized to fine coarse powder in a blender and sieved through 1 mm sieve.

Preparation of crude extracts

Floral extracts were prepared by serial extraction involving successive extraction with organic solvents of increasing polarity starting from a non-polar to a polar solvent (Hexane, Chloroform, Ethyl acetate and Ethanol). About 100 grams of weighed sieved petal powder was extracted with 1000 ml of each solvent twice with overnight incubation at room temperature for 48 hours. The individual extracts were filtered using Whatmann filter paper No. 1. The filtrate was processed in a vacuum evaporator under reduced pressure to recover the excess solvents for further use. The sticky extract obtained was dried in an oven at 32 ºC and stored in vials at 4 ºC for further analysis.

Materials and methods

Qualitative phytochemical screening of the prepared Hexane, Chloroform, Ethyl acetate and Ethanol flower extracts were performed as per the protocols stated by Sofowora [11], Trease [12], Evans [13], Mace [14], Kokate [15] and Harborne [16].

1. Test for Carbohydrates

Exactly 5 ml of distilled water was added to individual extracts and dissolved. The vortexed mixture was filtered and the filtrate was used to screen for the presence of Carbohydrates.

2. Fehling's test

The filtrate was hydrolysed with dilute HCl acid and neutalised with alkali. It is heated in water bath with the addition of 6-7 drops of Fehlings A & B solution. Appearance of red coloured precipitate indicates the presence of Carbohydrates.

3. Test for Glycosides

About 50 mg of extract was hydrolysed with concentrated HCl for a duration of 2 hours by keeping in a water bath. The solution was filtered and the filtrate was subjected to further tests.

4. Borntrager's test

About 3 ml of chloroform was added to 2 ml of filtrate and shaken well. On seperation of the two phase, chloroform layer was pipetted out and 10 % of ammonia solution was added in drops. Appearance of pink colour indicates the presence of Glycosides.

5. Test for Cardiac Glycosides

Keller-Kiliani test

About 0.5 g of extract was treated with 2 ml of glacial acetic acid and 2 drops of 5 % ferric chloride solution. The mixture was underlayered with 1 ml of concentrated H_2SO_4. Brown ring formation at the interface confirms the presence of Cardiac Glycosides.

6. Test for Anthraquinones

About 0.5 g of extract was boiled with 10 % of HCl in a water bath for few minutes. The mixture was filtered and cooled. equal volume of $CHCl_3$ was added in drops. It is further heated gently with the addition of 10 % NH_3. Appearance of rose pink colour indicates the presence of anthraquinones.

7. Test for Flavonoids

Alkaline reagent test

About 0.5 g of extract was treated with few drops of NaOH solution. Appearance of intense yellow colour which disappears on addition of dilute acid confirms the presence of flavonoids.

8. Test for Alkaloids

Mayer's test

Extracts were dissolved in dilute HCl individually and filtered. The filtrate was treated with 2-3 drops of Mayer's reagent (Potassium Iodide). Formation of yellowish green or creamy white precipitate indicates the presence of alkaloids.

9. Test for Tannins

About 1 g of extract was treated with 2 ml of 5 % ferric Chloride solution. Formation of dark blue or greenish black colour confirms the presence of Tannin.

10. Test for Steroids

Salkowski's test

About 1 gm of extract was treated with 2 ml of Chloroform and 2 ml of Cocentrated H_2SO_4 and shaken well. Formation of red colour at the chloroform layer and greenish fluorescencent yellow colour at the acid layer indicated the presence of steroid (Terpenoid).

Liebermann Burchard test

To 2 g of extract, 1 ml of Liebermann-Burchard reagent was added and shaken well. Formation of blue-green colour indicates the presence of triterpenoids.

11. Test for Phenols

About 1 g of extract was treated with 2 ml of distilled water followed by the addition of few drops of 10 % ferric chloride solition. The formation of blue or green colour confirms the presence of phenol.

12. Test for Amino acid

Ninhydrin test

To 2 g of plant extract was treated with 2-3 drops of 0.2% Ninhydrin reagent and heated for 5 minutes in a water bath. Formation of blue colour indicates the presence of protein.

Results

The preliminary phytochemical tests performed and the results obtained with hexane, chloroform, ethyl acetate and ethanol extracts of the flowers of *Rosa bourboniana* cultivated under organic and inorganic supplements are shown in Table. Positive results were obtained for all the phytochemical constituents tested. The ethanol extract was tested positive for carbohydrates in all the three plots. In the T1 and the T3 plots only a moderate level of activity was observed while in the T2 plot the activity was in mild level. It was completely absent in hexane, chloroform and ethyl acetate extract. An appreciable amount of glycoside was observed in the T1 plot, while it was in moderate level in the T2 plot and it was very mild in the T3 plot and completely absent in the other extracts.

In the T1 plot cardiac glycoside was tested positive with mild level in the chloroform extract and in moderate level in the ethanol extract. Whereas in the T2 plot mild level was noticed in the chloroform and ethanol extract, while a mild level of cardiac glycoside was observed in the ethanol extract of the T3 plot. Anthraquinones were moderately present in the ethyl acetate extract and in mild level in the ethanol extract while it was in mild quantities in both the ethyl acetate and ethanol extracts. Whereas it was tested positive only in the ethyl acetate extract of the T3 plot. Flavonoids were found in moderate level for hexane extract while it was in appreciable amount in the ethanol extracts of the T3 plot. But in the T2 plot the hexane extract was tested with mild level while the ethanol extract was found to be with moderate level, whereas only a mild level for flavonoids were observed in the hexane and ethanol extracts of petals of *Rosa bourboniana.*

The level of alkaloid was in appreciable amount in the ethyl acetate extract and it was in moderate level in the ethanol extract of the T1 plot. The T1 plot was tested with an appreciable amount of alkaloids in the ethyl acetate extract and in mild level in the ethanol extract. While the T2 plot was recorded with a mild level of alkaloid in the ethyl acetate and ethanol extracts of the T2 plot.

Tannin was observed in all the extracts except the hexane extract of all the three plots. The chloroform extract of the T1 plot showed a moderate level of tannin content while it was in mild level in the T2 and the T3 plot in the same extract. Whereas, the ethyl acetate and ethanol extract of all the three plots showed mild level of tannins. The ethyl acetate extract of the T1 plot showed an appreciable amount of steroids, while that of the T2 plot and T3 plot showed only mild levels while the ethanol extracts of all the three plots showed only mild levels of steroid content in the flowers of *Rosa bourboniana*.

The hexane, chloroform and ethanol fractions of the flowers from all the three selected plots showed positive results for the presence of phenols and mild level of phenol was observed in the hexane extract of all the three plots. While a moderate level of phenol content was observed in the chloroform extracts of the T1 plot and the T2 plot, the same was in mild level in the T3 plot. The ethanolic fractions from the T1 plot were recorded with an appreciable amount of phenols while only a moderate level was observed in the T3 plot and a mild level in the T2 plot. The amino acid level of flowers of *Rosa bourboniana* was in mild level in the hexane extract of the T1 plot and the T2 plot. While interestingly it does not show any result for the hexane extract of the T3 plot. The ethyl acetate extract from the flowers of the T1 plot showed an appreciable amount of amino acid while the level was in moderate level in the other two plots.

Table. Comparative preliminary qualitative analysis of the flowers of *Rosa bourboniana* from the Organic (T1), Inorganic (T2) and Control (T3) plots

Phytochemical constituents	Observations											
	Hexane extract			Chloroform extract			Ethyl acetate extract			Ethanol extract		
	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3
Carbohydrate	++	+	+	-	-	-	-	-	-	++	+	++
Glycoside	+++	++	+	-	-	-	-	-	-	-	-	-
Cardiac glycoside	-	-	-	-	+	+	-	-	-	++	+	+
Anthraquinone	-	-	-	-	-	-	++	+	+	-	+	+
Flavonoid	++	+	+	-	-	-	-	-	-	+++	++	+
Alkaloid	-	-	-	-	-	-	+++	+	++	++	+	+
Tannin	-	-	-	++	+	+	+	+	+	+	+	+
Steroid	-	-	-	-	-	-	+++	++	+	+	+	+
Phenol	+	+	+	++	++	+	-	-	-	+++	+	++
Amino acid	+	+	-	-	-	-	+++	++	++	-	-	-

+++ Appreciable amount
++ Moderate level
+ Mild level
- Absent

Discussion

Biologically active phytochemicals are naturally occurring chemical compounds found in plants. Phytochemicals work as a defense mechanism and are known to protect plant from various pathogens. Recent researches have proved that such phytochemicals can even protect human beings from various diseases in a broad range of spectrum. Plants are rich in wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, glycosides, saponins and flavonoids. These metabolites are found in varying quantities in various plant species and show considerable variation according to their geographical distribution. These phytochemicals are known to possess antibacterial, antifungal, antioxidant, anti-inflammatory and anticancer properties.

Preliminary phytochemical screening of the flowers of *Rosa bourboniana* from the three selected plots of study (T1, T2 and T3) were tested positive for the presence of different secondary metabolites in different qualitative variance. The positively tested phytochemicals includes carbohydrates, glycosides, cardiac glycosides, anthraquinones, flavonoids, alakaloids, tannins, steroids, phenols and aminoacids in their respective solvents. Levels of secondary metabolites were higher in the flowers from the vermicompost amended plot (T1) than that grown in the inorganic (T2) and control (T3) plots.

In the present study petals of *Rosa bourboniana* showed positive results for the presence of carbohydrates, glycosides, cardiac glycosides, flavonoids, alkaloids, tannins, steroids, phenols and aminoacids within hexane, chloroform, ethyl acetate and ethanolic fractions. Previous studies with aqueous and ethanolic fractions of petals of common *Rosa damascena* were tested positive for the presence of carbohydrates, tannins, proteins, aminoacids, alkaloids, steroids, flavonoids and glycosides [17]. Seldom reports are available with the phytochemistry of *Rosa bourboniana.* These phytochemicals are known to play an important role in the branch of medicine. Flavonoids are known to carry out antioxidant, protective effects and inhibit the initiation, promotion and progression of tumor cells [18] and also take part in the modulation of estrogen levels in humans [19].

Phenolics are known to suppress the growth and development of pathogens. They also carry out antioxidant activity and a wide range of pharmacologic activities which includes anticancer, antioxidant and the inhibition of platelet aggregation [20]. Steroids are the compounds of importance in pharmacy due to their role in sex hormones [21]**.** Steroids as such are directly implicated in the reduction of risks of coronary heart and neurodegenerative diseases in postmenopausal women [22].

The preliminary phytochemical screening of petals of *Rosa bourboniana* showed significantly higher rates for the presence of various phytochemicals. Since phytochemical screening gives the exact profile of the phytochemical quality of plants the results obtained with the higher content of phytochemical constituents in the flowers from the vermicompost amended plot (T1) confirms the positive role of vermicompost in modifying the phytochemical content of *Rosa bourboniana* comparing to that of the chemical fertilizer. Levels of phytochemicals are found to be lower in

the flowers from the chemical fertilizer amended plot (T2) and slightly higher than the control plot (T3).

Surprisingly the alkaloid content of flowers from the control plot is found to be higher than that of the plot amended with chemical fertilizer which strongly confirms the destructive nature of chemical fertilizer and concludes that plants grown under the influence of chemical fertilizer is poor in alkaloid content and a rich content of alkaloids is observed in the flowers from the plants grown with vermicompost (T1).

The presence of rich qualities of phytochemicals in the flowers of organic plot is attributed to the rich content of chlorophyll in the leaves of the plant. Since a rich network of chlorophyll synthesizes rich contents of metabolites necessary for plant metabolism supplemented through nutrient rich fertilizer. Vermicompost rich in nutrients like N, P, K, Ca, Mg, Fe, Mn and Zn has a positive effect on the plant growth, yield, soil fertility and soil microbes. Increase in the photosynthetic pigment content with the supplement of nutrient vermicompost was observed in red chilly [23]. Similar such findings were also observed in the leaves of pistachio (*Pistacia vera*) seedlings in which the observed photosynthetic rates accompanied by leaf area index (LAI) were better in vermicompost treatment comparing to the control without vermicomposts [24].

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