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EFFECT OF *WITHANIA SOMNIFERA* AND *SOLANUM NIGRUM* ON DROUGHT INDUCED STRESS IN WHEAT

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ABSTRACT:

In the following study, experiments were conducted to investigate the effects of *Withania somnifera* (WS) and *Solanum nigrum* (SN) (Solanaceae family) leachates on water deficit stress in Wheat. Drought stress caused significant decrease in growth parameters: (% seed germination shoot and root length, fresh and dry weight of shoots and roots) and photosynthetic pigments (chl a, chl b, carotenoids and total pigments). On the other hand, drought stress caused significant increase in antioxidant activity and osmolytes in wheat.

Withania somnifera and *Solanum nigrum* leachates were applied to the experimental pots containing wheat seeds at two different concentration(5% and 10%) enhanced all the above parameters than that of the control plants and drought stressed plants. The total phenolics and Flavanoid content of the leachates of WS and SN were also assessed which was in correlation with the antioxidant activity in the leachates. The study highlights the leachates from WS and SN not only enhanced the drought tolerance of wheat but also increased the yield parameters. The comparative study of both WS and SN show WS has more pronounced effect on the tolerance of drought stress and yield parameters in Wheat.

Keywords: *Withania somnifera Solanum nigrum* drought wheat abiotic stress

INTRODUCTION:

In natural environmental conditions plants growth and development are not only influenced by soil but are also affected by abiotic factors like drought, heat, heavy metal. In 2013, approximately 65 million ha of wheat production was affected by drought stress [1] . Drought is the most severe abiotic stress factor limiting plant growth and crop production [2] Drought stress induces several physiological, biochemical and molecular responses in several crop plants, which would help them to adapt to such limiting environmental conditions [3]. It inhibits the photosynthesis of plants, causes changes of chlorophyll contents and damages the photosynthetic apparatus [4]. It also inhibits the photochemical activities and decreases the activities of enzymes in the Calvin cycle [5].

Drought stress affects respiration, translocation, ion uptake, carbohydrates, nutrient metabolism and growth promoters. It imbalances reactive oxygen species (ROS) productions and the antioxidant defense system causing the accumulation of ROS which induces oxidative stress to biological molecules like protein, membrane lipids and disruption of DNA strands. [7] Among the common responses in plants to abiotic stresses is the production of different types of organic solutes which include small molecules called osmoprotectant and antioxidant such as proline [8] and soluble sugar [9] during stress. Wheat (Triticum aestivum L.) is one of the important rabi cereal crops cultivated in most parts of the world. It is the second important rabi cereal crop being next to rice in India [10]. Wheat is an annual plant belonging to family Graminae. It is cultivated almost in all parts of the world except in tropical regions. Among rabi cereals, wheat ranks second in acreage and production[11] . It can be grown in a extensive range of agro-climatic environments, but, many of these environments have drought stress as one of the major challenge to their production and productivity[12].

Withania somnifera and Solanum nigerum ,two weeds of Solnaceae family possess many medicinal and agronomically important traits like antioxidant activity, antibacterial[13,14]. The phytochemicals are reported to be present in almost all plant parts, including stems leaves, flowers, buds, pollen grains, seeds, fruits, roots,and rhizomes. The extracts of the SN contain many polyphenolic compounds. The leaves are rich in polyphenols, including phenolic acids and flavones [15]. *Solanum nigrum* leaves extract was able to reduce oxidative stress, extract had potential in preventing/alleviating stress-induced diseases, involving oxidative damage to cellular constituents especially the brain[16]. Antioxidant activity exhibited can be atrributed to the presence of the above-mentioned polyphenolic compounds present in the stem and leaves [17].

There is a necessity to study plant interaction to explore the potential to alleviate water deficit stress and their influence on plant yield .This study highlights the use of two weeds from Solanceae family and their effect on different growth parameters of wheat under drought stress.

MATERIAL & METHODS:

Plant material:

Triticum aestivum **(**Wheat) Lokwan variety seeds were used for study, were obtained from the Local market Ahmednagar. Plants from Solanaceae family ie *Solanum nigrum(SN)* and *Withania somnifera (WS)* were collected from local areas and authenticated .

The leachates of *Solanum nigrum(SN)* and *Withania somnifera (WS)* were prepared and analyzed for their Total phenolics, total Flavanoid content and antioxidant assays.

2.1 Determination of total phenol content (TPC) and total flavonoid content (TFC) and Antioxidant assays

The amount of total phenol and flavonoid content was determinedby Folin-Ciocalteu's reagent method and aluminium chloride colorimetric method respectively [18]. The antioxidant activity of the extracts was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity[19].

2.2 Plant cultivation and induced drought stress treatments:

Matured seeds were surface sterilized with 70 % ethanol for 2 min, followed by 10 min in 5 % sodium hypochlorite (v/v) and rinsed with sterile water for four to six times and then grown in pots containing equal amounts of homogeneous soil.

The seeds were sown at 2-3 cm depth in each pot on and when emergence was complete (~7days) the seedling density was reduced to 10 seedlings / pot. After 7 weeks from sowing, the plants were divided into four groups (5 pots/ group) and treated as follows:

- 1st Pot was without any treatments and served as control (Normal or well watering irrigation (W2D- watered after 2 days).
- 2nd Pot was subjected to drought stress (W6D- watered after 6 days) and 5% SN leachates was added.
- 3rd Pot was subjected to drought stress (W6D) and sprayed by 10% SN leachates.

- 4th Pot was subjected to drought stress (W6D) and sprayed by 5% WS Leachates
- 5th Pot was subjected to drought stress (W6D) and sprayed by 10% WS Leachates

When the developed plants reached 14 weeks, plants were carefully uprooted from the soil of each treatment where samples were analyzed.

Morphological traits of treated and untreated *Solanum nigrum(SN)* and *Withania somnifera (WS)* plants were measured. Plant height was measured with a manual scale. The plants were uprooted taken to the laboratory to measure plant height, shoot and root fresh and dry weights. Dry weight was measured after drying in an oven at 60 _C for 72 h.

2.2 A Determination of photosynthetic pigments:

Chlorophyll a, Chlorophyll b and Carotenoids were determined in soybean leaves. The spectrophotometric method recommended by was used [20]. The pigment contents were calculated as mg g-1 fresh weight of leaves.

2.2B Assay of non enzymatic antioxidant:

Ascorbic acid contents: Content of ascorbic acid was measured by recording absorbance at 530 nm.

Tocopherols contents: The absorbance of α-tocopherol was recorded at 520 nm against ethanol as a blank [21].

2.2 C Assay of osmolyte compounds:

Proline content was estimated by the method of Bates *et al* [22]. **Soluble sugars** were determined based on the method of phenol sulfuric acid as described by Dubois *et al* [23]. Pure glucose was used as standard.

Results

The effects of drought (D) stress on *Solanum nigrum(SN)* and *Withania somnifera (WS)* plants were analyzed for various morphological, growth, physiological, biochemical, phytochemical, parameters.

Changes in growth parameters: 1. Shoot and root length:

Water deficit stress caused significant decrease in shoot and root lengths in Wheat. The wheat provided with leachates of WS and SN show significant increase in length. The Shoot length has increased upto 45% in wheat provided with 5% leachates of WS and has increased upto 81.25 % in the wheat provided with 10% leachates of WS. The root length, Fresh weight shoot, dry weight shoot , fresh weight root and dry weight root show increase over control.

RESULT & DISCUSSION:

Drought stress significantly affects plant growth parameters like morphological, biochemical, fresh weight and dry weight. In present study the plant growth parameters of wheat (shoot and root length, fresh and dry weights of shoots and roots) decreased significantly with increasing drought stress as compared with control plants. The reduction in shoot and root length in response to water deficit might be due to either decrease in cell elongation, cell turgor, cell volume and eventually cell growth [24] and because of blocking up of xylem and phloem vessels . Treatment with WS and SN leachates caused increment in growth parameters. Drought stress caused significant change in the fresh and dry weight of shoots and roots of wheat as compared with control plants. Similar results were obtained by Prasad et al [25] who reported that both fresh and dry weights of shoots and roots of wheat decreased on exposure to heat and drought stress. For overcoming the stress induced deleterious effects plants increase the synthesis of phenolics, flavonoids, alkaloids, terpenoids, steroids, tannins, saponins, glycosides, and xanthoprotein [26].This is evident from the above study which shows increase in total flavanoid and phenolic content of WS and SN. It is believed that increased metabolite synthesis strengthens the non-enzymatic antioxidant system by altering peroxidation kinetics and maintaining the membrane fluidity [27]. The content of non enzymatic antioxidants increased in case of water deficit stress induced in wheat.

The reduction in chlorophyll content under drought stress has been considered a typical symptom of oxidative stress and may be the result of pigment photo-oxidation and chlorophyll degradation.

Inaddition, applying wheat plants with WS and SN Leachates lead to stimulation of the total photosynthetic pigments contents in leaves as compared with control plants There are very few reports on the use of weeds to stimulate the photosynthetic sytem and antioxidant mechanism in wheat. Morever the study also highlight the use of different concentration on leachates which may contribute to the allelopathic interaction of weeds and plants.

Ascorbic acid is one of the strongest non-enzymatic antioxidants that provide better protection against drought stress, regulation of cell elongation, protecting proteins and lipids and protecting cells against oxidative stress [28]. The increase in α - tocopherol content which observed under drought stress may be due to increase the activation of the expression genes responsible for the synthesis of tocopherols in plants [29]. In addition, spraying plants with leachates of WS and SN showed significant increase ascorbic acid, α- tocopherol contents of shoots above that of the corresponding controls.

The proline and soluble sugars content in shoots of wheat plants significantly increased under drought stress. ascorbic acid decreased the accumulation of proline under drought stress. Proline can act as a signaling molecule to modulate mitochondrial functions, influence cell proliferation or cell death and trigger specific gene expression, which can be essential for plant recovery from stress [30] .

The increase in amount of osmolytes indicate Plant is under stress condition. Application of WS and SN leachates increased fresh and dry weight of wheat plants. dry weight of roots, stems and leaves under drought stress. Withania somnifera(WS) is more potent and has a significant positive effect on growth parameters in wheat than Solanum nigerum(SN) compared to control plants.

CONCLUSION:

The present data suggest that leachtaes of WS and SN could trigger the activation of antioxidants in plants, which persists in the plants to alleviate the oxidative damage, leading to improvements in physiological attributes for the plants growth under drought conditions. WS and SN leachates can be used to alleviate the adverse effect of drought stress. The WS leachtate is more effective than SN.

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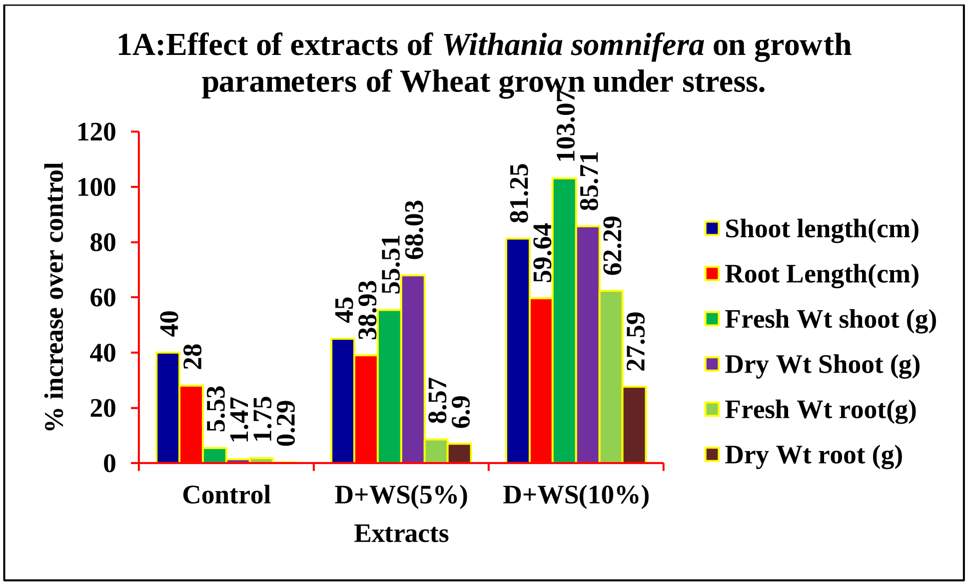


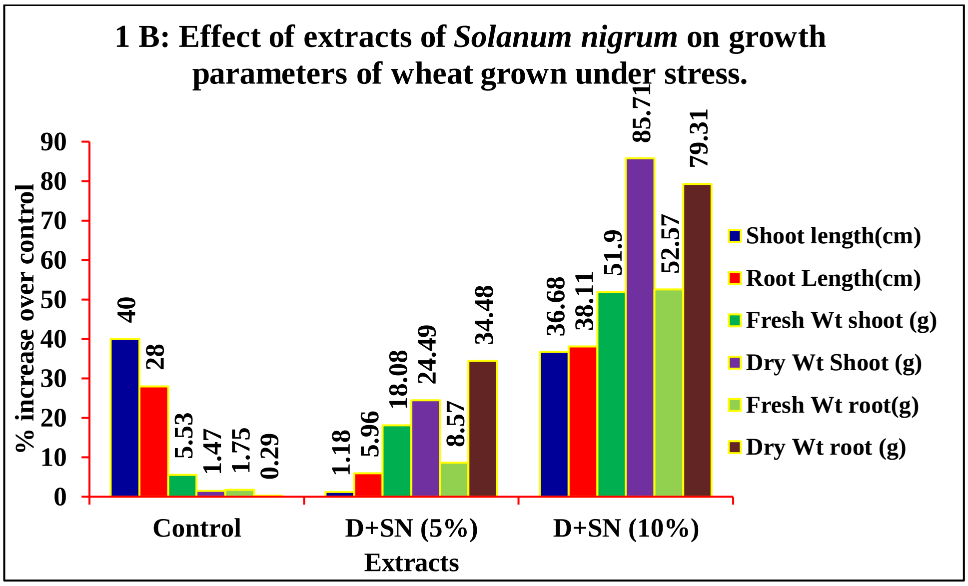
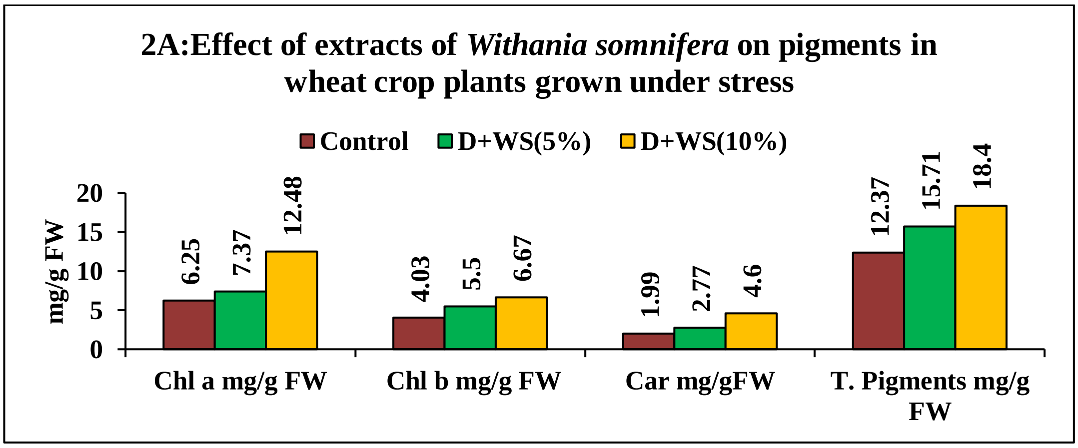


Fig 1: Comparative effect of SN and WS at 5% and 10 % on morphological parameters in wheat at drought stress



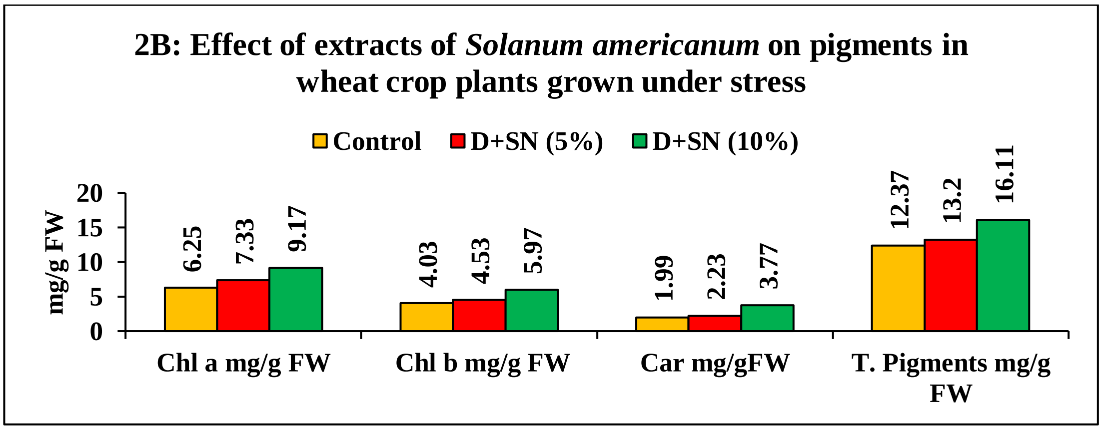


fig 2: Comparative effect of SN and WS at 5% and 10 % on photosynthetic pigments wheat at drought stress.

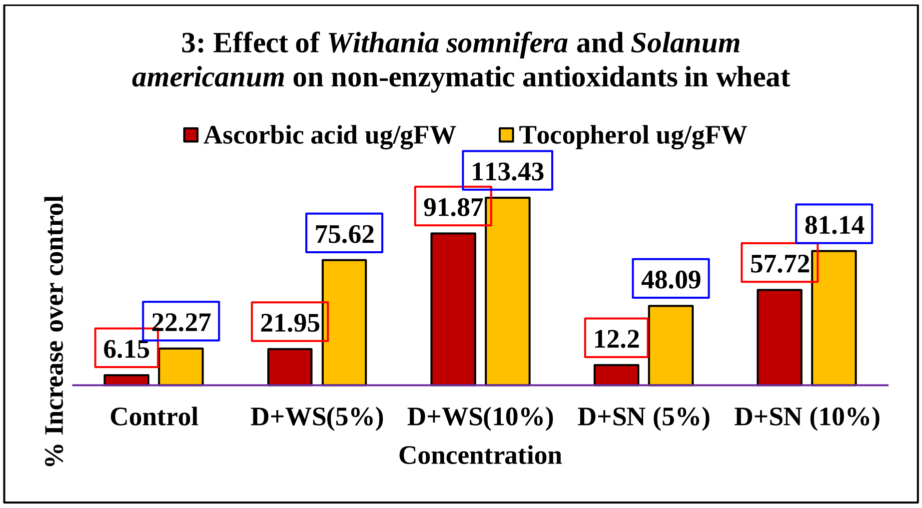
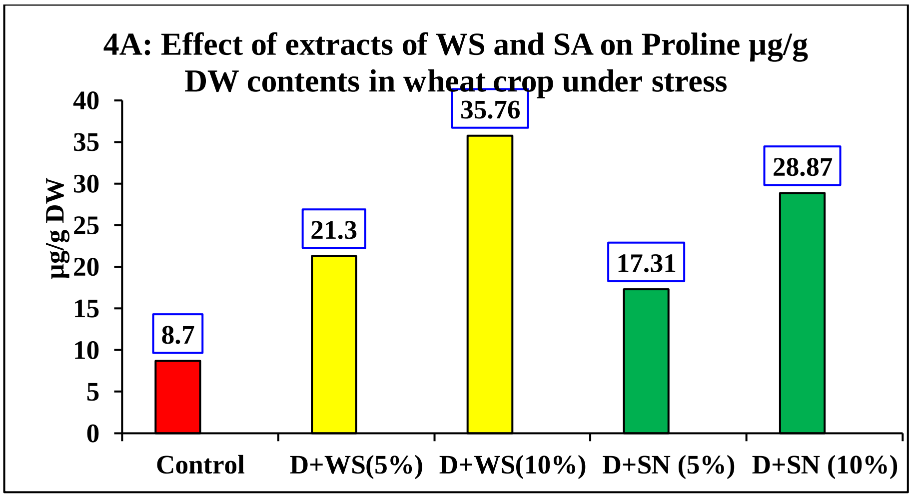


Fig 3: Comparative effect of SN and WS at 5% and 10 % on non enzymatic antioxidants wheat



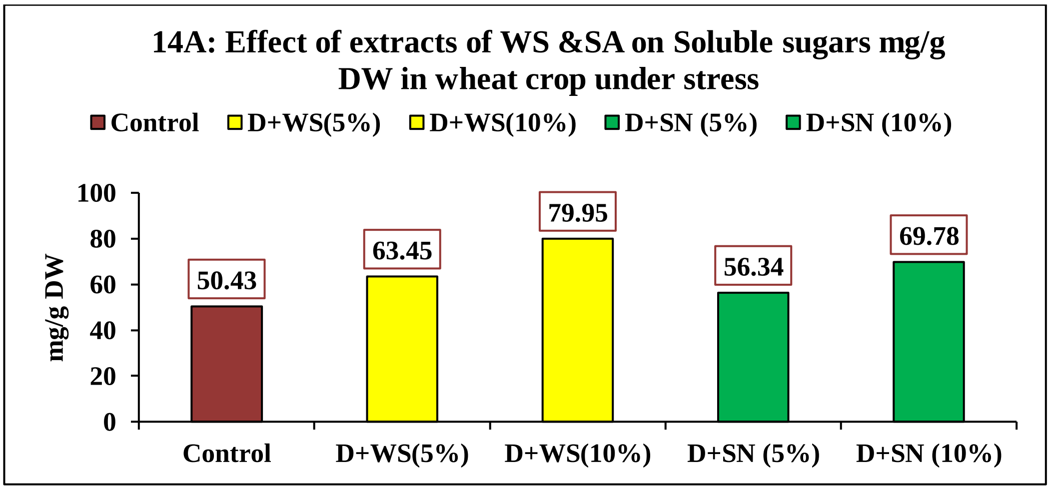
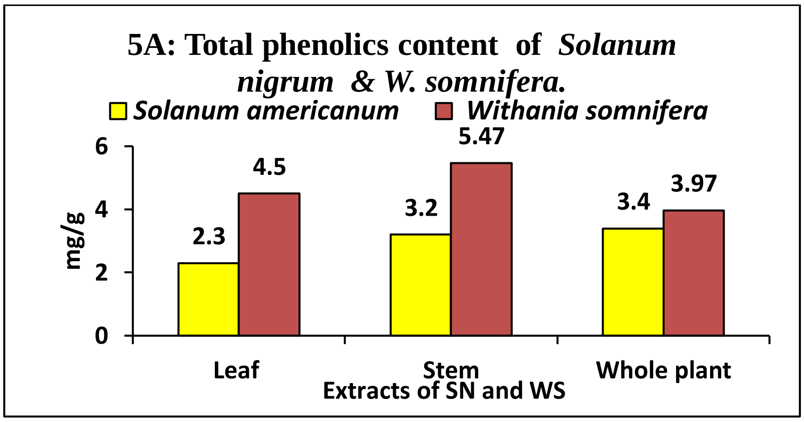


Fig 4: Comparative effect of SN and WS at 5% and 10 % on proline and soluble sugars in wheat under drought stress



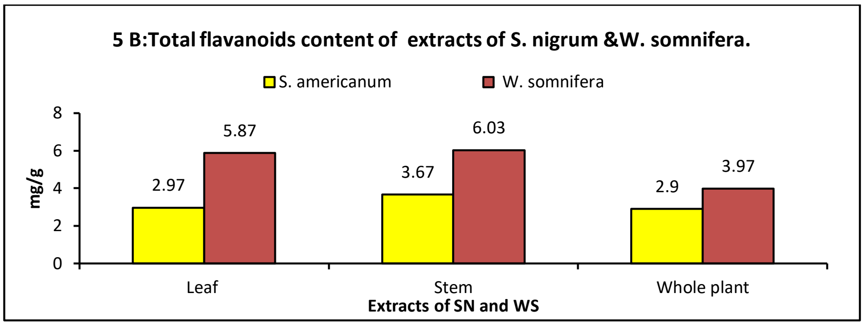
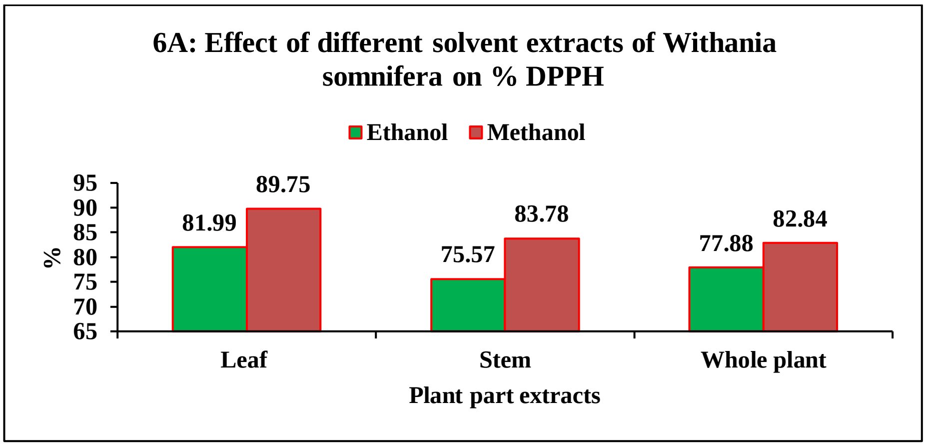


Fig 5: Comparative account of TFC and TPC in WS and SN



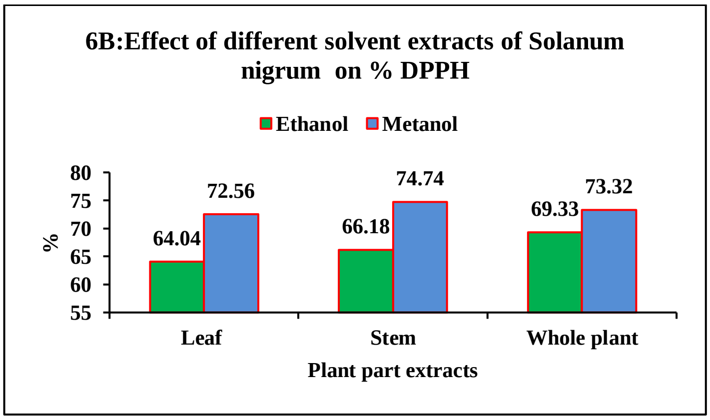


Fig 6. Effect of different solvents extracts of in WS and SN on % DPPH